Biolife Manual

Manual of Microbiological Culture Media

4th Edition

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4th Edition

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Section 1

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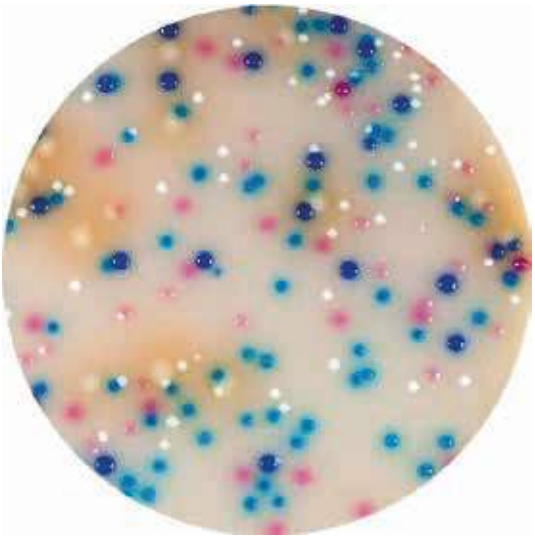


BIOLIFE & MICROBIOLOGY

ABOUT THIS MANUAL

The first edition of Biolife Manual in Italian was published in 1979. It was followed by the first English edition in 1985. Second editions were released in 1985 (Italian) and in 1991 (English). The third editions were published in 1998 (Italian) and 2003 (English).

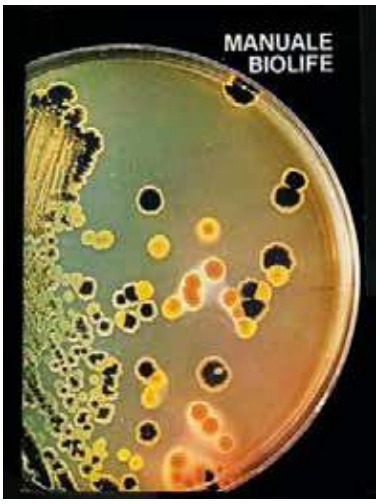
Since the last edition many improvements have been made in the Biolife products portfolio and the updating of technical sheets/instructions for use.
We have decided to summarized all these updates and improvements in this new Manual which is the 4th edition.

The 4th edition is organized in 3 Sections:

- The 1st Section provides general information about the company, product range, regulatory affairs, documentation provided to the users, and general information about culture media: their history, components, general directions for preparation, limitations, warning and precautions, storage conditions. This section also includes workflows and isolation schemes for the fields of clinical microbiology and food, water, environmental, pharmaceutical and cosmetic microbiology.
- The 2nd Section includes the monographs of microbiological culture media listed in alphabetical order. The monographs refer to dehydrated culture media but also contain information about selective supplements and enrichments required for their preparation and the corresponding ready-to-use media, if available. Some monographs are accompanied by photographic documentation about the cultural response of isolates. Product descriptions contain the following sections: intended use, composition-typical formula, principle of the method and explanation of the procedure, direction for preparation, physical characteristics, specimens, test procedure, reading and interpretation, user quality control, limitation of the method, storage conditions, references, product availability and packaging. Information concerning materials required but not supplied and the performance characteristics are provided in the Instructions for Use (eIFU) which can be downloaded, for each reference, from web site www.biolifeitaliana.it
- The 3rd Section incudes technical information and indications for the use of selective supplements, enrichments, raw materials of biological origin (agar and peptones) and various products for bacterial identification with biochemical tests.

The appendix of the Manual summarises the GHS hazard pictograms that provide general warnings on the use of Biolife products.

Biolife Manual 4th edition is intended to serve as a technical resource of information regarding specific products and procedures as well as to assist the customer in ordering.
For additional information, contact our Technical Service or your local Biolife Distributor.
Please let us know if we have met your expectations. Your friendly criticism will help us correct mistakes.



The cover of the first edition in Italian (1979)

Edited by: Biolife Italiana S.r.l.
Edition 4.0, April 2023.

ABOUT BIOLIFE ITALIANA

Biolife Italiana is a manufacturing company belonging to the Mascia Brunelli-Biolife group.
Biolife Italiana is operating in the *in vitro* diagnostic field for microbiology while Mascia Brunelli is operating since 1947 in production of IVDs for haematology, clinical chemistry, immunology and manufactures class 3 haemostatic medical devices for surgery.
Founded as a small Production Laboratory in the centre of Milan in 1968, today Biolife Italiana occupies an area of approximately 4000 sqm and includes class 1000 sterile rooms with separate filtration systems for the production of medical devices and *in vitro* diagnostic devices. In 2014 a new and modern Dehydrated Culture Media Production Unit was established, covering an area of about 600 sqm, with high productivity plans for blending and grinding powdered raw materials and packaging of dehydrated media in bottles and drums. Moreover, the production facility of freeze-dried supplements has been implemented in a new clean area with new lyophilisation plants.
These new production units, together with the expertise and experience of the involved staff, make Biolife one of the most qualified companies in the world in the field of culture media and supplements production, both in terms of quality and quantity.

Biolife Italiana is committed to:
- fully meet the needs of its customers in terms of pre- and after-sales support
- establish a process of continuous improvement of its products and services
- maintain and improve the market share
- identify, develop, manufacture and distribute new advanced and specialised products to meet the demands of new analytical methods in microbiology

Biolife's policy is strongly committed to the implementation of the following objectives: Training, R&D, Production, Delivery.




Since its beginning in 1968, Biolife's goal has always been to develop high quality culture media, in harmony with the technological development of the day and capable of solving the diagnostic problems of microbiologists.
Here are some milestones on a path of growth that has lasted over 50 years.

1968	Born of the company. Devising of a complete range of dehydrated media, including some formulations first available worldwide by Biolife such as Hektoen Enteric Agar, Giolitti & Cantoni Broth, Staphylococci 110 Medium.
1982	Biolife has been a pioneer supplying freeze-dried antibiotics containing selective supplements for microbiological culture media.
1985	First in Europe, Biolife proposed Mac Conkey Agar with MUG, as powdered medium, ready-to-use plate and ready-to-use bi-plate combined with Blood Agar (Biosector 3).
1986	Biolife devised and patented the first biochemical screening test for *Salmonella* colonies, based on the fluorogenic detection of C8 esterase enzyme (MUCAP Test).
1992	Development of the first chromogenic and fluorogenic medium for the enumeration of *Escherichia coli* and coliform bacteria in water: C-EC Agar.
1996	With the collaboration of two microbiologists, Franco Ottaviani and Marco Agosti, Biolife developed the first chromogenic culture medium that allowed the differentiation on primary isolation medium of pathogenic *Listeria monocytogenes* from non-pathogenic spp.: Agar Listeria Ottaviani & Agosti (ALOA).
1998	The same principle of detection of C_8 esterase enzyme of MUCAP Test has been exploited for the development, of a chromogenic plating medium, Chromogenic Salmonella Agar, that demonstrated very high specificity and sensitivity for the detection of *Salmonella* spp., including S. Typhi, and lactose positive strains. It was the first worldwide available medium based on the chromogenic magenta caprylate substrate.
1999	Development of a chromogenic medium for the isolation and differentiation of *C. albicans* (Chromalbicans Agar).
2000	Biolife and the sister company Mascia Brunelli developed and proposed on the market a complete range of immunochromatographic tests for the diagnosis of infectious diseases.
2006	Devise of a chromogenic medium for the simultaneous and differential enumeration of *Enterobacteriaceae* and *E. coli* in food (SENECA).
2012	Biolife formulated a chromogenic medium for the detection and differentiation of the main *Candida* spp. of clinical interest (Chromogenic Candida Agar).
2013	Development of a chromogenic medium for the detection and differentiation of *S. agalactiae* (Chromogenic Strepto B).
2016-2022	Biolife formulated chromogenic media for the detection and differentiation of ESBL producing *Enterobacteriaceae* (Chromogenic ESBL) and Carbapenemase producing *Enterobacteriaceae* (Chromogenic CRE). Biolife formulated chromogenic media for the detection of *Bacillus cereus*, *Cronobacter sakazakii*, *Yersinia enterocolitica, Lactobacillus acidophilus.*

How to contact Biolife

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OVERVIEW OF MICROBIOLOGY PRODUCTS AND SERVICES

Biolife's Product Portfolio includes:

Dehydrated Culture Media

More than 400 formulations for clinical and industrial Microbiology. The formulations meet the requirements of International Standards such as ISO, IDF, FDA-BAM, APHA, USDA-FSIS, EP-JP-USP harmonized methods. Biolife Italiana offers a wide range of chromogenic and fluorogenic media.

Selective Supplements and Enrichments

Freeze dried and liquid supplements ensure an optimal and standardized culture media preparation and therefore guarantee better productivity and selectivity performances in the microbiological isolation procedures

Ready-to-use media in plates, tubes and flasks

Manufactured in clean rooms under laminar flow according to the highest standards. Our products range include 90 mm, and 150 mm plates, bi- and tri-plates, 55 mm plates for MF technique, tubes and flasks.

Rapid Tests and Kits

Wide range of accurate, rapid and user friendly immunochromatographic tests and haematological stains.
Employing highly specific antibodies, developed by our R&D Department, a wide range of rapid latex agglutination kits are available for the confirmatory tests of key pathogens present in clinical specimens and food, water, environmental samples.

Customised Media Service

Biolife offers one of the most comprehensive ranges of culture media for microbiology, developed during our more than 50 years of activity. However, for special applications, customers can interface with Biolife for formulations prepared on request.
Our technical service is available for close collaboration with customers to develop and produce special culture media for specific applications.

The contacts and relationships with our foreign Distributors and worldwide Customers are managed by our International Sales and Marketing Organisation which includes technicians with experience and knowledge in culture media and microbiology.

REGULATORY AFFAIRS AND DOCUMENTATION

Biolife's processes and products are strictly regulated by the Quality Management System, implemented since 2003 and now compliant with ISO 9001[1] (Registration n° D2001500013) and ISO 13485[2] (Registration n° D2001500012) for design, manufacturing and distribution of *in vitro* diagnostic devices.
The *in vitro* diagnostic devices meet the requirements EU Regulation 2017/746.[3]
Responsible of the Quality Management System is the Quality Assurance & Regulatory Affair Department that, in cooperation with the Scientific and Technical Department, plays a key role in internal and supplier audits, qualification of suppliers, development and validation of products and production processes, quality control and quality assurance, and in the application of the EU Regulation 2017/746.
Both the Departments contribute to guarantee a complete traceability of product components with the aid of in-house computerised logistic system.
Our customers can be assisted with a competent support in a continually evolving international regulation system.
The following documentation is available for our customers, scientists and researchers:

Quality Control Certificates

Biolife provides a Certificate of Analysis (CoA) for each batch of products manufactured and released on the market. The CoA is a summary of the results obtained in the Quality Control Laboratory, applying procedures for the evaluation of culture media, supplements and ancillary products, according to EN 12322[4], ISO 11133[5], the European Pharmacopoeia[6], guidelines for products designed for clinical microbiology such as CLSI document[7] and the Australian Guidelines for the Quality Assurance of medical microbiological culture media[8,9]. The main data provided by CoA are the basic information of the product such as:

- catalogue number, product name, batch number and expiry date,
- data about colour, clearness, gel strength (for dehydrated solid media), pH,
- assessment of biocontamination for ready-to-use media within strictly defined specifications,
- productivity rate, selectivity ratio and specificity data obtained with a panel of test strains selected within International Microbial Collections as well as clinical or food isolates,
- incubation condition (time, temperature, atmosphere),
- physical and chemical parameters, microbial content, amino nitrogen (AN), total nitrogen (TN) and AN/TN ratio, for peptones and other raw materials of biological origin.

The Quality Control Certificate may be downloaded from the Biolife website www.biolifeitaliana.it, by entering the batch number on the home page.

Transmissible Spongiform Encephalitis (TSE) Statement

It is known that raw materials of animal origin used for the production of culture media can be a potential vehicle for transmissible spongiform encephalitis (TSE) and other animal infectious diseases.
Biolife manufactures dehydrated and ready-to-use culture media and supplements that comply with EMA/410/01/rev31[10] the European Pharmacopoeia (EP) recommendations[11], the WHO/EMC/ZOO 097.3 report[12], the FDA document CFR 9418[13], the Regulation (EC) n° 1069/2009[14], with the aim of minimising the risk of transmission of spongiform encephalitis and other infectious animal diseases.

For raw materials of animal origin, our suppliers provide a certificate of suitability and/or a declaration on the geographical origin of the animals, the slaughterhouse authorisations, and the infectivity class of the raw materials, preferring the use of raw materials of category B (low-infectivity tissues) or category C (tissues with no detected infectivity).
Biolife makes available to customers, on the web site www.biolifeitaliana/download, the multi-comprehensive document "TSE Statement for minimizing the risk of TSE in animal derivatives", describing all the raw materials of animal origin used for the production of dehydrated and ready-to-use media and supplements, with details, for each raw material, related to the tissue or materials, the country of origin of animals, the infectivity class, the availability of the Certificate of Suitability, notes regarding the production facility, the manufacturing process, the veterinary controls.
The same TSE statement is available, upon request, for each product and/or for each manufactured batch, thanks to a comprehensive traceability system of raw materials, semi-finished and end products. Where applicable, the Certificate of Suitability of animal based raw materials, GMO free certificates, kosher and halal certificates, are supplied to our customers, upon request.

Safety Data Sheets
Dehydrated culture media and supplements may contain hazardous and possibly toxic components. Biolife has classified all manufactured products in compliance with Regulation (EC) No 1272/2008[15] and has issued Safety Data Sheets (SDS) according to Regulation 1907/2006/CE[16]
The SDS provides, in a total of 16 sections, information on the company, identification of the substance or mixture, information on the preparation, information on the composition, the identification of hazardous ingredients, first aid measures, firefighting measures, accidental release measures, handling and storage, exposure controls and personal protection, physical and chemical properties, toxicological information, ecological information, disposal considerations, stability and reactivity, information on transport, regulatory information, other information.
Safety data sheets are available on the Biolife website www.bolifeitaliana.it, by entering the reference number or the name of the product on the home page. A table with GHS hazard pictograms providing generic warnings for the use of Biolife products is included in the appendix of this Manual. As legislation and classification are subject to frequent revision, for more accurate, complete and up-to-date information on hazards and risks, please refer to the product labels and safety data sheets at www.biolifeitaliana.it.

Instructions for Use
Since its early years, Biolife has paid attention to providing customers with technical data sheets summarising product characteristics, which were organised in the first edition of the Biolife Manual in 1979.
For all Biolife products (IVDs and non-IVDs), we have now undertaken a path to transform the technical data sheets into Instructions for Use (IFUs), drafted in accordance with EU Regulation 2017/746 by 2021[3] and ISO 18113[17].
The new IFUs provide, in a total of 16 sections, information on the intended use, composition, principle of the method and explanation of the procedure, directions for medium preparation, physical characteristics, material provided with catalogue number and package information, , materials required but not provided, samples that can be analysed, test procedure, results reading and interpretation, suggested user quality control, performances characteristics, limitation of the method, precautions and warning, storage condition and shelf life, consulted references.
Updated IFUs, are available on the Biolife website www.bolifeitaliana.it, by entering the reference number or product name on the home page and are included in the Section 3 of this Manual.

Label and packaging
The label of a dehydrated culture medium contains information on the name of the product, catalogue number and the pack size, lot number and expiry date, typical composition, directions for preparation, required supplements, acceptable pH range, storage condition, health and safety information according to Regulation (EC) No 1272/2008[15], and where applicable CE and IVD markings.
The label includes the phrase "the formula may be adjusted and/or supplemented to meet the required performances criteria". It is a standard practice for manufacturers of culture media to adjust and/or supplement the formula to achieve productivity, specificity and selectivity results in compliance with the specifications, established on the base of the intended use, company knowledge and relevant literature. This usual practice is also codified and regulated by ISO 11133:2014[5] "*It is sometimes the case that a particular ingredient (peptones and meat or yeast extracts, agar, buffering substances, bile salts, bile extract, deoxycholate, antibacterial dyes, indicator dyes, antibiotics) specified in the formulation has to be modified to achieve constant and consistent performances of the medium.*
The labels of ready-to-use media and supplements provide all the information described above with the exception of the composition, due to the small size of the labels, and some information is provided with symbols in compliance with ISO 15223.[18]

Validation of shelf life and transport temperature
All our products are tested for conformity to the specifications with a real-time stability test at the end of the shelf life, with the products stored under the recommended conditions.
Moreover, the products have been subdivided in class of risk and tested for the temperature changes to which products may be subjected during production or transport (thermal shock test).
On request, we provide data and declarations to our customers.

References
1. ISO 9001: 2015 Quality management systems — Requirements
2. ISO 13485:2016 Medical devices — Quality management systems — Requirements for regulatory purposes
3. Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU.
4. EN 12322 In vitro diagnostic medical devices - Culture media for microbiology - Performance criteria for culture media.
5. ISO 11133:2014 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media.
6. European Pharmacopoeia, current edition
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
8. The Australian Society for Microbiology. Australian Guidelines for the Quality Assurance of medical microbiological culture media 2nd edition, 2012
9. The Australian Society for Microbiology. Australian Guidelines for the Quality Assurance of medical mycological culture media, 2012
10. EMA/410/01 rev.3: "Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products;
11. Chapter 5.2.8. of European pharmacopoeia rev. 1 "Minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products";
12. FDA CFR 94.18 "Bovine spongiform encephalopathy; importation of edible products derived from bovines";
13. WHO/EMC/ZOO 097.3 "Report of a WHO Consultation on Medicinal and other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies";
14. REGULATION (EC) No 1069/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) N 1774/2002 (Animal by-products Regulation).
15. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006
16. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
17. ISO 18113-2:2009 In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 2: In vitro diagnostic reagents for professional use
18. ISO 15223-1:2016 Medical devices — Symbols to be used with medical device labels, labelling and information to be supplied — Part 1: General requirements

GENERAL INFORMATION ABOUT CULTURE MEDIA

THE HISTORY OF CULTURE MEDIA

Fannie and Walther Hesse

The discovery and development of culture media were the key factors for the development of Microbiology in the 19th and 20th centuries. The origins and evolution of microbiological culture media was made possible by challenging the theory of spontaneous generation and can be traced to the 19th Century. During this pioneering time bacteriologists attempted, with varying degrees of success, to grow microorganisms either directly using the food or material on which the microorganism first developed.[1]
Here below are summarized the main milestones of the wonderful historical path of the development microbiological culture media.

1817 The birth of microbial culture can be traced back to1817 when Bartolomeo Brizi, an Italian pharmacist, described the observations and experiments on a typical food of northern Italy known as "polenta", corn flour boiled in water and salt, on which purpurin stains would appear at warm temperature and moisture. He discovered that the colour was due to a bacterial contamination and called the observed strain *Serratia* in honour of and Italian physicist named Serratia, and chose *marcescens* for the sp. name after the Latin word for decay. He clarified the 500-year-long mystery of the communion bread coloured in red, assumed to be a substance derived from the blood of Christ.[1,2,3]

1860 Louis Pasteur, a French biologist, microbiologist and chemist, developed a culture medium "yeast soup'" containing the basic requirements for microbial growth. His objective was to create a fermentation medium to demonstrate that each fermentation (alcoholic, acetic, lactic …) was associated with the development of a particular microorganism. This medium contained: nitrogen (ammonium salts), a carbon source (sugar) and vitamins (ash).[1,2]

1872 Ferdinand Cohn refined Pasteur's liquid medium with different types of sugars to create a more versatile basal medium for the cultivation of new bacteria.

1881 Robert Koch demonstrated optimal growth of bacteria when they were incubated in a broth composed of fresh beef serum or meat extract and solidified with coagulated egg albumin, starch past, aseptically cut slice of a potato, gelatine.

1881 Walther Hesse joined Robert Koch's laboratory in a post-doctoral position. When culturing the organisms, he used a gelatine-containing medium capable of solidifying. Frustratingly, the medium had a tendency to melt during the summer months, thus ruining the experiments. Legend has it that went on a picnic with his wife Angelina Fannie and noticed that the jellies and puddings that she had brought along, did not melt in the hot summer weather. When asked why this was so, Lina (as she was called) replied that they contained agar, and that she had been shown the trick by a Dutch neighbour, recently emigrated from the island of Java.[4]

1882 Taking advantage of the discovery of Walther Hesse and his wife Fannie, Robert Koch introduced agar-agar in microbiological culture media.

1884 Friedrich Loeffler created "Löeffler serum", a coagulated blood serum, used for the detection of *Corynebacterium diphtheriae*. His description of the diphtheria bacillus, published in 1884, was the originating cause of an antitoxin treatment. The formulation of this nutrient medium is still widely used today (Loeffler's Medium).

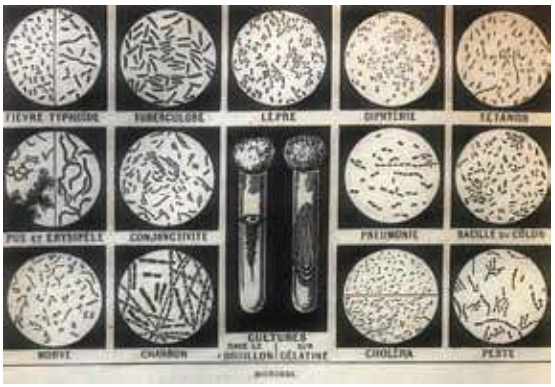
1885 Paul Ehrlich discovered the antimicrobial effects of some chemical product. He invented the precursor technique to Gram staining bacteria.

1887	Julius Richard Petri, working as assistant of Robert Koch, is generally credited with inventing the device known as the Petri dishes, which is named after him. He was involved in modifying the flat glass plate, common to laboratories, and produced a new type of culture dish for media. There is some dispute, however, concerning whether Petri invented the 'Petri dish', or whether it was in act invented earlier by Emanuel Klein, a Slovenian scientist working in England.[1] For many years glass dishes were used, mainly until the mid-1960s, where advances with injection moulding technology, led to Petri dishes being manufactured out of clear polystyrene plastic.
1988	First step towards diagnostic media: Martinus Beijerinck developed an elective medium without nitrogenous compounds to isolate the root nodule bacterium *Rhizobium* which is capable of fixing atmospheric nitrogen.
1890	Raymond Jacques Sabouraud crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for their isolation and classification.[5]
1892	Emmanuel Merck was reportedly the first to manufacture peptones for the preparation of culture media.
1897	Charles Adolphe Wurtz proposed to use the differences in the metabolisms of sugars of pure cultures and formulated a litmus lactose agar*: B. coli* formed red colonies and *B. typhi* colonies remained blue in colour.[6]
Early 1900s	Microbiologists realized that enteric organisms had distinct patterns of carbohydrate utilization and that those enteric organisms that could not utilize lactose were most likely pathogenic to humans. They began to develop media that could differentiate between lactose-fermenting and non-lactose-fermenting organisms.
1900, 1905	Alfred Theodore MacConkey, working at the University of Liverpool, described a medium with bile salts for the growth and differentiation of the *Bacillus coli communis* and the *Bacillus typhi abdominalis* in faecal samples.[7,8]
1902	Wilhelm von Drigalski and Heinrich Conradi, collaborators of Robert Koch, discovered the inhibitory activity of the crystal violet on the growth of Gram-positive bacteria and developed a selective and differential medium for the isolation of *B. typhi*.[9]
1903	S. Endo introduced the first isolation medium for the recovery of pathogenic enteric organisms, called fuchsin sulphite infusion agar. This medium allowed the isolation of these pathogens, but did not inhibit the normal, non-pathogenic enteric organisms also present in feces.[10]
1906	Jules Bordet and Octave Gengou optimized a culture media and succeeded in isolating and cultivating a bacterium, later called *Bordetella pertussis* which causes whooping cough, a deadly disease in young children.[11]
1912	Digestive Ferment Company, in USA, manufactured Bacto Peptone, the first product aimed at developing the microbiological market of culture media.[12]
1912	Churchman showed that derivatives of triphenylmethane, such as gentian violet and brilliant green dyes, were inhibitory to bacteria, particularly Gram-positives and that crystal violet causes some inhibition of fungi.[1]
1916	J. E. Holt-Harris and Oscar Teague introduced Eosin Methylene Blue (EMB) agar, later modified by Levine in 1918. EMB media allowed a visual distinction between *Bacillus coli* (now known as *Escherichia coli*), other non-pathogenic lactose-fermenting enteric Gram-negative rods, and the *Salmonella* and *Shigella* genera .[13]
1916	O. Teague and A.W. Clurman determined that brilliant green dye inhibited most of the non-pathogenic enteric Gram-negative rods. Their medium, brilliant green agar, enhanced the recovery of *Salmonella* from patients with typhoid fever.[10,14]
1919	James Brown developed blood agar to study the haemolysis reaction of *Streptococcus* and *Staphylococcus* spp.[2] However the history of blood agar is uncertain: the inclusion of blood as a nutritive supplement in culture media may pre-date the use of agar[15]; in their 1903 Manual of Bacteriology, Muir and Ritchie[16] list its inclusion before they discuss “agar-agar” as a replacement for gelatine as a solidifying agent.
1919	Edward Rosenow[17] proposed Brain Hearth Infusion media, later modified by Russell Haden[18], as general purposes rich media.
1923	L. Muller described a liquid medium with iodine and sodium thiosulphate which react together to form tetrathionate. Tetrathionate reductase producing bacteria (e.g. *Salmonella*) can grow in presence of tetrathionate.[19]
1931-1932	E. Löwenstein[20] developed an egg coagulated medium containing congo red and malachite green for the detection of *Mycobacterium tuberculosis*. Lowenstein’s formulation has been modified by K.A. Jensen[21] in 1932, suppressing the congo red, changing the concentration of the magnesium citrate and potassium phosphate and increasing the malachite green.
1935	Einar Leifson described deoxycholate media. He used deoxycholic acid and its salts as the inhibitory agent.[22]
1936	Leifson[23] utilized the studies of Klett[24] and Guth[25] about the selective inhibitory effects of selenite and its use for the culture of typhoid organisms, fully investigated selenite activity to formulate the liquid medium selenite broth and to promote its wide use as an enrichment medium for the isolation of *Salmonella* spp.
1941	J.H. Mueller and J. Hinton developed Mueller Hinton Agar (MHA) for the isolation of pathogenic *Neisseria* spp..[26] Nowadays, it is more commonly used for the routine susceptibility testing of non-fastidious microorganism by the Kirby-Bauer disk diffusion technique.
1945	Johnston[27] described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 hours. This medium was later modified by Carpenter and Morton[28] using GC Medium Base with the addition of haemoglobin and a yeast concentrate (chocolate agar).
1948	Chapman[29] formulated media for isolation and differentiation of staphylococci based on early work by Gordon[30] of 1903, indicating that the fermentation of mannitol could be used as a mean of differentiating pathogenic from non-pathogenic staphylococci, and by 1942 Koch’s discovery[31] that the presence of 7.5% sodium chloride in media inhibited the growth of most organisms except staphylococci.
1950s	Better understanding of microbial biochemical pathways.[2]
1960s	Addition of antibiotics in culture media to improve the inhibition of non-target bacteria.[2]
1964	Thayer and Martin proposed a selective medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*, incorporating haemoglobin, yeast supplement B, polymyxin B and ristocetin into GC Agar.[32]
1965	Welton I. Taylor developed xylose lysine decarboxylase agar for the enhanced recovery of *Shigella* spp.[33]
1968	Sylvia King and William I. Metzger, working at the Hektoen Institute in Chicago, formulated Hektoen Enteric (HE) agar with the goal to increase the recovery of *Shigella* spp. from mixed cultures.[34]
1977-1978	Joseph McDade discovered *Legionella pneumophila*. Feeley *et al*. developed a specific selective medium with charcoal, yeast extract, cysteine and ferric pyrophosphate that was a major breakthrough for experimental and clinical investigators.[35]
1979	J.P. Butzler and M.B Skirrow proposed new selective media for the isolation of *Campylobacter* spp. from faeces.[36]
1979	Kilian and Bulow[37] described a medium (PGUA agar) for the identification of *E. coli* in primary cultures of urine sample, ushering in the era of culture media containing enzyme substrates for bacterial identification directly on the primary isolation plates.
1983-1985	Barry Marshall isolated *H. pylori* from gastric and duodenal ulcers; Goodwin et al. devised the first selective medium specifically intended for the isolation of *H. pylori*.[38]
1984	Trepeta and Edberg[39] modified the classic formulation of MacConkey agar with the addition of 4-methylumbelliferyl-β-D-glucuronide (MUG), developing the prototype of fluorogenic media.
1990-2022	In the last 30 years, thanks to academic and IVDs companies’ researches, a wide range of chromogenic culture media has been devised and made commercially available, providing useful tools for clinical and industrial microbiology. Chromogenic media utilize synthetic chromogenic enzyme substrates in order to specifically target pathogenic species (or groups of species) based on their enzyme activity.[40] They include:

Chromogenic media for enteric pathogens and for the detection of specific target-organisms in food, water, environmental samples: *Salmonella, Shigella, Clostridium difficile*, *Listeria monocytogenes, Cronobacter sakazakii, Bacillus cereus, Campylobacter, Enterobacteriaceae, Escherichia coli, coliforms*., Shiga Toxin producing *E. coli*, *Vibrio* spp. *Yersinia enterocolitica.*
Chromogenic media for detection of non-enteric pathogens: *Candida, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus agalactiae*, urinary tract pathogens.
Chromogenic media for detection of antibiotics resistant bacteria: Methicillin-Resistant *Staphylococcus aureus*, Vancomycin-Resistant Enterococci, Extended-Spectrum-β Lactamase-Producing *Enterobacteriaceae*, Carbapenemase-Producing *Enterobacteriaceae*.

References

1. Sandle, T. (2011): 'History and development of microbiological culture media', The Journal (Institute of Science and Technology), Winter 2010-2011, pp10-14
2. Bonnet M, Lagier JC, Raoult D, Khelaifia S. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. New Microbes and New Infections 2020; 34: 100622 https://doi.org/10.1016/j.nmni.2019.100622
3. Microbe wiki. Serratia marcescens https://microbewiki.kenyon.edu/index.php/Serratia_marcescens
4. Wikipedia: https://en.wikipedia.org/wiki/Walther_Hesse; page last edited on 22 May 2020
5. Sabouraud R. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralite des trichophytons de l'homme. Ann Dermatol Syphil1892; 3:1061-1087.
6. Wurtz. 1897. Techniques bactériologiques. Masson, Paris.
7. MacConkey AT. Note on a new medium for the growth and differentiation of the Bacillus coli communis and the Bacillus Typhi abdominalis. The Lancet, July 07, 1900; vol 156, Issue 4010, P20.
8. MacConkey A. 1905. Lactose-fermenting bacteria in feces. J. Hyg. 5:333–378
9. Merck Microbiological Manual, 12th ed.
10. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010.
11. Bordet, J., and D. Gengou. 1906. Le microbe de la coqueluche. Ann. Inst. Pasteur 20:731.
12. Mohapatra A. Culture media history and types. Slides share; Mar 15, 2018
13. Holt-Harris, J. E., and O. Teague. 1916. A new culture medium for the isolation of Bacillus typhosa from stools. 18:596–600.
14. Teague, O., and A. W. Clurman. 1916. An improved brilliant green medium for the isolation of typhoid bacilli from stools. J. Infect. Dis. 18:647–652.
15. Buxton T. Blood agar plates and hemolysis protocols. ASMScience, 2005
16. Robert M, Ritchie J. 1903. Manual of Bacteriology. The MacMillan Company, London, 1903.
17. Rosenow EC. Studies on elective localization. J Dent Research 1919; 1:205-49.
18. Hayden RL. Elective localization in the eye of bacteria from infected teeth. Arch Int Med1923; 32:828-49.
19. Leifson desoxycholate
20. M Lowenstein, E. 1931. Die Zachtung der Tuberkelba zillen aus dem stramenden Blute. Zentalbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig. 120:127.
21. Jensen, K. A. (1932). Rinzuchtung und Typenbestim mung von Tuberkelbazillentammen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig, 125, 222-239
22. Muller L. (1923) *C. R. Soc. Biol. (Paris)* 89. 434-443.
23. Leifson E. New selenite selective enrichment medium for isolation of typhoid and paratyphoid (salmonella) bacilli. A. J Hyg 1936; 24:423
24. Klett A. (1900) Zeitsch. fÍr Hyg. und Infekt. 33. 137-160.
25. Guth F. (1916) Zbl. Bakt. I. Orig. 77. 487-496.
26. Mueller JH, Hinton J. A protein-free medium for primary isolation of the gonococcus and meningococcus. Proc Soc Exp Biol Med 1941; 48:330-333
27. Johnston. J. J Vener Dis Infor 1945; *26*:239.
28. 2 Carpenter, C.M., and H.E. Morton. 1947. An improved medium for isolation of the gonococcus in 24 hours. Proc. N.Y. State Assoc. Public Health Labs. *27*:58-60.
29. Chapman, G. H. 1945. The significance of sodium chloride in studies of staphylococci. J. Bacteriol. 50:201–203.
30. Gordon, M. H. 1903-04. Reports of some characters by which various streptococci and staphylococci may be differentiated and identified. Local British Government Board, Rept. Med. Officer. 33:388–430.
31. Koch, F. E. 1942. Electivnährboden für Staphylokokken. Zentr. Bakt. Parasitenk. I Orig.149:122–124.
32. Thayer JD, E. Martin Jr E. A selective medium for cultivation of N. gonorrhoeae and N. meningitidis. Pub. Health Rep. 1964; 79:49.
33. Taylor WI. Isolation of shigellae I. Xylose lysine agars; new media for isolation of enteric pathogens. Am J Clin Pathol 1965; *44*:471-475
34. King S, WI Metzger WI. A new plating medium for the isolation of enteric pathogens: I. Hektoen enteric agar. Appl Microbiol 1968; 16:577–578.
35. Feeley, J.C., G.W. Gorman, R.E. Weaver,D. C.Mackel,andH.W.Smith.1978.Primary isolation media for Legionnaires disease bacterium. J.Clin.Microbiol.8:320-325
36. Butzler, J. P. and Skirrow, M. B. (1979) Campylobacter enteritis. Clin. Gastroenterol.8, 737–765
37. Kilian, M. and Bulow, P. (1979) Use of a b-glucuronidase detecting agar medium (PGUA agar) for the identification of Escherichia coli in primary cultures of urine samples. Acta Path Microbiol Scand 8, 271–276.
38. Goodwin, C. S., Blincow, E. D., Warren, J. R., Waters, T. E., Samderson, C. R. and Easton, L. (1985) Evaluation of cultural techniques for isolating Campylobacter pyloridis from endoscopic biopsies of gastric mucosa. J. Clin. Pathol.38, 1127–1131.
39. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172
40. Perry JD. A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. Clin Micr Rev 2017; 30:449



Taken from: Le Nouveau Petit Larousse Illustré" ed. 1939

INGREDIENTS OF CULTURE MEDIA

A culture medium is, more or less, a combination of complex nutrient substrates formulated for the cultivation of microorganisms.
There are many different uses of culture media, which can be summarised as follows:

- Maintenance of microorganisms in culture and subcultures.
- Isolation and/or enumeration of microorganisms in foodstuffs, water, dairy products, cosmetics, pharmaceutical products etc.
- Isolation of pathogens that cause infections.
- Identification of microorganisms for therapeutic, epidemiological and taxonomic purposes.
- Determination of the sensitivity of microorganisms to antimicrobial agents.
- Study of the biochemical and physiological characteristics of microorganisms and their ability to adapt to different environments.
- Bulk cultivation of microorganisms for the production of antibiotics, enzymes, toxins, antisera, vaccines etc.
- Evaluation of the biological activity of pharmaceutical preparations (antibiotics, vitamins, amino acids, disinfectants) and the specific activity of drugs.
- Sterility testing of pharmaceutical products, materials and medical equipment.

The formulations of all Biolife culture media are published in the second section of this Manual and the components can be divided into several classes: peptones, carbohydrates, indicators, salts, selective agents, solidifying agents, enrichments, enzymatic substrates.

A – PEPTONES AND EXTRACTS

Peptones and Extracts are nutrients derived from meat, casein, soybean, yeast cells, liver, malt, gelatine. Peptones are produced by enzymatic hydrolysis using pancreatic enzymes such as trypsin, pepsin and enzymes extracted from papaw fruit, and by acid hydrolysis using strong inorganic acids such as hydrochloric and sulphuric acid. Enzymatic hydrolysis breaks the protein molecule at specific points and tends to preserve the amino acid and vitamin content of the original raw material; acid hydrolysis breaks all the peptide bonds and produces free amino acids; it tends to destroy the vitamin and part of the amino acid content. Meat infusions and extracts, obtained by protein coagulation by heating are very similar to peptones. Peptones and Extracts provide nitrogen, carbon, minerals, peptides and amino acids for the microbial growth. The peptones used in Biolife media are highly standardised, water-soluble and ensure excellent reproducibility of their basic characteristics.

B - CARBOHYDRATES

Carbohydrates are added to culture media as a source of energy to increase the growth rate of organisms; they are present as fermentable substrates in combination with pH indicators for microbial differentiation. The most commonly used carbohydrates in culture media are glucose, lactose, mannitol and sucrose.

C – INDICATOR SUBSTANCES

There are essentially three types of indicator substances: pH indicators, oxidation-reduction indicators, and hydrogen sulphide indicators. Among the pH indicators, phenol red, neutral red, bromothymol blue and bromocresol purple have largely replaced the more widely used substances of the past, such as litmus and Andrade's indicator. The pH indicator is used to reveal the formation of acids from carbohydrates and the formation of bases (ammonium ions) from peptones, single amino acids or amines.
Among the above-mentioned indicators, phenol red is the most sensitive as it reveals even very small pH variations in the culture media and is the most widely used. The oxidation-reduction indicators used in culture media are methylene blue and resazurin, which take on particular colourations in their oxidised state, when there is oxygen present in the media: methylene blue changes from colourless to blue, and resazurin, from colourless to pink.
The hydrogen sulphide indicators are usually ferrous salts (ferric citrate, ferric sulphate, ferric ammonium sulphate, ferric ammonium citrate): the hydrogen sulphide produced by bacteria from sodium thiosulphate, reacts with the ferrous salts to produce ferrous sulphide that precipitates in the centre of the colony (characteristic colonies with black centres).

D- SALTS

The salts in the culture media provide the metals required for microbial growth (Mg, Mn, Fe, Ca, Zn, Cu), buffer the medium (potassium and sodium phosphates) and maintain an adequate osmotic balance in the culture medium (NaCl).

E - SELECTIVE AGENTS

The selective agents are chosen and added to culture media to suppress the growth of unwanted organisms, favouring the growth of one or a few types of targets microorganisms.
The first selective agents used in microbiology were dyes and they are still present for this purpose in some formulations. Crystal violet is used in MacConkey agar to inhibit the growth of Gram-positive bacteria; brilliant green is used in combination with bile salts to inhibit Gram-positive bacteria and stimulate the growth of *Salmonella*. Among the substances of biological origin, bile salts are the most widely used selective agents. They are present both in mixtures (Bile salts, Bile salts No.3) and as pure substances (sodium deoxycholate) to inhibit the growth of Gram-positive bacteria in media for the isolation of intestinal pathogens.
A new class of selective agents, which is finding wide use in both clinical and industrial microbiology, are antibiotics. These substances, alone or in a mixture, included in the powdered medium or supplied separately in lyophilised form, have the advantage of selecting with greater specificity the bacterial species to be isolated.
Another class of extremely varied and diversified selective agents are the organic and inorganic salts. Sodium chloride at high concentrations inhibits both Gram-negative and Gram-positive bacteria, with the exception of staphylococci. Sodium azide at different concentrations in culture media is used for the selective isolation of streptococci and enterococci. Sodium selenite in a buffered medium, stimulates the growth of *Salmonella* and is an inhibitor for Gram-positives bacteria. Sodium citrate, sodium tellurite, sodium tetrathionate and sodium lauryl sulphate also belong to this class of ingredients.

F - SOLIDIFYING AGENTS

The main solidifying agent used in culture media for microbiology is agar-agar. Originally used as a solidifying agent in foods by the people of the island of Java, it was introduced into bacterial isolation techniques in the late 1800s by Dr Walther Hesse, a medical officer from Saxony, who was studying microbial contamination of air. Taking advantage of the discovery of Walther Hesse, Robert Koch introduced agar-agar in microbiological culture media giving a great impetus to the technique of isolating microorganisms in a pure culture.
Agar is extracted from agarophyte seaweeds, mainly *Gelidium, Gracilaria, Pterocladia* and *Eucheuma*. Agar with different properties can be obtained depending on the location of the algae cultivation and the extraction techniques. The agar obtained from algae cultivated on the Atlantic coasts has a stronger solidifying power than agar obtained from algae cultivated on the coasts of the Pacific coasts.
Agar in culture media has the sole role of a solidifying agent and has no nutritional properties towards microorganisms.

G – ENRICHMENTS

To improve the fertility properties of culture media for the cultivation of fastidious microorganisms *(Neisseria, Haemophilus,* etc.) various enrichments are added to the culture media, usually after autoclaving and cooling the base to 50°C. Blood and animal serum are the most commonly used enrichments. Depending on the type of microbial research to be conducted, haemoglobin, albumin, egg yolk, whole eggs, chemically defined enrichment solutions are used.

H – ENZYMATIC SUBSTRATES

By incorporating synthetic or natural substrates, which can be cleaved in culture media by specific microbial enzymes, a considerable improvement has been obtained in the identification of some microbial species and genera. Depending on the substrate, the cleavage of these products by microbial enzymes can lead either to the development of fluorescence, diffuse coloration or to the formation of a coloured precipitate in the centre of the colonies or the formation of an opaque or clarifying halo around the colonies. This visible signal is always of great value for the immediate identification of the colonies. For some media, identification occurs at the species level and, for others at the genus or group level. The specificity of the detection is related to the specificity of the detected enzymatic activity and to the culture media formulation. To increase the specificity and sensitivity of microbial detection in chromogenic culture media, enzyme substrates must be used in combination with optimised reagents, nutrient compounds and inhibitory substances.

DIRECTIONS FOR MEDIA PREPARATION

Culture media, from dehydrated bases, should be prepared according to the instructions given in the second section of this Manual and on the label of each product. In order to obtain accurate and consistent results when using dehydrated culture media, it is recommended to keep in mind a few simple rules regarding their storage, reconstitution and sterilisation. Drawbacks, such as pH variation after sterilisation and imperfect transparency of the culture medium, are sometimes due to errors in their preparation.

A- RECONSTITUTION OF CULTURE MEDIA

Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Safety Data Sheets to apply appropriate precautions during handling.
Complete instructions for the preparation of culture media are given in the second section of this Manual and on the label of each product. However, in general, it is advisable to follow these simple rules:

- ✓ Use fresh or freshly boiled water obtained by distillation, demineralisation, deionisation or reverse osmosis, with a microbial contamination not exceeding 10^2 CFU/mL and a conductivity no more than 25 micro siemens and preferably below 5 micro siemens.
- ✓ Use well rinsed glassware, both for reconstituting the medium and for autoclaving; use a container with a volume of at least two and a half times the amount of suspension.
- ✓ To dissolve the culture medium, accurately weigh the desired amount of medium into a container, reading from the label the amount of powder per litre of water. Weighing should be accurate to the decigram. Avoid inhaling the powder and prolonged contact with the skin.
- ✓ Add half the required amount of water. Shake the flask vigorously until a suspension is obtained, then add the remaining water, washing the sides of the flask well to remove any traces of medium.

The components of liquid media that are actually soluble in water at room temperature result in clear solutions in the process described up to this point. However, even for broths it is advisable to heat them slightly to completely dissolve their components. Media containing agar, gelatine and cystine must be brought to the boil to ensure complete dissolution. The usual method is to heat them directly over a flame. To limit denaturation of the components of the medium as much as possible, stir the suspension continuously and, as soon as the solution starts to boil, remove the heat source immediately and allow the solution to boil on the hot wire gauze for a few minutes. The complete dissolution of the medium is indicated by the perfect transparency of the solution flowing down the walls of the vessel.

When calculating the quantity of medium to prepare, bear in mind that:
When calculating the amount of medium to be prepared, keep in mind that:

- ✓ In the 90 mm diameter Petri dishes, 18-22 mL of medium should be dosed. In the 55 mm Petri dishes dispense 8-10 mL of medium
- ✓ For the sensitivity test, 25 mL of medium should be dispensed into the 90 mm diameter plates and 60 mL into the 140 mm diameter plates. These volumes correspond to a layer approximately 4 mm thick.
- ✓ In the 18 x 140 mm tubes, if a short layer and a long bottom is required, 8 mL of medium should be used. If a long bottom is required, the quantity should be reduced to 5 mL.

B- STERILISATION OF CULTURE MEDIA

For culture media that withstand temperatures above 100°C, the safest and most commonly used sterilisation method is steam under pressure obtained in perfectly sealed equipment such as autoclaves or auto-preparators. When the air in the autoclave has been completely replaced by steam, there is a relationship between pressure and temperature. For volumes up to one litre, sterilisation in an autoclave generally takes place at 121°C (1 atmosphere) for 15 minutes. For culture media containing carbohydrates, temperatures between 115 and 118 °C are sometimes recommended. For complete sterilisation, the whole volume should be exposed to the stated temperature for 15 minutes; large volumes may require 20-30 minutes or more. It is always advisable to sterilise culture media in small quantities, in containers filled to no more than 1/3 of their full volume and closed with hydrophobic cotton or metal caps previously loosened by half a turn. For sterilisation, it is recommended to always follow the times, temperatures and procedures indicated on the label of the culture medium. Autoclaves should be checked periodically to ensure that the sterilisation temperature has been reached. Vials or strips with *B. stearothermophilus* spores or strips impregnated with thermo-active substances that change colour according to temperature are used for this purpose.

C- DISPENSING CULTURE MEDIA

The general rule must be to keep the culture medium sterile during all operations.
Plates must be sterilised by gamma irradiation. The ideal working condition is to operate under a laminar flow cabinet with disinfected surfaces. To prepare the plates, the culture medium must be cooled to a temperature of approximately 45-50°C. After the addition of any enrichment (blood, serum, eggs, etc.) dispensing is carried out using essentially three types of systems: manual, semi-automatic and automatic. The manual system consists of pouring the medium directly from the container to the dishes.
The semi-automatic system uses a peristaltic pump with a tube that, on the one hand, sucks from the container and, on the other, distributes the medium to the plates. Timed peristaltic pumps are available on the market, in which the dispensing time (and thus the volume of liquid) and the waiting time between dispensing can be fixed. If this system is used, the vessel containing the culture medium must be prepared for autoclaving with a silicone tube of the correct size and length that passes through the raw cotton plug and enters the Erlenmeyer flask by about ten centimetres (not the culture medium) connected to a glass tube. The outside of the flask should be covered with gauze, cotton and hydrophobic paper. The addition of enrichments can also be carried out using a similar system
The third dispensing system uses a fully automatic equipment, which takes the dishes from specially equipped "magazine", opens their lids, dispenses the medium and stacks the prepared plates.

FACTORS INFLUENCING THE QUALITY OF CULTURE MEDIA

Faults: wrong pH, turbidity, unusual precipitates, darkening, soft gel, poor bacterial growth, and poor selectivity.

Possible causes:
1. Wrong weighing of the dehydrated medium, caused by an operator's manual error or by the use of a malfunctioning scale.
2. Use of powder from bottles that have been left open and may have deteriorated due to exposure to heat, humidity, oxidisation or other environmental factors.
3. Inaccurate measurement of the quantity of water quantity or use of purified water obtained from a malfunctioning apparatus.
4. Use of unclean glassware with traces of detergent or other chemicals.
5. Use of poorly mixed or incompletely dissolved agar based media.
6. Overheating of culture media during the preparation, sterilisation or its stay in the water bath.
7. Incorrect pH measurement, with excessive addition of alkali or acids in media where the pH needs to be corrected. To measure the pH, transfer a sample into a beaker, let it cool to about 20-25°C and then measure by immersing the electrode 2-3 cm.
8. Addition of an unsatisfactory enrichment to the culture medium (e.g. contaminated blood) or addition of enrichment when the culture medium is too hot (inactivation of the added substance) or too cold (solidification of the culture medium with uneven dispersion of the additive).

GENERAL PRECAUTIONS AND WARNINGS

- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Some dehydrated culture media and many selective supplements are classified as hazardous according to current European legislation (see the Appendix of this Manual). Before the use, consult the Safety Data Sheets to apply appropriate precautions during handling and for disposal.
- Ready-to-use culture media in plates, tubes and flasks are generally not classified as hazardous.
- The majority of dehydrated and ready-to-use culture media contain raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the products do not contain any transmissible pathogen. Therefore, it is recommended that the culture media be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Dehydrated and ready-to-use culture media with CE mark and IVD symbol on the labels are *in vitro* diagnostics, for professional use only; they must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated and ready-to-use culture media without CE mark and IVD symbol on the labels are for microbiological control and, for professional use only; they must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Culture media in whose intended use explicitly refers to the microbiological examination of clinical specimens of human origin but are not CE-IVD-marked, do not fulfil the requirements of the EU Regulation 2017/746.
- The medium base and the supplement described together in the instructions of use shall be used in association according to the described directions.
- The user is responsible for the production and quality control processes of the self-prepared culture media and the validation of the shelf life of the finished products, depending on the type (plates/tubes/bottles), the supplements added and the storage method (temperature and packaging).
- Apply Good Manufacturing Practice in the production process of prepared media
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as powder medium and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused media and supplements and the sterilized plates or tubes inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture media and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana S.r.l. (complaint@biolifeitaliana.it) and, if applicable, the relevant Authorities, of any serious incident occurring in connection with the use of the *in vitro* diagnostics and other products.
- The information provided in the instructions for use of Section 2 of this Manual has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

PRECAUTIONS AND WARNINGS SPECIFIC FOR READY-TO-USE PLATES
- Each ready-to use plate is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of the specifications defined on the Quality Control Certificate.

PRECAUTIONS AND WARNINGS SPECIFIC FOR READY-TO-USE TUBES
- Depending of the formulation, ready-to-use media in tubes, are subjected to a terminal sterilisation by autoclaving or by filtration.
- Each ready-to use tube is for single use only. Do not transfer or subdivide the tube contents in other containers, unless otherwise stated in the instruction for use.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.

PRECAUTIONS AND WARNINGS SPECIFIC FOR READY-TO-USE FLASKS
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the agar-based culture medium is liquefied, it cannot be solidified and dissolved a second time.
- Depending of the formulation, ready-to-use flasks are subject to terminal sterilization by autoclaving or by filtration.

PRECAUTIONS AND WARNINGS SPECIFIC FOR SELECTIVE SUPPLEMENTS AND ENRICHEMENTS

- The medium base and the supplement described together in the instructions of use of Section 2 shall be used in association according to the described directions.
- Be careful when opening the metal ring to avoid injury.

GENERAL LIMITATIONS ON THE USE OF CULTURE MEDIA

The culture media will sometimes contain dead organisms that will be evident on a Gram staining. Other sources of non-viable organisms include slides, staining reagents, dipping oil or original sample.

Culture media for biochemical, serological and confirmatory tests require inoculation with pure cultures.

Organisms differ in humidity, temperature, atmosphere (oxygen levels) and incubation time requirements. These requirements must be observed in order to successfully grow the desired organism.

Sometimes selective media can allow the growth of some strains of organisms that are intended to be inhibited; on the contrary, some strains can be inhibited which are destined to grow. It is therefore advisable to use a non-selective medium in parallel with a selective one to increase the chances of recovery of a pathogen.

Changes in the expected results may be due to improper collection, storage and transportation of the sample, improper execution of procedures, improper exposure to temperatures or atmospheres, microbial mutation or variation, interference with other chemicals or inhibitors.

Incubators that have recently been cleaned and disinfected are known to inhibit crop growth due to toxic fumes and vapours.

Culture media and supplements used for the examination of samples of human origin are intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

MATERIALS REQUIRED BUT NOT SUPPLIED FOR CULTURE MEDIA PREPARATION AND USE

Purified water, autoclave, water-bath, Erlenmeyer flasks, sterile Petri dishes, microbiological tubes.
Incubator, pH-meter and laboratory equipment as required.
Sterile loops, needs, swabs, pipettes.
Controlled atmosphere generators and jars, membrane filtration system.
Animal blood, selective supplements, ancillary reagents.

STORAGE CONDITIONS AND SHELF LIFE: GENERAL DIRECTIONS

DEHYDRATED MEDIA

Dehydrated culture media are hygroscopic and are sensitive to moisture, heat and light. Storage conditions are given in the monographs in sections 2 and 3 and on the product label and must be observed.

- ✓ Write the date of receipt in the laboratory on the label.
- ✓ With few exceptions, dehydrated media should be stored at +10°C/+30°C in a dry place, away from direct sunlight, autoclaves, incubators or other heat sources. Where indicated store at +2°C/+8°C.
- ✓ Check the expiry date on the label. The expiry date refers to a product stored in a closed bottle under ideal storage conditions.
- ✓ Use the First In/First Out rule and do not open a new bottle until the previous one has been emptied. Note the date of opening the bottle on the label.
- ✓ If properly stored, they may be used up to the expiry date. Do not use beyond this date.
- ✓ Avoid opening the bottles in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap were damaged or in case of evident deterioration of the powder (colour changes, hardening, presence of large lumps).

SELECTIVE SUPPLEMENTS

Upon receipt, store the product in the original packaging at +2°C/+8°C or at the temperature indicated on the label, away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once opened and reconstituted, the resulting solution should be used immediately, unless otherwise stated in the instruction for use. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

READY-TO-USE PLATES

Upon receipt, store in their original packaging at +2°C/+8°C or at the temperature indicated on the label, away from direct light. If properly stored, the plates may be used up to the expiry date; do not use beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

READY-TO-USE TUBES AND FLASKS

Upon receipt, store in their original pack at +2°C/+8°C or at the temperature indicated on the label, away from direct light. If properly stored, the tubes and flasks may be used up to the expiry date; do not use beyond this date. Before use, check the integrity of the screw cap. Do not use tubes or flasks with signs of deterioration (e.g., microbial contamination, atypical colour).

TABLE OF APPLICABLE SYMBOLS

The symbols printed on product packaging comply with ISO 15223-1 and the European regulation IVDR 2017/746

SYMBOL	SIGNIFICANCE	DCM	SUPPLEMENT	PLATES	TUBES/FLASKS
REF or REF	Catalogue number	X	X	X	X
LOT	Batch code	X	X	X	X
IVD	*In vitro* Diagnostic Medical Device	X	X	X	X
	Manufacturer	X	X	X	X
	Use by	X	X	X	X
	Temperature limitation	X	X	X	X
	Contents sufficient for <n> tests	X	X	X	X
	Consult Instructions for Use	X	X	X	X
	Store away from direct light	X	X	X	X
	Store in a dry place	X			
	Fragile, handle with care		X	X	X

CULTURE MEDIA FOR CLINICAL MICROBIOLOGY

Specimens	Infectious diseases
Ear swab, middle ear effusion	Otitis external, acute localised otitis externa, acute diffuse otitis externa, chronic otitis externa, malignant otitis externa, otitis media, acute otitis media, chronic suppurative otitis media

Culture media	Ready-to-use media REF	Dehydrated media and supplements REF	Organisms
Chocolate agar with bacitracin	541519	401520+4240009+4240010 +412271	*H. influenzae, M. catarrhalis, S. pneumoniae*. Other organisms in pure growth may be significant
Chocolate agar	541520	401520+4240009+412271	Any organism
Columbia CNA Agar	541361	4011361+defibrinated sheep blood	Lancefield group A *Streptococcus*, *S. aureus*
Schaedler Blood Agar with metronidazole 5µg disc	549989 +19MTZ50		Anaerobes
MacConkey Agar	541670	401670	*Enterobacteriaceae*, Pseudomonads
Sabouraud Dextrose Agar	542005	402005	Fungi
Blood agar	541151	402150+ defibrinated sheep blood	*M. catarrhalis, S. pneumoniae*

Specimens	Infectious diseases
Eye swabs, canalicular pus, aqueous and vitreous humour, corneal scrapings, contact lens cases and cleaning fluid	Conjunctivitis, keratitis, endophthalmitis, acute post-operative endophthalmitis, glaucoma filtering-bleb-associated endophthalmitis, chronic endophthalmitis, post-traumatic endophthalmitis, endogenous endophthalmitis, orbital cellulitis, canaliculitis, blepharitis

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
Chocolate agar	541520	401520+4240009+412271	*H. influenzae* Lancefield group A, B, C and G streptococci, *Moraxella* spp., *N. gonorrhoeae, N. meningitidis, P. aeruginosa, S. aureus, S. pneumoniae,* other organisms
Blood agar	541151	402150+ defibrinated sheep blood	Lancefield group A, B, C and G streptococci, *P. aeruginosa, S. aureus, S. pneumoniae*. Other organisms
Modified Thayer Martin (MTM) medium	541522	401520+4240009+412271 +4240008	*N. gonorrhoeae*
Columbia CNA Agar	541361	4011361	Lancefield group A *Streptococcus*, *S. aureus*
Schaedler Blood Agar	549989 +19MTZ50		Anaerobes, Actinomycetes
CLED Agar	541901	4012901	*Enterobacteriaceae*
Sabouraud Dextrose Agar	542005	402005	Fungi

Specimens	Infectious diseases
Mouth swab, saliva and oral rinse	Oral mucositis, Erythematous and pseudomembranous candidiasis, Angular cheilitis and peri-oral infections, Staphylococcal mucositis, Oral ulceration, Abscess and deep-seated infections, Osteomyelitis, Vincent's angina

Culture media	Ready-to-use media REF	Dehydrated media and supplements REF	Organisms
Blood agar	541151	402150+ defibrinated sheep blood	Group A, strep, *S. aureus*, Coliforms
Chromogenic Candida Agar	548005	408005	*Candida* spp.
CLED Agar or Mac Conkey Agar	541901 or 541670	4012901 or 401670	Coliforms and non-fermentative Gram-negative
Sabouraud Dextrose Agar	542005	402005	*C. albicans*, non-albicans yeasts

Specimens	Infectious diseases
Nose swab, antral washout, sinus aspirate and sinus washout	Nasal Carriage, Sinusitis, Acute Sinusitis, Community Sinusitis, Nosocomial Sinusitis, Chronic Sinusitis, Fungal Sinusitis

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
Blood agar	541151	402150+ defibrinated sheep blood	*S. aureus,* Lancefield group A Streptococcus
Sabouraud Dextrose Agar	542005	402005	Fungi
Chocolate agar	541520	401520+4240009+412271	β-haemolytic streptococci, *Enterobacteriaceae, H. influenzae, M. catarrhalis, Pseudomonads, S. aureus, S. anginosus* group, *S. pneumoniae*
Schaedler Blood Agar	549989		*Fusobacterium* spp., *Peptostreptococcus* spp. *Propionibacterium* spp. *Prevotella* spp.

Specimens	Infectious diseases
Throat swab, posterior pharyngeal swab, nasopharyngeal swab, pharyngeal washings, pus aspirate, oropharyngeal swab, throat gargle	Pharyngitis (also known as sore throat), Tonsillitis, Epiglottitis, Laryngitis

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
Columbia CNA Agar	541361	4011361+defibrinated sheep blood	Lancefield group A, C and G streptococci, *A. haemolyticum*
Blood agar	541151	402150+ defibrinated sheep blood	*S. aureus*
Modified Thayer Martin (MTM) medium	541522	401520+4240009+412271 +4240008	*N. gonorrhoeae, N. meningitidis*
Chocolate agar	541520	401520+4240009+412271	*H. influenzae*
Sabouraud Dextrose Agar	542005	402005	Fungi
Schaedler Selective Blood Agar	549990		*F. necrophorum*
Serum Tellurite Agar	549998		*C. diphtheriae*

Specimens	Infectious diseases
High vaginal swab (HVS), vaginal discharge, vulval swab, labial swab, cervical swab, endocervical swab, penile swab, urethral swab, genital ulcer swab, semen, screening swabs for *N. gonorrhoeae*, aspirates from bartholin's gland, fallopian tube, tuboovarian abscess, pouch of Douglas fluid, intra-uterine contraceptive device (IUCD), products of conception	Sexually Transmissible Infections, Vaginal Infections (other than STIs), Other Infections of the Female Genital Tract, Infections (other than STIs) of the Male Genital Tract

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
Blood agar	541151	402150+ defibrinated sheep blood	*S. aureus*, Lancefield Groups A, C and G streptococci. Other organisms may be significant e.g. Lancefield group B streptococci in pregnancy.
Sabouraud Dextrose Agar	542005	402005	Yeasts
Trichomonas CPLM Selective Broth	5513311		*T. vaginalis*
Schaedler Blood Agar	549989 +19MTZ50		Anaerobes
CLED Agar	541901	4012901	*Enterobacteriaceae, Pseudomonads*
ALOA	541605	401605+423501	*Listeria*
Chocolate agar	541520	401520+4240009+412271	*H. influenzae*
Modified Thayer Martin (MTM) medium	541522	401520+4240009+412271 +4240008	*N. gonorrhoeae*

Specimens	Infectious diseases
Faeces	Enteritis

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
Campylobacter Blood Free Agar (Karmali)	541283	401283+4240035	*Campylobacter* spp.
XLD Agar	542206	402206	*Salmonella* spp., *Shigella* spp.
Selenite Broth	552025	402025	*Salmonella* spp.
m-TSB	512155M3	402155M	*E. coli* O157
Sorbitol Mac Conkey Agar + Cefixime Tellurite Supplement		401669S + 42ISEC	*E. coli* O157
Bacillus Cereus Selective Agar (PEMBA)	541112	401112+4240001+42111601	*B. cereus, B. subtilis, B. licheniformis*
Mannitol Salt Agar	541665	401665	*S. aureus*
Clostridium Difficile Selective Agar		401308+4240006	*C. difficile*
Alkaline Peptone Water		401032	*V. cholerae, V. parahaemolyticus*
TCBS Agar	542106	402106	*V. cholerae, V. parahaemolyticus*
CIN agar	549997	401302+4240011	*Y. enterocolitica, Y. pseudotuberculosis, Yersinia spp.*
Schaedler Selective CNA Blood Agar	549907		*C. perfringens*

Specimens	Infectious diseases
Bag urine, pad urine, catheter urine, prostate massage/secretions, clean catch urine, suprapubic aspirate, cystoscopy urine, ureteric urine, ileal conduit urine, urostomy urine, mid-stream urine, nephrostomy urine	Urinary tract infection

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
CLED Agar or Chromogenic Urine Agar IV	541901 549810G	4012901 409810G	*Enterobacteriaceae*, Enterococci, Lancefield Group B streptococci, Pseudomonads, *S. saprophyticus.* Other coagulase-negative staphylococci, *S. aureus*
Selenite Broth	552025	402025	S. Typhi, S. Paratyphi
Sabouraud Dextrose Agar	542005	402005	Fungi
Schaedler Blood Agar	549989		Anaerobes, Streptococci
Chocolate agar	541520	401520+4240009+412271	Fastidious organisms

Specimens	Infectious diseases
Skin, nail, hair	superficial fungal infections

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
Sabouraud Dextrose Agar with CAF CEX	542008		Dermatophytes and yeasts
Sabouraud Dextrose Agar with CAF	542006	402006	Dermatophytes, moulds and yeasts
Malt Agar		401645	Encourages mould sporulation
Dermatophyte Selective Medium-DTM	541369	4013691 + 4240024	Helps to distinguish dermatophytes

Specimens	Infectious diseases
Bronchial aspirate, transthoracic aspirate, bronchoalveolar lavage, transtracheal aspirate, bronchial brushings, protected catheter specimens, bronchial washings, endotracheal tube specimens, sputum – expectorated	Pneumonia, Lung abscess, Cystic fibrosis, Mycobacterial disease, Legionella disease, Nocardia and Actinomyces infections, Fungal infections

Culture media	Ready-to-use REF	Dehydrated media and supplements REF	Organisms
Chocolate agar with bacitracin	541519	401520+4240009+4240010 +412271	*H. influenzae*. Other organisms in pure growth may be significant
Blood agar	541151	402150+ defibrinated sheep blood	*M. catarrhalis, S. aureus, S. pneumoniae*. Other organisms in pure growth may be significant
Sabouraud Dextrose Agar	542005	402005	Fungi
CLED Agar or MacConkey agar	541901 541670	4012901 401670	*Enterobacteriaceae, Pseudomonads*
Mannitol Salt Agar	541665	401665	*S. aureus*
Burkholderia Cepacia Selective Agar	541153	401153+4240073	*B. cepacia* complex
Legionella Selective Agar (GCPC)	549995	401582+423210+423215	*Legionella* spp.

References

1. Public Health England. (2014). Investigation of Ear Infections and Associated Specimens. UK Standards for Microbiology Investigations. B 1 Issue 9Public Health England. (2017). Investigation of Bacterial Eye Infections. UK Standards for Microbiology Investigations. B 2 Issue 6.1
2. Public Health England. (2015). Investigation of Superficial Mouth Samples. UK Standards for Microbiology Investigations. B 4 Issue 7
3. Public Health England. (2015). Investigation of Throat Related Specimens. UK Standards for Microbiology Investigations. B 9 Issue 9
4. Public Health England. (2017). Investigation of Genital Tract and Associated Specimens. UK Standards for Microbiology Investigations. B 28 Issue 4.6.
5. Public Health England. (2014). Investigations of Faecal Specimens for Enteric Pathogens. UK Standards for Microbiology Investigations. B 30 Issue 8.1.
6. Public Health England. (2019). Investigation of urine. UK Standards for Microbiology Investigations. B 41 Issue 8.7
7. Public Health England. (2016). Investigation of Dermatological Specimens for Superficial Mycoses. UK Standards for Microbiology Investigations. B 39 Issue 3.1.
8. Public Health England. (2019). Investigation of bronchoalveolar lavage, sputum and associated specimens. UK Standards for Microbiology Investigations. B 57 Issue 3.5.

CULTURE MEDIA FOR ANTIMICROBIAL SENSITIVITY TEST

Application	Culture media	Powder	90 mm plates	150 mm plates
AST of common, aerobic, rapidly growing bacteria	MUELLER HINTON AGAR II	401740	541740	501740P
AST of fastidious organisms (EUCAST)	MUELLER HINTON AGAR F (MHA-F)		541740F	
AST of fastidious organisms (*S. pneumoniae*)	MUELLER HINTON BLOOD AGAR SHEEP		541743	501743P
AST of fastidious organisms (*N. gonorrhoeae*)	MUELLER HINTON CHOCOLATE AGAR		541742	
AST of fastidious organisms (*Haemophilus*)	HAEMOPHILUS TEST MEDIUM		549901	
AST of fungi	RPMI AGAR		54RPMI90	54RPMI15
AST of anaerobes	SUPPLEMENTED BRUCELLA AGAR		549850	
AST with isolated staphylococci strains and oxacillin discs or strips	MUELLER HINTON AGAR + 2% NaCl		549860	
Detection of MRSA strains	OXACILLIN SALT SCREEN AGAR		549510	
Detection of VRE strains	VANCOMYCIN SCREEN AGAR		549520	

References

1. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100.
2. Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. Approved Standard 7th ed. CLSI document M11–A7.
3. The European Committee on Antimicrobial Susceptibility Testing. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 8.0 (January 2020). http://www.eucast.org.
4. Pfaller et al. Evaluation of Etest method for determining fluconazole susceptibilities of 402 clinical yeast isolates by using three different agar media. JCM, vol. 36, no. 9, p. 2586-2589, 1998
5. Pfaller M.A. et al. In vitro susceptibility testing of filamentous fungi: comparison of Etest and reference microdilution methods for determining itraconazole MICs. JCM, vol. 38, no. 9, p. 3359-3361, 2000.
6. Szekely A. et al. Comparison of Etest and broth microdilution for antifungal drug susceptibility testing of moulds. JCM, vol. 37, no. 5, p. 1480- 1483, 1999.

CULTURE MEDIA FOR THE FOOD CHAIN TESTING

SAMPLE PREPARATION

ISO 6887-1:2017; ISO 6887-2:2017; ISO 6887-3:2017; ISO 6887-4:2017; ISO 6887-5:2020; ISO 6887-6:2013; ISO 7218:2007

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Tubes	Flasks
PEPTONE SALT SOLUTION	MAXIMUM RECOVERY DILUENT	401691	551691	5116912 5116913
BUFFERED PEPTONE WATER	BUFFERED PEPTONE WATER	401278/401278C	551278	5112782 5112783
PEPTONE SOLUTION	TRYPTONE	412290		

ENUMERATION OF MICROORGANISMS AT 30°C

ISO 4833-1:2013; ISO 4833-2:2013

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Tubes	Flasks
PLATE COUNT AGAR (PCA)	TRYPTIC GLUCOSE YEAST AGAR	402145	542145	552145	5121502 5121503
OVERLAY MEDIUM	AGAR BIOS LL	411030			
PLATE COUNT AGAR (PCA)+SKIMMED MILK	PLATE COUNT AGAR WITH SKIM MILK	401918			

ENUMERATION OF PSICOTHROPHIC MICRORGANISMS

ISO 17410:2019

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Tubes	Flasks
PLATE COUNT MEDIUM	TRYPTIC GLUCOSE YEAST AGAR	402145	542145	552145	5121502 5121503

BACILLUS CEREUS: COLONY-COUNT TECHNIQUE AT 30°C

ISO 7932:2004

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
MYP AGAR	BACILLUS CEREUS AGAR BASE MYP	401111			5111112
	BACILLUS CEREUS ANTIMICROBIC SUPPL.		4240001		
	EGG YOLK EMULSION		42111601 42111605 42111600		
	BACILLUS CEREUS SELECTIVEAGAR MYP			541112M	
SHEEP BLOOO AGAR	BLOOD AGAR SHEEP-ISO FORMULATION (pH7,0-7,2)			541156P	

BACILLUS CEREUS: DETERMINATION OF LOW NUMBERS

ISO 21871:2006

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
TRYPTONA SOYA POLYMYXIN BROTH (TSPB)	TRYPTIC SOY BROTH	402155			5121552
	BACILLUS CEREUS ANTIMICROBIC SUPPL.		4240001		
POLYMYXIN PYRUVATE EGG YOLK MANNITOL BROMOTHYMOL BLUE AGAR	BACILLUS CEREUS AGAR BASE PEMBA	401112			5111122
	BACILLUS CEREUS ANTIMICROBIC SUPPL.		4240001		
	EGG YOLK EMULSION		42111601 42111605 42111600		

	BACILLUS CEREUS SELECTIVE AGAR PEMBA			541112	
MANNITOL EGG YOLK POLYMYXIN AGAR (MYP)	BACILLUS CEREUS AGAR BASE MYP	401111			5111112
	BACILLUS CEREUS ANTIMICROBIC SUPPL.		4240001		
	EGG YOLK EMULSION		42111601 42111605 42111600		
	BACILLUS CEREUS SELECTIVEAGAR MYP			541112M	
SHEEP BLOOO AGAR	TRYPTIC SOY AGAR	402150			5121502 5121503
	BLOOD AGAR SHEEP			541151	

CAMPYLOBACTER: DETECTION METHOD

ISO 10272-1:2017+A1:2023

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
BOLTON BROTH	CAMPYLOBACTER ENRICH. BASE BROTH-BOLTON	401286B			
	LYSED HORSE BLOOD		90HLX100		
	BOLTON BROTH SUPPLEMENT		4240025		
PRESTON BROTH	NUTRIENT BROTH N°2 -pH 7,4	401812S2			
	LYSED HORSE BLOOD		90HLX100		
	PRESTON ANTIMICROBIC SUPPLEMENT II		4240022		
MODIFIED CHARCOAL CEFOPERAZONE DEOXYCHOLATE AGAR (MCCD AGAR)	CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON	401282			
	BOLTON CCDA ANTIMICROBIC SUPPLEMENT		4240020		
	CAMPYLOBACTER BLOOD FREE AGAR (CCDA)			541113	
SECOND SELECTIVE PLATING MEDIUM	CAMPY. BLOOD FREE MEDIUM BASE KARMALI	401283			
	KARMALI SELECTIVE SUPPLEMENT		4240035		
	CAMPYLOBACTER BLOOD FREE AGAR KARMALI			541283	
COLUMBIA BLOOD AGAR	COLUMBIA BLOOD AGAR BASE	401136			511362/3
	COLUMBIA BLOOD AGAR SHEEP			541136	
OXIDASE REAGENT	OXIDASE TEST STRIP				191040ST
HYDROLISIS OF HIPPURATE REAGENT	HIPPURATE HYDROLYSIS - HIP				2456711*
INDOXYL ACETATE DISCS	INDOXYL ACETATE - LAC				2459511*

CAMPYLOBACTER: COLONY COUNT METHOD

ISO 10272-1:2017+A1:2023

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks/ Reagents
MODIFIED CHARCOAL CEFOPERAZONE DEOXYCHOLATE AGAR (mCCD AGAR)	CAMPY. BLOOD FREE MEDIUM BASE BOLTON	401282			
	BOLTON CCDA ANTIMICROBRIC SUPPL.		4240020		
	CAMPYLOBACTER BLOOD FREE AGAR (CCDA)			541113	
COLUMBIA BLOOD AGAR	COLUMBIA BLOOD AGAR BASE	401136			5111362 5111363
	COLUMBIA BLOOD AGAR SHEEP			541136	
OXIDASE REAGENT	OXIDASE TEST STRIP				191040ST
HYDROLISIS OF HIPPURATE REAGENT	HIPPURATE HYDROLYSIS - HIP				2456711*
INDOXYL ACETATE DISCS	INDOXYL ACETATE - IAC				2459511*
OXIDASE REAGENT	OXIDASE TEST STRIP				191040ST

CLOSTRIDIUM PERFRINGENS: COLONY COUNT TECHNIQUE

ISO 7937:2004

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Tubes	Flasks
SULFITE-CYCLOSERINE AGAR (SC)	TSC AGAR BASE	402158			
	D-CYCLOSERINE ANTIMICROBIC SUPPL.		4240002		
FLUID THIOGLYCOLLATE MEDIUM	THIOGLYCOLLATE MEDIUM USP	402137		552137	5121372
LACTOSE SULFITE MEDIUM (LS)	LACTOSE SULFITE MEDIUM	401579			
NITRATE MOTILITY MEDIUM	MOTILITY NITRATE CP MEDIUM	401726			
LACTOSE GELATIN MEDIUM	LACTOSE GELATIN MEDIUM	401576			

COLIFORMS: COLONY-COUNT TECHNIQUE

ISO 4832:2006

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Tubes	Flasks
CRYSTAL VIOLET NEUTRAL RED BILE LACTOSE (VRBL) AGAR	VIOLET RED BILE AGAR	402185	542185		5121852/3
BRILLIANT GREEN LACTOSE BILE BROTH	BRILLIANT GREEN BILE BROTH 2%	401265		551265	

COLIFORMS: MOST PROBABLE NUMBER TECHNIQUE

ISO 4831:2006

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Tubes
LAURYL SULFATE TRYPTOSE BROTH	LAURYL PEPTO BIOS BROTH	401580	
BRILLIANT GREEN LACTOSE BILE BROTH	BRILLIANT GREEN BILE BROTH 2%	401265	551265

CRONOBACTER SPP.

ISO 22964:2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
BUFFERED PEPTONE WATER (BPW)	BUFFERED PEPTONE WATER	401278			551278	5112782 5112783
	BPW CASEIN	401278C				
CRONOBACTER SELECTIVE BROTH (CSB)	CRONOBACTER SCREENING BROTH BASE	401355				
	VANCOMYCIN CSB SUPPLEMENT		4240057C			
CHROMOGENIC CRONOBACTER ISOLATION (CCI) AGAR	CHROMOGENIC CRONOBACTER ISOLATION AGAR	408030		548030		
TRYPTONE SOYA AGAR (TSA)	TRIPTIC SOY AGAR	402150		542150	552150	5121502 5121503
OXIDASE REAGENT	OXIDASE TEST STRIP					191040ST
MR / VP BASE MEDIUM	MRVP MEDIUM	4017352				
VOGES -PROSKAUER (VP) REAGENT	VOGES-PROSKAUER-VP (DIATABS)					2457711*

ESCHERICHIA COLI: MOST PROBABLE NUMBER TECHNIQUE

ISO 7251:2005

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Tubes	Flasks/ Reagents
LAURYL SULFATE BROTH	LAURYL PEPTO BIOS BROTH	401580		
EC BROTH	EC BROTH	401425	551425	
PEPTONE WATER INDOLE FREE	PEPTONE-TRYPTONE WATER	401891	551891	5518912
INDOLE REAGENT	KOVACS' REAGENT			19171000 19171001

E. COLI β-GLUCURONIDASE POSITIVE: COLONY-COUNT TECHNIQUE AT 44 DEGREES USING MEMBRANES

ISO 16649-1:2018;

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Tubes
MINERALS MODIFIED GLUTAMATE MEDIUM	MINERALS MODIFIED GLUTAMATE MEDIUM	401737		551737 551737D
	SODIUM GLUTAMATE		412364	

E. COLI β-GLUCURONIDASE POSITIVE: COLONY-COUNT TECHNIQUE AT 44 DEGREES

ISO 16649-2:2001

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
TRYPTONE BILE GLUCURONIDE AGAR	TRYPTONE BILE X-GLUC AGAR (TBX)	402156		542156 49 2156	5121562

E. COLI β-GLUCURONIDASE POSITIVE: DETECTION WITH MOST PROBABLE NUMBER TECHNIQUE

ISO 16649-3:2015

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Tubes
MINERALS MODIFIED GLUTAMATE MEDIUM	MINERALS MODIFIED GLUTAMATE MEDIUM	401737		551737 551737D
	SODIUM GLUTAMATE		412364	

ESCHERICHIA COLI O157: DETECTION

ISO 16654:2001-AMD 2:2023

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Supplement	Flasks/ Reagents
MODIFIED TRYPTONE SOYA BROTH WITHNOVOBIOCIN	TRYPTIC SOY BROTH MODIFIED (m-TSB)	402155M			
	NOVOBIOCIN ANTIMICROBIC SUPPLEMENT			4240045	
TC-SMAC	MAC CONKEY SORBITOL AGAR	401669S			
	CEFIXIME TELLURITE O157 SUPPLEMENT			42ISEC	
NUTRIENT AGAR	NUTRIENT AGAR	401810	541810		5118102
TRYPTONE TRYPTOPHANE MEDIUM	TRYPTONE TRYPTOPHANE MEDIUM	402165			552165
INDOLE REAGENT	KOVACS' REAGENT				19171000 19171001

ENTEROBACTERIACEAE: DETECTION

ISO 21528-1:2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Tubes	Flasks/ Reagents
BUFFERED PEPTONE WATER (ENRICHMENT AND DILUENT)	BUFFERED PEPTONE WATER	401278		551278	5112782 5112783
	BPW CASEIN	401278C			
VIOLET RED BILE GLUCOSE (VRBG) AGAR	VIOLET RED BILE GLUCOSE AGAR	402188	542188		5121882 5121883
NUTRIENT AGAR	NUTRIENT AGAR n° 3	401814			
GLUCOSE O/F MEDIUM	GLUCOSE OF MEDIUM	401525		551525	
OXIDASE REAGENT	OXIDASE TEST STRIP				191040ST

ENTEROBACTERIACEAE: COLONY-COUNT

ISO 21528-2:2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Tubes	Flasks/ Reagents
VIOLET RED BILE GLUCOSE (VRBG) AGAR	VIOLET RED BILE GLUCOSE AGAR	402188	542188		5121882 5121883
NUTRIENT AGAR	NUTRIENT AGAR n° 3	401814			
GLUCOSE O/F MEDIUM	GLUCOSE OF MEDIUM	401525		551525	
OXIDASE REAGENT	OXIDASE TEST STRIP				191040ST

LACTOBACILLUS ACIDOPHILUS: COLONY-COUNT TECHNIQUE AT 37 °C

ISO 20128:2006 IDF 192:2006

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder
MRS/CLINDAMYCIN/CIPROFLOXACIN AGAR (MRS/CL/CIP AGAR)	MRS AGAR WITH TWEEN 80	401728
	CLYNDAMICIN AND CIPROFLOXACIN	NA

LISTERIA MONOCYTOGENES AND *LISTERIA* SPP.: DETECTION

ISO 11290-1:2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
HALF FRASER BROTH	FRASER BROTH BASE	401495				
	FRASER HALF SELECTIVE SUPPLEMENT		4240044			
	LISTERIA FRASER HALF BROTH				551594	5115942 5115943
	LISTERIA FRASER BROTH BASE HALF CONCENTRATION	401594				
	LISTERIA FRASER SUPPLEMENT (FERRIC AMMONIUM CITRATE)		4240056 42185056 42185056A			
FRASER BROTH	FRASER BROTH BASE	401495				
	FRASER SELECTIVE SUPPLEMENT		4240043			
	LISTERIA FRASER BROTH				551596	5115962 5115963
	LISTERIA FRASER BROTH BASE	401596				
	LISTERIA FRASER SUPPLEMENT (FERRIC AMMONIUM CITRATE)		4240056 42185056 42185056A			
AGAR LISTERIA ACC.TO OTTAVIANI AGOSTI (ALOA)	AGAR LISTERIA ACC.TO OTTAVIANI AGOSTI (ALOA®)	401605				
	ALOA® SELECTIVE ENRICHMENT SUPPL.		423501			
	ALOA® AGAR LISTERIA ACC.TO OTTAVIANI AGOSTI			541605 501605P		
SECOND SELECTIVE MEDIUM	LISTERIA PALCAM AGAR BASE	401604				
	PALCAM SELETIVE SUPPLEMENT		4240042			
	LISTERIA SELECTIVE AGAR (PALCAM) or			541604		
	LISTERIA OXFORD AGAR BASE	401600				
	OXFORD ANTIMICROBIC SUPPLEMENT		4240038			
	LISTERIA SELECTIVE AGAR (OXFORD)			541600		
HYDROGEN PEROXIDE SOLUTION	NA					
MOTILITY AGAR	MOTILITY MEDIUM	401714			551714	
BLOOD AGAR (pH7,2+/-0,2)	BLOOD AGAR BASE N°2 PH 7,2	401156P		541156P		
	SANGUE DEFIBRINATO DI MONTONE		90SDX50			
PHOSPHATE BUFFERED SALINE	NA					
RED BLOOD CORPUSCLE SUSPENSION	NA					
CAMP MEDIUM	BLOOD AGAR BASE N°2 - PH 7,2	401156P		541156P		
	SANGUE DEFIBRINATO DI MONTONE		90SDX50			
	BLOOD AGAR SHEEP -ISO FORMULATION			541156P		
CARBOHYDRATE UTILIZATION BROTH	FERMENTATION BROTH BASE	401488			521488X 521488R	
VOGES -PROSKAUER (VP) REACTION	VOGES-PROSKAUER-VP (DIATABS)					2457711
TSYEA	TRYPTIC SOY YEAST EXTRACT AGAR	402166		542166		

LISTERIA MONOCYTOGENES: ENUMERATION

ISO 11290-2:2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
BUFFERED PEPTONE WATER or	BUFFERED PEPTONE WATER or	401278			551278	5112782 5112783
	BPW CASEIN	401278C				
HALF FRASER BROTH (with or without selective agents)	FRASER BROTH BASE	401495				
	FRASER HALF SELECTIVE SUPPLEMENT		4240044			
	LISTERIA FRASER HALF BROTH				551594	5115942 5115943
AGAR LISTERIA ACC.TO OTTAVIANI AGOSTI (ALOA)	AGAR LISTERIA ACC.TO OTTAVIANI AGOSTI (ALOA®)	401605				
	ALOA® SELECTIVE ENRICHMENT SUPPL.		423501			
	ALOA®AGAR LISTERIA ACC.TO OTTAVIANI AGOSTI			541605 501605P		
TSYEA	TRYPTIC SOY YEAST EXTRACT AGAR	402166			542166	
HYDROGEN PEROXIDE SOLUTION	NA					
MOTILITY AGAR	MOTILITY MEDIUM	401714			551714	
BLOOD AGAR	BLOOD AGAR BASE N°2 - PH 7,2	401156P				
	SANGUE DEFIBRINATO DI MONTONE		90SDX50			
	BLOOD AGAR SHEEP -ISO FORMULATION (pH 7,0-7,2)			541156P		
CAMP MEDIUM	BLOOD AGAR BASE N°2 - PH 7,2	401156P		541156P		
	SANGUE DEFIBRINATO DI MONTONE		90SDX50*			
CARBOHYDRATE UTILISATION BROTH	FERMENTATION BROTH BASE	401488			521488R 521488X	
VOGES -PROSKAUER (VP) REACTION	VOGES-PROSKAUER-VP (DIATABS)					2457711*

MESOPHILIC LACTIC ACID BACTERIA: COLONY COUNT

ISO 15214:1998

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder
MRS MEDIUM (DE MAN, ROGOSA AND SHARPE) AT PH 5,7	MRS AGAR ISO FORMULATION WITH TWEEN 80 (pH to be adjusted)	401728S

PSEUDOMONAS: ENUMERATION IN MEAT AND MEAT PRODUCTS

ISO 13720:2010

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks/ Reagents
CEPHALOTHIN-SODIUM FUSIDATE-CETRIMIDE AGAR	PSEUDMONAS AGAR BASE	401960			
	CFC PSEUDOMONAS SUPPLEMENT		4240075		
	PSEUDOMONAS SELECTIVE AGAR			541959	
OXIDASE TEST	OXIDASE TEST STRIPS				191040ST

PSEUDOMONAS: ENUMERATION IN MILK AND MILK PRODUCTS

ISO/TS 11059:2009 IDF/RM 225:2009

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes
PENICILLIN AND PIMARICIN AGAR (PPA)	PSEUDMONAS AGAR BASE	401960			
	PP PSEUDOMONAS SUPPLEMENT		4240048		
NUTRIENT AGAR	NUTRIENT AGAR	401810		541810	5118102
GLUCOSE AGAR	PURPLE GLUCOSE AGAR	401970			551970

SALMONELLA: DETECTION METHOD

ISO 6579-1 :2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
BUFFERED PEPTONE WATER	BUFFERED PEPTONE WATER	401278			551278	5112782 5112783
	BUFFERED PEPTONE WATER CASEIN	401278C				
RAPPAPORT-VASSILIADIS MEDIUM WITH SOYA (RVS BROTH)	RAPPAPORT VASSILIADIS SOY (RVS) BROTH	401981			551981	5119812
MODIFIED SEMI-SOLID RAPPAPORT-VASSILIADIS (MSRV) AGAR	MSRV MEDIUM BASE + NOVOBIOCIN ANTIMICROBIC SUPPLEMENT (10 mg/L)	401982	4240045			
MULLER-KAUFFMANN TETRATHIONATE-NOVOBIOCIN (MKTTN) BROTH	MULLER KAUFFMANN TETRATHIONATE BROTH BASE ISO FORMULATION	401745				
	NOVOBIOCIN MKTT SELECTIVE SUPPLEMENT		4240047			
	IODINE SOLUTION		421501			
	MULLER KAUFFMANN TETRATHIONATE NOVOBIOCIN BROTH				551745	5117452
XYLOSE LYSINE DEOXYCHOLATE AGAR (XLD AGAR)	XLD AGAR ISO FORMULATION	402208		542208		
SECOND ISOLATION AGAR	CHROMOGENIC SALMONELLA AGAR BASE	405350				
	SALMONELLA SELECTIVE SUPPLEMENT		4240013			
	CHROMOGENIC SALMONELLA AGAR			545350		
NUTRIENT AGAR (with NaCl optional)	NUTRIENT AGAR	401810		541810		5118102
	NUTRIENT AGAR n°3	401814				
TSI AGAR	TRIPLE SUGAR IRON AGAR ISO FORM.	402141S				
UREA AGAR (CHRISTENSEN)	UREA AGAR BASE CHRISTENSEN	402175				
	UREA 40% SOLUTION		4240096 42211601			
	UREA AGAR				552175	
BETA GALATTOSIDASE REAGENT	ONPG -(BETA-GALATTOSIDASE)					2450311*
TRYPTONE TRYPROPHAN MEDIUM	TRYPTONE TRYPTOPHAN MEDIUM	402165			552165	
INDOLE REAGENT	KOVACS' REAGENT					19171000 19171001

SALMONELLA: DETECTION OF *SALMONELLA ENTERICA* SUBSPP. *ENTERICA* SEROVARS TYPHI AND PARATYPHI

ISO 6579-1 :2017-annex D; ISO 6579-1:2017/AMD 1:2020

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Tubes	Flasks
BUFFERED PEPTONE WATER	BUFFERED PEPTONE WATER	401278		551278	5112782 5112783
RAPPAPORT-VASSILIADIS MEDIUM WITH SOYA (RVS BROTH)	RAPPAPORT VASSILIADIS SOY (RVS) BROTH	401981		551981	5119812
MODIFIED SEMI-SOLID RAPPAPORT-VASSILIADIS (MSRV) AGAR	MSRV MEDIUM BASE + NOVOBIOCIN ANTIMICROBIC SUPPLEMENT (10 mg/L)	401982	4240045		
SELENITE CYSTINE MEDIUM (SC) (L-cystine 0.1g/1100mL)	SELENITE CYSTINE BROTH (L-cystine 0.01g/1000mL)	402026		552026 552026A	
	SELENITE CYSTINE BROTH BASE	402026B			
	SODIUM BISELENITE	4123651			
BISMUTH SULFITE AGAR (BS)	BISMUTH SULPHITE AGAR	4012102			

SALMONELLA: ENUMERATION BY A MINIATURIZED MOST PROBABLE NUMBER TECHNIQUE

ISO/TS 6579-2:2012

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks
MODIFIED SEMI-SOLID RAPPAPORT-VASSILIADIS MEDIUM (MSRV)	MSRV MEDIUM BASE	401982				
	NOVOBIOCIN ANTIMICROBIC SUPPLEMENT (10 mg/L)		4240045			
XLD AGAR	XLD AGAR ISO FORMULATION	402208		542208		
2nd PLATING MEDIUM	CHROMOGENIC SALMONELLA AGAR BASE	405350				
	SALMONELLA SELECTIVE SUPPLEMENT		4240013			
	CHROMOGENIC SALMONELLA AGAR			545350		5118102
NUTRIENT AGAR	NUTRIENT AGAR	401810	541810			
TSI AGAR	TRIPLE SUGAR IRON AGAR ISO FORM.	402141S				
UREA AGAR (CHRISTENSEN)	UREA AGAR BASE CHRISTENSEN	402175				
	UREA 40% SOLUTION		4240096 42211601			
	UREA AGAR				552175	

SALMONELLA: GUIDELINES FOR SEROTYPING OF *SALMONELLA* SPP

ISO/TR 6579-3:2014

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Flasks
NUTRIENT AGAR (with NaCl optional)	NUTRIENT AGAR	401810	541810	5118102
	NUTRIENT AGAR n°3	401814		

SHIGELLA: DETECTION OF SHIGELLA SPP.

ISO 21567:2004

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks
SHIGELLA BROTH	SHIGELLA BROTH BASE	402040				
	NOVOBIOCIN ANTIMICROBIC SUPPLEMENT		4240045			
MACCONKEY AGAR	MAC CONKEY AGAR	401670		541670		5116702
XLD AGAR	XLD AGAR ISO FORMULATION	402208		542208		
HEKTOEN ENTERIC AGAR	HEKTOEN ENTERIC AGAR	401541		541541		
NUTRIENT AGAR	NUTRIENT AGAR	401810		541810		5118102
TSI AGAR	TRIPLE SUGAR IRON AGAR ISO	402141S				
UREA AGAR (CHRISTENSEN)	UREA AGAR BASE CHRISTENSEN	402175				
	UREA 40% SOLUTION		4240096 42211601			
	UREA AGAR				552175	

SULFITE REDUCING BACTERIA: ENUMERATION

ISO 15213-1:2023

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder
IRON SULFITE AGAR	IRON SULFITE AGAR	401565

STAPHYLOCOCCI – ENUMERATION OF COAGULASE POSITIVE STAPHYLOCOCCI USING BAIRD PARKER AGAR

ISO 6888-1:2021

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
BAIRD PARKER AGAR MEDIUM	BAIRD PARKER AGAR BASE	401116				5111162
	EGG YOLK TELLURITE EMULSION 20%		423700 423701 423702			
	BAIRD PARKER EYT AGAR			541116		
BRAIN HEART INFUSION BROTH	BRAIN HEART INFUSION BROTH	401230			551230	
RABBIT PLASMA	COAGULASE PLASMA EDTA					429937 429936 429938

STAPHYLOCOCCI - ENUMERATION OF COAGULASE POSITIVE STAPHYLOCOCCI USING RPF AGAR

ISO 6888-2:2021

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
RABBIT PLASMA FIBRINOGEN AGAR MEDIUM	BAIRD PARKER AGAR BASE	401116			5111162
	RPF SUPPLEMENT II		423102 423102D		
	BAIRD PARKER RPF AGAR			513101	5131022

STAPHYLOCOCCI - DETECTION AND MPN TECHNIQUE FOR LOW NUMBERS

ISO 6888-3:2003

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
MODIFIED GIOLITTI AND CANTONI BROTH	GIOLITTI-CANTONI BROTH	401516				
	POTASSIUM TELLURITE 1% SOLUTION		42211501			
AGAR SOLUTION	AGAR BIOS LL	411030				
BAIRD PARKER AGAR MEDIUM	BAIRD PARKER AGAR BASE	401116				5111162
	EGG YOLK TELLURITE EMULSION 20%		423700 423701 423702			
	SULFAMEZATHINE SELECTIVE SUPPL.		423050			
	BAIRD PARKER AGAR			541116		
RABBIT PLASMA FIBRINOGEN AGAR MEDIUM	BAIRD PARKER AGAR BASE	401116				5111162
	RPF SUPPLEMENT II		423102 423102D			
	BAIRD PARKER RPF AGAR			543101		5131022
BRAIN HEART INFUSION BROTH	BRAIN HEART INFUSION BROTH	401230			551230	
RABBIT PLASMA	COAGULASE PLASMA EDTA					429937 429938 429936

VIBRIO: DETECTION OF *VIBRIO PARAHAEMOLYTICUS, VIBRIO CHOLERAE, VIBRIO VULNIFICUS*

ISO 21872-1:2017+Amd 1:2023

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder		Plates	Flasks/ Reagents
ALKALINE SALINE PEPTONE WATER (pH 8,6)	ALKALINE PEPTONE WATER	401032			
THIOSULFATE, CITRATE, BILE AND SUCROSE AGAR (TCBS)	TCBS KOBAYASHI AGAR	402106		542106	
OXIDASE REAGENT	OXIDASE TEST STRIP				191040ST
BETA GALATTOSIDASE REAGENT	ONPG -(BETA-GALATTOSIDASE)				2450311*
KOVACS' REAGENT	KOVACS' REAGENT				19171000 19171001

YEASTS AND MOULDS: COLONY COUNT TECHNIQUE IN PRODUCTS WITH WATER ACTIVITY GREATER THAN 0,95

ISO 21527-1:2008

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
DICHLORAN-ROSE BENGAL CHLORAMPHENICOL AGAR (DRBC)	DRBC AGAR BASE	4013932			5113933
	CHLORAMPHENICOL ANT SUPPLEMENT		4240003		
	DRBC CHLORAMPHENICOL AGAR	401393C			
	DRBC AGAR			541393	

YEASTS AND MOULDS: COLONY COUNT TECHNIQUE IN PRODUCTS WITH WATER ACTIVITY LESS THAN OR EQUAL TO 0,95

ISO 21527-2:2008

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
DICHLORAN 18 % MASS FRACTION GLYCEROL AGAR (DG18)	DG18 AGAR BASE	4013942			5113943
	CHLORAMPHENICOL ANTIMICROBIC SUPPLEMENT		4240003		
	DG18 CHLORAMPHENICOL AGAR	401394C			
	GLYCEROL		421015		
	DG 18 AGAR			541394	

YERSINIA ENTEROCOLITICA: DETECTION METHOD

ISO 10273:2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
PSB BROTH	YERSINIA PSB BROTH	402270				
ITC BROTH	YERSINIA ITC BROTH BASE	402265				
	TICARCILLIN IRGASAN ANTIMICROBIC SUPP.		4240060			
	POTASSIUM CHLORATE SUPPLEMENT		4240065			
CIN AGAR	CIN AGAR BASE	401302				
	YERSINIA SELECTIVE SUPPLEMENT	4240011				
	YERSINIA SELECTIVE AGAR			549997		
OPTIONAL CHROMOGENIC MEDIUM	CHROMOGENIC YERSINA AGAR BASE	408050				
	CHROMOGENIC YERSINIA SUPPLEMENT	4240095				
NUTRIENT AGAR (with NaCl optional)	NUTRIENT AGAR	401810		541810		5118102
	NUTRIENT AGAR N°3	401814				
TRYPTIC SOY BROTH (TSB)	TRYPTIC SOY BROTH	402155			552155	
UREA AGAR (CHRISTENSEN)	UREA AGAR BASE (CHRISTENSEN)	402175			552275	
	UREA 40% SOLUTION		42211601 4240096			
BILE AND ESCULIN AGAR (pH 6,6)	BILE ESCULIN AGAR (pH 6,4)	401017				
PHENYLALANINE (TRYPTOPHANE) DEAMIN. AG. (PH7,3)	PHENYLALANINE AGAR (pH 7,2)	401916			551916	
KOVACS' REAGENT	KOVACS' REAGENT					19171000 19171001

YERSINIA ENTEROCOLITICA: PROCEDURE FOR COLD ENRICHMENT

ISO 10273:2017 ANNEX D

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder
PSB BROTH	YERSINIA PSB BROTH	402270
CIN AGAR	CIN AGAR	401302

TOTAL MICROBIAL COUNT IN CORK STOPPERS

ISO 10718:2015

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder
M-GREEN	M-GREEN AGAR	401527

Note *: only for Italian market

References

1. ISO 6887-1:2017 Microbiology of the food chain -Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 1: General rules for the preparation of the initial suspension and decimal dilutions
2. ISO 6887-2:2017. Microbiology of the food chain -Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 2: Specific rules for the preparation of meat and meat products
3. ISO 6887-3:2017/Amd 1:2020 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products — Amendment 1: Sample preparation for raw marine gastropods
4. ISO 6887-4:2017. Microbiology of the food chain -Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 4: Specific rules for the preparation of miscellaneous products
5. ISO 6887-5:2020. Microbiology of the food chain -Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 5: Specific rules for the preparation of milk and milk products
6. ISO 6887-6:2013. Microbiology of food and animal feed -Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 6: Specific rules for the preparation of samples taken at the primary production stage
7. ISO 7218:2007. Microbiology of food and animal feeding stuffs -General requirements and guidance for microbiological examinations
8. ISO 4833-1:2013/Amd 1:2022 Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique — Amendment 1: Clarification of scope
9. ISO 4833-2:2013/Amd 1:2022 Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 2: Colony count at 30 °C by the surface plating technique — Amendment 1: Clarification of scope
10. ISO 17410:2019. Microbiology of the food chain -Horizontal method for the enumeration of psychrotrophic microorganisms
11. ISO 7932:2004/Amd 1:2020 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive Bacillus cereus — Colony-count technique at 30 degrees C — Amendment 1: Inclusion of optional tests
12. ISO 21871:2006. Microbiology of food and animal feeding stuffs -Horizontal method for the determination of low numbers of presumptive Bacillus cereus -Most probable number technique and detection method
13. ISO 10272-1:2017+A1:2023. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method
14. ISO 10272-2:2017+A1:2023. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 2: Colony-count technique
15. ISO 7937:2004. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of Clostridium perfringens -Colony-count technique
16. ISO 4832:2006. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of coliforms -Colony-count technique
17. ISO 4831:2006. Microbiology of food and animal feeding stuffs -Horizontal method for the detection and enumeration of coliforms -Most probable number technique
18. ISO 22964:2017. Microbiology of the food chain -Horizontal method for the detection of Cronobacter spp.
19. ISO 7251:2005. Microbiology of food and animal feeding stuffs -Horizontal method for the detection and enumeration of presumptive Escherichia coli -Most probable number technique
20. ISO 16649-1:2018. Microbiology of the food chain -Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli -Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide
21. ISO 16649-2:2001. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli -Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide
22. ISO 16649-3:2015. Microbiology of the food chain -Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli -Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide
23. ISO 16654:2001+AMD 2: 2023. Microbiology of food and animal feeding stuffs -Horizontal method for the detection of Escherichia coli O157-Inclusionof performances testing of all culture media and reagents
24. ISO 21528-1:2017. Microbiology of the food chain -Horizontal method for the detection and enumeration of Enterobacteriaceae -Part 1: Detection of Enterobacteriaceae
25. ISO 21528-2:2017. Microbiology of the food chain -Horizontal method for the detection and enumeration of Enterobacteriaceae -Part 2: Colony-count technique
26. ISO 20128:2006 [IDF 192:2006]. Milk products -Enumeration of presumptive Lactobacillus acidophilus on a selective medium -Colony-count technique at 37 degrees C
27. ISO 11290-1:2017. Microbiology of the food chain -Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -Part 1: Detection method
28. ISO 11290-2:2017. Microbiology of the food chain -Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -Part 2: Enumeration method
29. ISO 15214:1998. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of mesophilic lactic acid bacteria -Colony-count technique at 30 degrees C
30. ISO 13720:2010. Meat and meat products -Enumeration of presumptive Pseudomonas spp.
31. ISO/TS 11059:2009 [IDF/RM 225:2009]. Milk and milk products -Method for the enumeration of Pseudomonas spp.
32. ISO 6579-1:2017. Microbiology of the food chain -Horizontal method for the detection, enumeration and serotyping of Salmonella -Part 1: Detection of Salmonella spp.
33. ISO 6579-1:2017/AMD 1:2020. Microbiology of the food chain -Horizontal method for the detection, enumeration and serotyping of Salmonella -Part 1: Detection of Salmonella spp. -Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC
34. ISO/TS 6579-2:2012. Microbiology of food and animal feed -Horizontal method for the detection, enumeration and serotyping of Salmonella -Part 2: Enumeration by a miniaturized most probable number technique
35. ISO/TR 6579-3:2014. Microbiology of the food chain -Horizontal method for the detection, enumeration and serotyping of Salmonella -Part 3: Guidelines for serotyping of Salmonella spp.
36. ISO 21567:2004. Microbiology of food and animal feeding stuffs -Horizontal method for the detection of Shigella spp.
37. ISO 15213-1:2023. Microbiology of the food chain - Horizontal method for the detection and enumeration of Clostridium spp. Part 1: Enumeration of sulfite-reducing Clostridium spp. by colony-count technique
38. ISO 6888-1:1999/AMD 2:2018. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other spp.) -Part 1: Technique using Baird-Parker agar medium -Amendment 2: Inclusion of an alternative confirmation test using RPFA stab method
39. ISO 6888-1:2021 Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Method using Baird-Parker agar medium
40. ISO 6888-2:2021 Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 2: Method using rabbit plasma fibrinogen agar medium
41. ISO 6888-3:2003. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other spp.) -Part 3: Detection and MPN technique for low numbers
42. ISO 21872-1:2017+Amd 1:2023. Microbiology of the food chain — Horizontal method for the determination of Vibrio spp. — Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus — Amendment 1: Inclusion of performance testing of culture media and reagents
43. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of yeasts and moulds -Part 1: Colony count technique in products with water activity greater than 0,95
44. ISO 21527-2:2008. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of yeasts and moulds -Part 2: Colony count technique in products with water activity less than or equal to 0,95
45. ISO 10273:2017. Microbiology of the food chain -Horizontal method for the detection of pathogenic Yersinia enterocolitica
46. ISO 10718:2015 Cork stoppers — Characterization of a low-in-germs stopper, through the enumeration of colony-forming units of yeasts, moulds and bacteria, capable of both being extracted and growing in alcoholic medium

CULTURE MEDIA FOR WATER TESTING

CAMPYLOBACTER: DETECTION AND ENUMERATION

ISO 17995:2019

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates
COMPLETE PRESTON BROTH	NUTRIENT BROTH N°2	401812S2		
	PRESTON ANTIMICROBIC SUPPLEMENT II		4240022	
	CAMPYLOBACTER GROWTH SUPPLEMENT		4240021	
	LYSED HORSE BLOOD		90HLX100	
COMPLETE BOLTON BROTH	CAMPY. ENRICH. BASE BROTH-BOLTON	401286B		
	BOLTON BROTH SUPPLEMENT	4240025		
COMPLETE MODIFIED-CHARCOAL CEFOPERAZONE DESOXYCHOLATE AGAR (m-CCDA)	CAMPY. BLOOD FREE MEDIUM BASE BOLTON	401282		
	BOLTON CCDA ANTIMICROBRIC SUPPL.		4240020	
	CAMPYL.BLOOD FREE AGAR (CCDA)			541113

CLOSTRIDIUM PERFRINGENS: ENUMERATION BY MEMBRANE FILTRATION METHOD

ISO 14189:2013

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks/ Reagents
TRYPTOSE SULFITE-CYCLOSERINE AGAR (SC)	TSC AGAR BASE	402158			
	D-CYCLOSERINE ANTIMICROBIC SUPPL.		4240002		
	TSC AGAR			492158	
BLOOD AGAR	COLUMBIA BLOOD AGAR	401136		541136	
ACID PHOSPHATASE REAGENT	ACID PHOSPHATASE REAGENT				192010

CULTURABLE MICROORGANISM: COLONY COUNT

ISO 6222:1999

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates
YEAST EXTRACT AGAR	YEAST EXTRACT AGAR	402275	492275

ENTEROCOCCI: DETECTION AND ENUMERATION OF INTESTINAL ENTEROCOCCI BY MEMBRANE FILTRATION METHOD

ISO 7899-2:2000

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates
SLANETZ BARTLEY AGAR	SLANETZ BARTLEY AGAR	402046		492046
	SLANETZ BARTLEY AGAR w/o TTC	402047		
	TTC 1% SOLUTION		42111801	
BILE AESCULIN AZIDE AGAR	BILE AESCULIN AZIDE AGAR ISO FORM.	401018		491018

ESCHERCHIA COLI AND COLIFORM BACTERIA: ENUMERATION BY MEMBRANE FILTRATION METHOD

ISO 9308-1:2014 - Amd 12-2016

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Tubes	Flasks
CHROMOGENIC COLIFORM AGAR (CCA)	CHROMOGENIC COLIFORM AGAR ISO FORM.	401297	491297		
TRYPTONE SOYA AGAR	TRYPTIC SOY AGAR	402150	542150	552150	5121502 5121503

ESCHERCHIA COLI AND COLIFORM BACTERIA: ENUMERATION BY MOST PROBABLE NUMBER METHOD

ISO 9308-2:2012

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Tubes	Flasks/ Reagents
LACTOSE BROTH	LACTOSE BROTH	401575		551575/D	
MAC CONKEY BROTH	MAC CONKEY BROTH PURPLE	401675			
IMPROVED FORMATE LACTOSE GLUTAMATE MEDIUM	MINERALS MODIFIED GLUTAMATE MEDIUM	401982			
	SODIUM GLUTAMATE		412364		
LAURYL TRYPTOSE (LACTOSE BROTH)	LAURYL PEPTO BIOS BROTH	401580			
BRILLIANT GREEN LACTOSE (BILE) BROTH	BRILLIANT GREEN BILE BROTH 2%	401265		551265	

EC MEDIUM	EC BROTH	401425		551425	
TRYPTONE WATER	PEPTONE-TRYPTONE WATER	401891		551891	5118912

LEGIONELLA: ENUMERATION

ISO 11731: 2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks/ Reagents
BUFFERED CHARCOAL YEAST EXTRACT AGAR MEDIUM (BCYE)	LEGIONELLA BCYE AGAR BASE	401582			
	LEGIONELLA BCYE α-GROWTH SUPPL.		423210		
	LEGIONELLA AGAR			549945	
BUFFERED CHARCOAL YEAST EXTRACT MEDIUM WITHOUT L-CYSTEINE (BCYE - CYS)	LEGIONELLA BCYE AGAR BASE	401582			
	LEGIONELLA BCYE α-GROWTH SUPPL. W/O CYSTEINE		423212		
	LEGIONELLA AGAR W/O CYSTEINE			549943	
BUFFERED CHARCOAL YEAST EXTRACT MEDIUM WITH SELECTIVE SUPPLEMENTS (BCYE+AB)	LEGIONELLA BCYE AGAR BASE	401582			
	LEGIONELLA BCYE α-GROWTH SUPPL.		423210		
	LEGIONELLA AB SELECTIVE SUPPL.		423225		
	LEGIONELLA AB SELECTIVE AGAR			549947	
GLYCINE VANCOMYCINE POLYMIXINE B CYCLOHEXIMIDE (GVPC) AGAR	LEGIONELLA BCYE AGAR BASE	401582			
	LEGIONELLA BCYE α-GROWTH SUPPL.		423210		
	LEGIONELLA GVPC SELECTIVE SUPPL.		423215		
	LEGIONELLA SELECTIVE AGAR			549995 499995	
MODIFIED WADOWSKY YEE (MWY) AGAR (with anysomicin)	LEGIONELLA BCYE AGAR BASE	401582			
	LEGIONELLA BCYE α-GROWTH SUPPL.		423210		
	LEGIONELLA MWY SELECTIVE SUPPL. (ISO)		423220		
	LEGIONELLA MWY SELECTIVE AGAR (ISO)			549948	
GLYCEROL BROTH	NUTRIENT BROTH	401815			
	GLYCEROL		421015		
SEROLOGICAL REAGENTS	LEGIONELLA RAPID LATEX TEST				96271050

PSEUDOMONAS AERUGINOSA: DETECTION AND ENUMERATION - METHOD BY MEMBRANE FILTRATION

ISO 16266:2006

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
PSEUDOMONAS AGAR BASE/CN-AGAR	PSEUDOMONAS AGAR BASE	401960				
	CN PSEUDOMONAS SUPPLEMENT		4240046			
	GLYCEROL		421015			
	PSEUDOMONAS CN SELECTIVE AGAR			491960-541960		
KING'S B MEDIUM	PSEUDOMONAS AGAR F	401961		541961		
ACETAMIDE BROTH	ACETAMIDE BROTH	4010101			5510101	
NUTRIENT AGAR	NUTRIENT AGAR W/NACL	401811				
OXIDASE TEST	OXIDASE TEST STRIPS					191040ST

SALMONELLA: DETECTION METHOD

ISO 19250:2010.

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
BUFFERED PEPTONE WATER	BUFFERED PEPTONE WATER	401278			551278	5112782/3
RAPPAPORT-VASSILIADIS MEDIUM WITH SOYA (RVS BROTH)	RAPPAPORT VASSILIADIS SOY (RVS) BROTH	401981			551981	5119812
XYLOSE LYSINE DEOXYCHOLATE AGAR (XLD AGAR)	XLD AGAR ISO FORMULATION	402208		542208		
NUTRIENT AGAR	NUTRIENT AGAR	401810		541810		
TRIPLE SUGAR/IRON AGAR (TSI AGAR)	TRIPLE SUGAR IRON AGAR ISO FORM.	402141S				
UREA AGAR (CHRISTENSEN)	UREA AGAR BASE CHRISTENSEN	402175				
	UREA 40% SOLUTION		4240096 42211601			
	UREA AGAR				552175	
L-LYSINE DECARBOXYLASE MEDIUM	-	NA				
SELENITE CYSTINE BROTH	SELENITE CYSTINE BROTH	402026			552026	5120262
MULLER-KAUFFMANN TETRATHIONATE-NOVOBIOCIN BROTH (MKTTN)	MUELLER KAUFFMANN TETRATHIONATE BROTH BASE ISO FORMULATION	401745				
	NOVOBIOCIN MKTT SELECTIVE SUPPLEMENT		4240047			
	IODINE SOLUTION		421501			
	MUELLER KAUFFMANN TETRATHIONATE NOVOBIOCIN BROTH				551745	5117452

SULFITE REDUCING ANAEROBES CLOSTRIDIA: METHOD BY MEMBRANE FILTRATION

ISO 6461-2:1986

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
SULPHITE IRON AGAR or TRYPOSE-SULFITE AGAR	TSC AGAR BASE	402158				

References

1. ISO 17995:2019. Water quality — Detection and enumeration of thermotolerant Campylobacter spp
2. ISO 14189:2013. Water quality — Enumeration of Clostridium perfringens — Method using membrane filtration
3. ISO 6222:1999. Water quality — Enumeration of culturable micro-organisms — Colony count by inoculation in a nutrient agar culture medium
4. ISO 7899-2:2000. Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method
5. ISO 9308-1:2014. Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora
6. ISO 9308-1:2014/AMD 1:2016. Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora — Amendment 1
7. ISO 9308-2:2012. Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 2: Most probable number method
8. ISO 11731:2017. Water quality — Enumeration of Legionella
9. ISO 16266:2006. Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration
10. ISO 19250:2010. Water quality — Detection of Salmonella spp.
11. ISO 6461-2:1986. Water quality — Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) — Part 2: Method by membrane filtration

CULTURE MEDIA FOR COSMETICS TESTING

PREPARATION OF THE SAMPLE AND SUITABILITY TEST

ISO 18415:2017.

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
Diluent for the microbial suspension (tryptone sodium chloride solution)	MAXIMUM RECOVERY DILUENT	401691			551691	5116912 5116913
Eugon LT100 broth	EUGON BROTH	401643				
	EUGON LT SUPPLEMENT		421540			
	EUGONLT SUP BROTH				551583	5115832
Fluid casein digest-soy lecithin-polysorbate 20 medium (FCDLP 20)	TAT BROTH BASE	402100				
	TWEEN 20		42120501			
Soybean-casein-digest-lecithin-polysorbate 80 medium (SCDLP 80 broth)	TRYPTIC SOY BROTH	402155			552155	5121552 5121553
	TWEEN 80		42120502			
	LECITHIN		41EAT0242			
Agar medium for suitability test Soybean-casein digest agar medium (SCDA) or tryptic soy agar (TSA)	TRYPTIC SOY AGAR	402150		542150	552150	5121502 5121503

ENUMERATION AND DETECTION OF AEROBIC MESOPHILIC BACTERIA

ISO 21149:2017

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Tubes	Flasks/ Reagents
Soybean–casein digest agar medium (SCDA) or tryptic soy agar (TSA)	TRYPTIC SOY AGAR	402150	542150	552150	5121502 5121503

ENUMERATION OF YEAST AND MOULD

ISO 16212:2017.

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks/ Reagents
Sabouraud dextrose chloramphenicol agar medium (SDCA)	SABOURAUD DEXTROSE AGAR + CAF	402006		542006	552006	5120062
Agar medium for cultivation of reference strain: Sabouraud dextrose agar medium (SDA)	SABOURAUD DEXTROSE AGAR	402005		542005		5120052 5120053
Other culture media: Potato dextrose agar medium with antibiotics	POTATO DEXTROSE AGAR	401935				
	CHLORAMPHENICOL ANTIMICROBIC SUPPLEMNENT		4240003			

DETECTION OF *ESCHERICHIA COLI*

ISO 21150:2015

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Flasks/ Reagents
Selective agar medium for isolation of Escherichia coli: MacConkey agar medium	MAC CONKEY AGAR	401670	541670	5116703
Selective agar medium for confirmation of Escherichia coli: Levine eosin-methylene blue agar medium	LEVINE EMB BLUE AGAR	401595		

DETECTION OF *PSEUDOMONAS AERUGINOSA*

ISO 22717:2015

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Plates	Flasks/ Reagents
Selective agar medium for isolation of Pseudomonas aeruginosa: Cetrimide agar medium	PSEUDOMONAS SELCTIVE AGAR	401963	541963	5119632
Agar medium for confirmation of Pseudomonas aeruginosa: Pseudomonas agar medium for detection of pyocyanin (Pseudomonas agar P)	PSEUDOMONAS AGAR P	401962		

DETECTION OF *STAPHYLOCOCCUS AUREUS*

ISO 22718:2015

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Flasks
Selective agar medium for isolation of Staphylococcus aureus: Baird Parker agar medium	BAIRD PARKER BASE AGAR	401116			5111162
	EGG YOLK TELLURITE EMULSION 20%		423700 423701 423702		
	BAIRD PARKER AGAR			541116	
Another selective agar medium: Mannitol-salt agar medium (Chapman agar)	MANNITOL SALT AGAR	401665		541665	
Another selective agar medium: Vogel-Johnson agar medium	VOGEL JOHNSON AGAR	402192			

DETECTION OF *CANDIDA ALBICANS*

ISO 18416:2015

ISO CULTURE MEDIA	BIOLIFE CULTURE MEDIA	Powder	Supplement	Plates	Tubes	Flasks
Selective agar medium for isolation of Candida albicans: Sabouraud dextrose chloramphenicol agar	SABOURAUD DEXTROSE AGAR CAF	402006		542006	552006	5120062
Other culture media: Potato dextrose agar medium with antibiotics	POTATO DEXTROSE AGAR	401935				
	CHLORAMPHENICOL ANTIMICROBIC SUPPLEMENT		4240003			

References

1. ISO 18415:2017/Amd 1:2022 Cosmetics — Microbiology — Detection of specified and non-specified microorganisms — Amendment 1
2. ISO 21149:2017/Amd 1:2022 Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria — Amendment 1
3. ISO 16212:2017/Amd 1:2022 Cosmetics — Microbiology — Enumeration of yeast and mould — Amendment 1
4. ISO 21150:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Escherichia coli — Amendment 1
5. ISO 22717:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa — Amendment 1
6. ISO 22718:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Staphylococcus aureus — Amendment 1
7. ISO 18416:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Candida albicans — Amendment 1

CULTURE MEDIA FOR PHARMACEUTICAL PRODUCTS TESTING

TOTAL COUNT

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder	Plates	Tubes	Flasks
Preparation of the sample	BUFFERED SODIUM CHLORIDE PEPTONE SOLUTION	PHARMACOPOEIA DILUENT	401395		551395N	511395N2
	CASEIN SOJA BEAN DIGEST BROTH	TRYPTIC SOY BROTH	402155		552155	5121552
	BUFFERED PEPTONE MEDIUM	BUFFERED PEPTONE WATER	401278		551278	5112782 5112783
Membrane Filtration Method Poured Plate method Surface Spread Method						
Total aerobic microbial count	CASEIN SOJA BEAN DIGEST AGAR	TRYPTIC SOY AGAR	402150	542150		5121503
	R2A	R2A	401996			5119963
Total yeasts/moulds count	SABOURAUD DEXTROSE AGAR	SABOURAUD DEXTROSE AGAR	402005	542005		5120052 5120053

BILE TOLERANT GRAM-NEGATIVE BACTERIA

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder	Tubes	Flasks
Sample preparation and pre-incubation	CASEIN SOJA BEAN DIGEST BROTH	TRYPTIC SOY BROTH	402155	552155	5121552 5121553
Test for absence	ENTEROBACTERIA ENRICHMENT BROTH MOSSEL	ENTEROBACTERIA ENRICHMENT BROTH MOSSEL EP	401467		
	VIOLET RED BILE GLUCOSE AGAR (VRBG) EP	VIOLET RED BILE GLUCOSE AGAR (VRBG) EP	402189	542189	
Quantitative test	ENTEROBACTERIA ENRICHMENT BROTH MOSSEL	ENTEROBACTERIA ENRICHMENT BROTH MOSSEL EP	401467		
	VIOLET RED BILE GLUCOSE AGAR (VRBG) EP	VIOLET RED BILE GLUCOSE AGAR (VRBG) EP	402189		

ESCHERICHIA COLI

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder	Plates	Tubes	Flasks
Sample preparation and pre-incubation	CASEIN SOJA BEAN DIGEST BROTH	TRYPTIC SOY BROTH	402155		552155	5121552 5121553
Selection and subculture	MAC CONKEY BROTH	MAC CONKEY BROTH EP	401679			
	MAC CONKEY AGAR	MAC CONKEY AGAR	401670	541670		5116703

SALMONELLA

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder	Plates	Tubes	Flasks
Sample preparation and pre-incubation	CASEIN SOJA BEAN DIGEST BROTH	TRYPTIC SOY BROTH	402155		552155	5121552 5121553
Selection and subculture	RAPPAPORT VASSILIADIS SALMONELLA ENRICHMENT BROTH	RAPPAPORT VASSILIADIS SALM. ENR. BROTH EP	401979			
	XYLOSE, LYSINE, DESOXYCHOLATE AGAR	XLD AGAR	402206	542006		

PSEUDOMONAS AERUGINOSA

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder	Plates	Tubes	Flasks
Sample preparation and pre-incubation	CASEIN SOJA BEAN DIGEST BROTH	TRYPTIC SOY BROTH	402155		552155	5121552 5121553
Selection and subculture	MANNITOL SALT AGAR	MANNITOL SALT AGAR	401665	541665		

CLOSTRIDIA

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder
Selection and subculture	REINFORCED MEDIUM FOR CLOSTRIDIA	CLOSTRIDIUM BROTH	401304
	COLUMBIA AGAR	COLUMBIA AGAR EP	401134

CANDIDA ALBICANS

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder	Plates	Tubes	Flasks
Sample preparation and pre-incubation	SABOURAUD DEXTROSE BROTH	SABOURAUD BROTH	402000		552000	5120002
Selection and subculture	SABOURAUD DEXTROSE AGAR	SABOURAUD DEXTROSE AGAR	402005	542005		5120052 5120053

STERILITY TEST

TEST	PHARMACOPOEIA CULTURE MEDIUM	BIOLIFE CULTURE MEDIUM	Powder	Tubes	Flasks
Bacterial Sterility Test	FLUID THIOGLYCOLLATE MEDIUM	THIOGLYCOLLATE MEDIUM USP	402137	552137	
Mycotic Sterility Test	SOJA-BEAN CASEIN DIGEST MEDIUM	TRYPTIC SOY BROTH	402155	552155	5121552 5121553

References

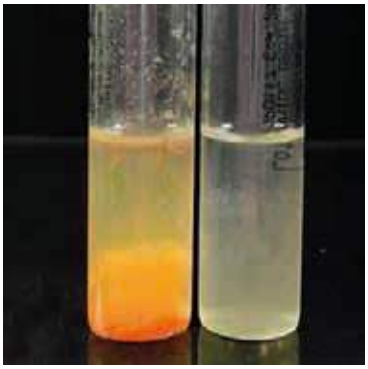
1. European Pharmacopoeia 11th Edition, 2022, Vol. 1. 2.6.1 Sterility test.
2. European Pharmacopoeia 11th Edition, 2022, Vol. 1. 2.6.12 Microbiological examination of non-sterile pharmaceutical products: microbial enumeration test.
3. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631

Section 2

Monographs of culture media

ACETAMIDE BROTH

Dehydrated and ready-to-use culture medium



Positive confirmation of *P. aeruginosa* at left and negative at right

INTENDED USE

For the confirmation test of *Pseudomonas aeruginosa* according to ISO 16266.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE TUBES

Potassium dihydrogen phosphate	1.0 g
Sodium chloride	0.2 g
Magnesium sulphate anhydrous	0.2 g
Acetamide	2.0 g
Sodium molybdate	5.0 mg
Iron sulphate heptahydrate	0.5 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Acetamide Broth contains acetamide as a sole source of carbon for the growth. It is recommended by ISO 16266 for the confirmation of presumptive *Pseudomonas aeruginosa* colonies isolated from water samples by membrane filtration method.[1]
Acetamide Broth is based on the ability of non-fermenting Gram-negative bacteria to deaminate the acetamide with production of ammonia which increases the pH of the medium indicated by the change in colour of the Nessler reagent added to the broth.
Potassium dihydrogen phosphate has a high buffering capacity, sodium chloride contributes to the osmotic balance, while magnesium sulphate, sodium molybdate and iron sulphate allow the selective growth of *Pseudomonas*.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 3.4 g in 1000 mL of cold purified water. Adjust the pH to 7.0 ± 0.5 at 25 °C with either hydrochloric acid or sodium hydroxide, if necessary. Dispense this mixture in 5 mL aliquots to culture tubes which are then capped and sterilized in an autoclave at 121 ± 3 °C for 15 min.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	colourless, fine, homogeneous, free-flowing powder
Prepared tubes appearance	straw coloured, limpid
Final pH at 20-25 °C	7.0 ± 0.5

SPECIMENS

Acetamide Broth is not intended for primary isolation from samples; it is inoculated with pure colonies from a culture on solid media.

TEST PROCEDURE

Subculture all, or if impracticable, as many as possible of the colonies cultivated on the selective medium requiring confirmation from the membrane filter to Nutrient Agar and incubate for 22 ± 2 h at 36 ± 2 °C. Check the subcultures for purity and test those that were initially reddish brown for the oxidase reaction.
Inoculate a tube of Acetamide Broth with the subculture and incubate at 36 ± 2 °C for 22 ± 2 h

READING AND INTERPRETATION

After incubation, add 1 to 2 drops of Nessler reagent and examine the tubes for the production of ammonia, characterized by the production of a colour varying from yellow to brick red depending upon concentration.
Count as confirmed *P. aeruginosa* all colonies which produce pyocyanin (blue/green pigment on Pseudomonas Agar F – King Medium B) or which are oxidase positive, fluoresce under UV radiation and are able to produce ammonia from acetamide.[1]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. aeruginosa ATCC 10145	34-38°C / 20-24 H/ A	growth with colour change of Nessler Reagent
E. coli ATCC 11775	35-37°C / 48 H/ A	no growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place. According to ISO 16266, the self-prepared tubes can be stored at +2°C +8°C in the dark for up to 3 months.
Ready-to-use tubes
Sore tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. ISO 16266:2006 Water quality – Detection and enumeration of *Pseudomonas aeruginosa* by membrane filtration.

PACKAGING

Product	Type	REF	Pack
Acetamide Broth	Dehydrated medium	40101012	500 g (146.8 L)
Acetamide Broth	Ready-to-use tubes	5510101	20 x 5 mL

IFU rev. 2, 2022/04

m-AEROMONAS SELECTIVE AGAR BASE (HAVELAAR)
AEROMONAS SELECTIVE SUPPLEMENT
Dehydrated culture medium and selective supplement

Aeromonas Selective Agar (Havelaar): *A. hydrophila* colonies on a membrane filter

INTENDED USE
Selective and differential medium for the detection of *Aeromonas* in finished water by membrane filtration.

COMPOSITION *
AEROMONAS SELECTIVE AGAR BASE (HAVELAAR)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	5.00 g
Yeast extract	2.00 g
Dextrin	11.40 g
Sodium chloride	3.00 g
Potassium chloride	2.00 g
Magnesium sulphate	0.10 g
Ferric chloride	0.06 g
Sodium deoxycholate	0.10 g
Bromothymol blue	0.08 g
Agar	13.00 g

AEROMONAS SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Ampicillin	5 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Aeromonas is a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria that morphologically resemble members of the family *Enterobacteriaceae*. The significance of *Aeromonas* species as human pathogens is getting increasing attention.[1] The organisms are ubiquitous in fresh and brackish water[2], are considered as potential food-poisoning agents [3] and are responsible for, and are implicated in, a number of intestinal and extra-intestinal infections in humans as well as other animals.[4]
m-Aeromonas Selective Agar Base supplemented with ampicillin, corresponds to the medium described by Havelaar et al.[5,6] Supplemented with ampicillin and vancomycin, it is recommended by United States Environmental Protection Agency (USEPA Method 1605), for the detection of *Aeromonas* in finished water by membrane filtration.[7]
Recovery from pure cultures and environmental samples is optimal and specificity is high.[5] The use of ampicillin suppresses adequately the background flora without having any decrease in the *Aeromonas* recovery. Strains sensitive to 10 mg/l of ampicillin appear to occur at a frequency of 1% or less.[5] Vancomycin increases selectivity properties due to its inhibitory action on Gram-positive bacteria.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 18.35 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and, under aseptic conditions, add the contents of one vial of Aeromonas Selective Supplement-Ampicillin (REF 4240012) reconstituted with 5ml of sterile purified water. Mix well and distribute into sterile 55 mm dishes.
USEPA methods require the addition of vancomycin hydrochloride: reconstitute one vial of Vancomycin Selective Supplement CSB (REF 4240057C) with 5 mL of sterile purified water; to 500 mL of medium prepared as described above with ampicillin, add 1 mL of Vancomycin Selective Supplement CBS (final concentration of vancomycin: 2 mg/L).

PHYSICAL CHARACTERISTICS

Aeromonas Selective Agar Base (Havelaar)	
Dehydrated medium appearance	grey-green, fine, homogeneous, free-flowing powder
Prepared plates appearance	green-light blue, limpid, limpid
Final pH at 20-25 °C	8.0 ± 0.2
Aeromonas Selective Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless, limpid

SPECIMENS
Samples representative of the drinking water distribution system. Consult the appropriate reference for sample collection, preservation, and storage.[7]

TEST PROCEDURE
Appropriate volumes or decimal dilutions of the samples are filtered using membrane filters (0.45 µm pore size), and the filters are transferred onto the medium surface. Incubate in aerobic conditions at 30°C[5] or 35°C[7] for 24 ± 2 hours

READING AND INTERPRETATION
After incubation, *Aeromonas* colonies show a visible yellow colour (dextrin fermentation). The detection of dextrin fermentation is considered to be highly specific and until now no dextrin negative *Aeromonas* strains have been found.[5]
Confirm the presumptive detection with standard biochemical tests: trehalose fermentation, oxidase test and production of indole. Any presumptive colony that is positive for oxidase, ferments trehalose, and produces indole is considered to be *Aeromonas*.[7]

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
A. hydrophila ATCC 7966	35°C / 24 H/ A	growth with yellow colonies
E. coli ATCC 11775	35°C / 24 H/ A	no growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- A very low percentage of *Aeromonas,* susceptible to 10 mg/L of ampicillin, may be inhibited on the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, suitable identification tests should be performed.

STORAGE CONDITIONS

Dehydrated medium

Sore at +10°C /+30°C away from direct light in a dry place.

Freeze-dried supplement

Sore the product in the original package at +2°C /+8°C away from direct light.

According to USEPA method[7], the prepared plates with ampicillin and vancomycin should be stored in a tight-fitting container (i.e., sealed plastic bag) at a temperature of 1 to 5°C for no longer than 14 days.

REFERENCES

1. Holmberg SC, Farmer JJ. Aeromonas hydrophila and Plesiomonas shigelloides as causes of intestinal infections Rev Inf Dis. 1984; 6: 633-639
2. Graf J (editor). (2015). Aeromonas. Caister Academic Press.
3. Palumbo SA, Maxino F, Williams AC Buchanan RL, Thayer DW.. Starch-Ampicillin Agar for the Quantitative Detection of Aeromonas hydrophila. App Environ Microbiol. 1985; 50:1027-1030.
4. Parker JL, Shaw JG. Aeromonas spp. clinical microbiology and disease. J Infect . 2011 Feb; 62(2):109-18.
5. Havelaar AH, During M, Versteegh JFM. Ampicillin-dextrin agar medium for the enumeration of Aeromonas species in water by membrane filtration. J App Bact 1988; 62: 279-287.
6. Havelaar AH, Vonk M. The preparation of ampicillin dextrin agar for the enumeration of Aeromonas in water. Letters App Bact 1988; 7: 169-171
7. United States Environmental Protection Agency (USEPA), Method 1605: Aeromonas in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V). October 2001.

PACKAGING

Product	Type	REF	Pack
m-Aeromonas Selective Agar Base	Dehydrated medium	4010192	500 g (13.6 L)
Aeromonas Selective Supplement-Ampicillin	Freeze-dried selective supplement	4240012	10 vials, each for 500 mL of medium

IFU rev. 2, 2022/04

AESCULIN BILE AZIDE AGAR

Dehydrated culture medium

Aesculin Bile Azide Agar: *Enterococcus faecalis*

INTENDED USE

Selective and differential medium for the isolation and differentiation of enterococci from food, water and environmental samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	17.00 g
Peptone	3.00 g
Yeast extract	5.00 g
Oxgall	10.00 g
Sodium chloride	5.00 g
Sodium citrate	1.00 g
Aesculin	1.00 g
Fe-ammonium citrate	0.50 g
Sodium azide	0.25 g
Agar	13.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Aesculin Bile Azide Agar is a selective and diagnostic medium for enterococci. Rochaix first reported in 1924[1] on the validity of the esculin hydrolysis reaction for the identification of enterococci. Media of this type have been studied and proposed since 1950, and some authors evaluated their efficacy in the clinical and hygienic field.[2]

Aesculin Bile Azide Agar is based on the formulation of Isenberg et al.[3] who, in 1970, modified the Rochaix formula by reducing the concentration of bile salts and adding sodium azide for the isolation of group D-streptococci and their differentiation from non-group D streptococci. According to current bacterial nomenclature, the designation 'group D streptococci' for faecal streptococci is not to be regarded as specific since the Lancefield group D antigen is produced by *Enterococcus*, *Pediococcus* and some streptococci.[4,5] Differentiation between group D-enterococci and group D non-enterococci (both are positive in the esculin test) can be made by the 6.5% NaCl tolerance test: the former group is tolerant to this salt concentration, the latter is inhibited by it.[5,6]

Tryptone, peptone and yeast extract provide nitrogen, carbon, vitamins, amino acids and trace elements for microbial growth; sodium azide and bile salts are selective agents that limit the growth of Gram-negative and Gram-positive bacteria other than streptococci; esculin is hydrolysed by enterococci to glucose and esculetin (6-7-dihydroxycoumarin): esculetin reacts with the iron salts in the medium, giving it a brown-black colour.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 55.7 in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121° C for 15 minutes. Do not exceed sterilisation time and temperature. Mix well and distribute in sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	tan with trace blue cast, limpid
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

Refer to applicable international standards and regulations for the collection of food and water samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

Prepare tenfold dilutions of the sample with peptone water. Within 3 hours from the sample preparation, spread 0.1 ml of the inoculum onto the plates. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium).

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
The appearance of enterococci colonies on Aesculin Bile Azide Agar after 24 hours of incubation at 37°C is characteristic: convex, translucent or whitish colonies, 1-2 mm in diameter with dark brown or black halo; incubation should not be prolonged beyond 24 hours, because extensive blackening of the medium makes reading difficult.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 29212	37°C/24 H/A	good growth, colonies with blackening
S. pyogenes ATCC 19615	37°C/24 H/A	partially inhibited
E. coli ATCC 25922	37°C/24 H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Uneven distribution of bacterial colonies or the presence of high microbial loads may interfere with the differentiation of positive colonies due to the spread of colour to adjacent colonies.
- Other microorganisms such as *Listeria* and *Aerococcus* and occasionally strains of *S. mutans* and *S. sanguis* may grow on Aesculin Bile Azide Agar with blackening of the medium. The esculin hydrolysis test cannot be used alone to identify enterococci but used in combination with other tests: Gram staining, catalase, growth in the presence of 6.5% NaCl. Gram-positive, esculinase-positive, catalase-negative cocci that grow in the presence of 6.5% NaCl are enterococci.[6]
- There are streptococci that grow in the presence of sodium azide but do not hydrolyse esculin; the presence of growth in the absence of blackening is not an identifying characteristic of enterococci.[6]
- Media with esculin, bile salts and sodium azide are more suitable for primary isolation of enterococci than for their differentiation, for which culture media without sodium azide (Bile Esculin Agar) are better.
- Although the intended use and method of use of the medium described here refers to the detection of enterococci in food and other materials and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of Bile Azide Agar for the detection of *Enterococcus* spp. in clinical specimens. Clinical applications should be validated by the user.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Sore at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin[6] the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to four weeks.

REFERENCES

1. Rochaix, A. Milieux a l'esculine pour le diagnostic differential des bacteries du groups strepto-enteropneumocoque. C R Soc Biol. 1924; 90:771-72.
2. Swan A. The use of a bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J Clin Pathol 1954; 7:160-3.
3. Isenberg HFD, Goldberg D, Sampson J. Laboratory studies with a selective Enterococcus medium. Appl Microbiol.1970;20:433-36.
4. Hardie D, J.M., Whiley R.A. Classification and overview of the genera Streptococcus and Enterococcus J App Microbiol Symposium Supplement, 1997¸83, 1S–11S
5. https://www.sciencedirect.com/topics/earth-and-planetary-sciences/streptococcus
6. MacFaddin, JF. Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD, 1985.

PACKAGING

Product	Type	REF	Pack
Aesculin Bile Azide Agar	Dehydrated medium	4010142	500 g (9 L)

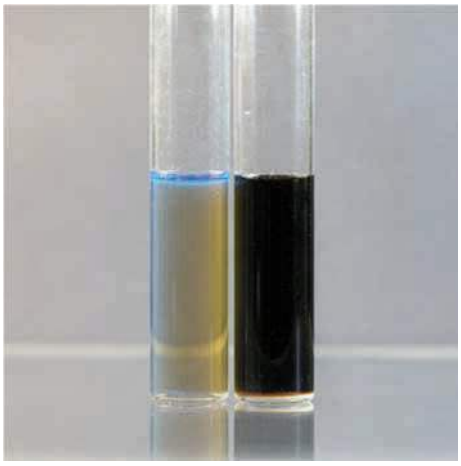
IFU rev. 1, 2022/04

AESCULIN BILE AGAR: see BILE AESCULIN AGAR

AESCULIN BILE AZIDE AGAR ISO FORMULATION: see BILE AESCULIN AZIDE AGAR ISO FORMULATION

AESCULIN BILE AZIDE BROTH

Dehydrated culture medium



Aesculin Bile Azide Broth: uninoculated tube and *Enterococcus faecalis* growth

INTENDED USE

Selective and differential medium for the isolation and differentiation of enterococci from food, water and environmental samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	17.00 g
Peptone	3.00 g
Yeast Extract	5.00 g
Oxgall	10.00 g
Sodium Chloride	5.00 g
Sodium Citrate	1.00 g
Aesculin	1.00 g
Fe-Ammonium Citrate	0.50 g
Sodium Azide	0.25 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Aesculin Bile Azide Broth is a selective and diagnostic medium for enterococci. It differs from solid plated medium only in the omission of agar. Enterococci grow in the presence of sodium azide and bile salts and also have the ability to hydrolyse esculin to glucose and aesculetin (6-7 dihydroxycoumarin). Rochaix first reported in 1924[1] on the validity of the esculin hydrolysis reaction for the identification of enterococci. Media of this type have been studied and proposed since 1950, and some authors evaluated their efficacy in the clinical and hygienic field.[2]
Aesculin Bile Azide Broth based on the formulation of Isenberg et al.[3] who, in 1970, modified the Rochaix formula by reducing the concentration of bile salts and adding sodium azide for the isolation of group D-streptococci and their differentiation from non-group D streptococci; the authors reported the results of a study on 250 'D streptococci' strains from clinical isolation, showing that the medium was not inhibitory for the strains. They also observed that on this medium only *Listeria monocytogenes* among 86 strains belonging to various Gram-positive and Gram-negative bacterial species grew with an appearance similar to that of D -streptococci.
According to current bacterial nomenclature, the designation 'group D streptococci' for faecal streptococci is not to be regarded as specific since the Lancefield group D antigen is produced by *Enterococcus*, *Pediococcus* and some streptococci.[4,5] Differentiation between group D-enterococci and group D non-enterococci (both are positive in the esculin test) can be made by the 6.5% NaCl tolerance test: the former group is tolerant to this salt concentration, the latter is inhibited by it.[5,6]
Tryptone, peptone and yeast extract provide nitrogen, carbon, vitamins, amino acids and trace elements for microbial growth; sodium azide and bile salts are selective agents that limit the growth of Gram-negative and Gram-positive bacteria other than streptococci; esculin is hydrolysed by enterococci to glucose and aesculetin (6-7-dihydroxycoumarin): aesculetin reacts with the iron salts in the medium, giving it a brown-black colour.
A more complete description of the characteristics of the faecal streptococci/intestinal enterococci group can be found in the data sheet of Azide Maltose Agar KF (401107).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42.7 g of broth in 1000 ml of cold purified water. Heat to dissolve, distribute into screw capped tubes and sterilise by autoclaving at 121° C for 15 minutes. Do not exceed sterilisation time and temperature.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	tan with trace blue cast, limpid
Final pH at 20-25 °C	7.1 ± 0.

SPECIMENS

Refer to applicable international standards and regulations for the collection of food and water samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

Prepare tenfold dilutions of the sample with peptone water. Within 3 hours from the sample preparation, spread 1 ml of the inoculum into the tubes. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium).

READING AND INTERPRETATION

The appearance of esculin-positive tubes is characterised by turbidity in the broth and the formation of a dark brown or black colour.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 29212	37°C/24 H/A	good growth, blackening of the broth
S. pyogenes ATCC 19615	37°C/24 H/A	partially inhibited
E. coli ATCC 25922	37°C/24 H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Other microorganisms such as *Listeria* and *Aerococcus* and occasionally strains of *S. mutans* and *S. sanguis* may grow on Aesculin Bile Azide Broth with blackening of the medium. The esculin hydrolysis test cannot be used alone to identify enterococci but used in combination with other tests: Gram staining, catalase, growth in the presence of 6.5% NaCl. Gram-positive, aesculinase-positive, catalase-negative cocci that grow in the presence of 6.5% NaCl are enterococci.[6]
- There are streptococci that grow in the presence of sodium azide but do not hydrolyse esculin; the presence of growth in the absence of blackening is not an identifying characteristic of enterococci.[6]
- Media with esculin, bile salts and sodium azide are more suitable for primary isolation of enterococci than for their differentiation, for which culture media without sodium azide (Bile Esculin Agar) are better.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Rochaix, A (1924) Milieux à l'esculine pour le diagnostic différentiel des bactéries du groupe strepto-entéro-pneumocoque. C.R. Soc. Biol. 90, 771.
2. Swan, A. (1954) - The use of a Bile, Aesculin Medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci) J. Clin. Path, 7, 160-163.
3. Isenberg, H.D., Goldberg, D. & Sampson, J. (1970) - Laboratory studies with a selective enterococcus medium. Appl. Micr., 20, 433-436.
4. Hardie, D. J.M., Whiley R.A. (1997) Classification and overview of the genera Streptococcus and Enterococcus RealiJ. App. Microbiol. Symposium Supplement, 83, 1S–11S
5. https://www.sciencedirect.com/topics/earth-and-planetary-sciences/streptococcus
6. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD

PACKAGING

Product	Type	REF	Pack
Aesculin Bile Azide Broth	Dehydrated medium	40101412	500 g (11.5 L)

IFU rev 1, 2022/04

ALKALINE PEPTONE WATER

Dehydrated culture medium

Alkaline Peptone Water; from left: uninoculated tube and *Vibrio parahaemolyticus*

INTENDED USE

Medium for enrichment of *Vibrio* spp. in food chain.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20 g
Sodium chloride	20 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Alkaline Peptone Water in the above formulation corresponds to the medium recommended by ISO 21872-1.[1] The preparation of the medium at half the concentration (20 g/L) corresponds to the FDA-BAM recommended formulation.[2] The medium is suitable for enrichment of *Vibrio* spp. in foods, animal feeding stuffs, environmental samples in the area of food production and food handling.[1,2]
The WHO[3] has identified that *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* are the major food-borne *Vibrio* spp. However, the method reported by ISO Standard can also be appropriate for the identification of other *Vibrio* spp. causing illness in humans. According to ISO 21872-1, the determination of potentially enteropathogenic species requires an analytical procedure divided into four steps: pre-enrichment and enrichment in Alkaline Peptone Water with differentiated incubation temperature at 37°C and 41.5°C, isolation on TCBS Agar and on a second medium of the user's choice, confirmation tests. Recovery of *V. parahaemolyticus* and *V. cholerae* from fresh food is enhanced by incubation at 41.5°C, while recovery of *V. vulnificus, V. parahaemolyticus* and *V. cholerae* from frozen, dried or salted food is enhanced by 37°C. Peptone povides nitrogen, carbon, amino acids for bacterial growth; the alkaline pH and high concentration of sodium chloride favour the growth of halophilic vibrios.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. If a medium according to FDA-BAM is required, weigh 20 g of powder in 1000 mL of cold purified water. Heat to complete solution, dispense into screw-capped tubes or bottles and autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	amber, fine, homogeneous, free-flowing powder
Prepared tubes appearance	colourless or pale yellow, limpid
Final pH at 20-25 °C	8.6 ± 0.1

SPECIMENS

Foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable international standards and regulations[1,2] for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

Prepare the initial suspension by suspending 25 g or mL of sample into 225 mL of Alkaline Peptone Water.

Incubate the initial suspension at 37 °C ± 1 °C for 6 h ± 1h (for detection of *V. parahaemolyticus* and *V. cholerae* in deep frozen, dried or salted products and *V. vulnificus* in all samples).
Incubate the initial suspension at 41.5 °C ± 1 °C for 6 h ± 1h (for detection of *V. parahaemolyticus* and *V. cholerae* in fresh products).
Transfer 1 mL of the pre-enrichment culture into tubes containing 10 mL of Alkaline Peptone Water and incubate at 41.5 °C for 18 h ± 1h for the detection of *V. parahaemolyticus* and *V. cholerae* and at 37 °C ± 1 °C for 18 h ± 1h for *V. vulnificus*.
Inoculate with a 1 µl sampling loop the surface of a TCBS agar plate so as to permit the development of well-isolated colonies. Proceed likewise with the chosen second selective isolation medium.
Incubate the TCBS Agar at 37°C for 24h ± 3h. Incubate the second medium according to the Instructions for Use.

READING AND INTERPRETATION

Bacterial growth in Alkaline Peptone Water is evidenced by the development of turbidity in the broth.
On TCBS Agar *V. cholerae* develops smooth, yellow (sucrose positive) colonies with a diameter of 1-2 mm, *V. parahaemolyticus* and *V. vulnificus* develop smooth, green (sucrose negative) colonies with a diameter of 2-3 mm.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
V. furnissii ATCC 11128	37°C / 18 h / A	good growth (turbidity)
V. parahaemolyticus ATCC 17802	37°C / 18 h / A	good growth (turbidity)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- For enhanced recovery of *V. vulnificus*, medium containing derivatives of cellobiose-polymyxin B-colistin and cellobiose-colistin has been shown to be effective.[2]
- *V. parahaemolyticus, V. cholerae* and *V. vulnificus* may be present in small numbers in the samples and are often accompanied by a much larger number of other microorganisms belonging to the *Vibrionaceae* family or to other families.[2]
- Although the intended use and method of use of the medium described here refers to the detection of *Vibrio* spp. in food and other materials and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of Alkaline Peptone Water for the examination of human clinical specimens.[4] Use simple concentration medium, incubate at 35°C for 18 hours and subculture onto TCBS Agar plate; occasionally vibrios grow with a shorter incubation (6 hours) and for these samples longer incubations cause overgrowth of contaminants masking the presence of *Vibrio* spp.[5] Clinical applications must be validated by the user.

STORAGE CONDITIONS

Sore at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. FDA (1995) Bacteriological Analytical Manual, Chapter 9, Vibrio, May 2004. Content current as of:10/31/2017
2. ISO 21872 (2017) Microbiology of the food chain — Horizontal method for the determination of Vibrio spp. —Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus.
3. FAO/WHO. 2001, Hazard identification, exposure assessment and hazard characterization of Campylobacter spp. in broiler chickens and Vibrio spp. in seafood, a joint FAO/WHO expert consultation, Geneva, Switzerland, 23–27 July 2001.
4. M.Lesmana et al. (1985) An evaluation of Alkaline Peptone Water for enrichment of Vibrio cholera in feces. Southeast Asian J. Trop. Med Pub. Healt. 16, 265.
5. Tarr C.L., Bopp C.A., Farmer III J.J. Vibrios and Related Organisms. In Manual of Clinical Microbiology, 11th ed. 2015, ASM Press.

PACKAGING

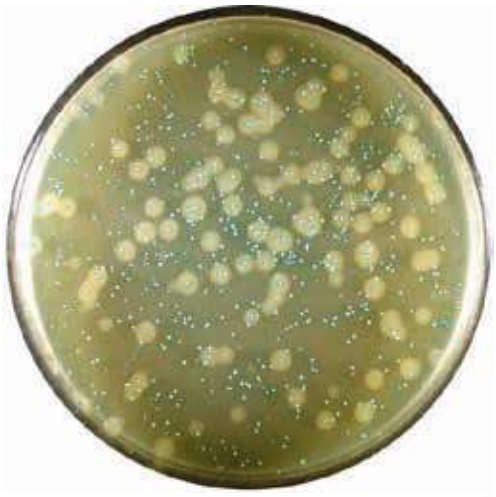
Product	Type	REF	Pack
Alkaline Peptone Water	Dehydrated medium	4010322	500 g (12.5 / 25 L)

IFU rev 1, 2022/04

ChromArt

ALOA®
AGAR LISTERIA ACC. TO OTTAVIANI & AGOSTI
ALOA® ENRICHMENT-SELECTIVE SUPPLEMENTS

Dehydrated culture medium, selective supplement and enrichment, ready-to-use media



ALOA:
colonies of *L. monocytogenes* and *L. innocua*

INTENDED USE

For the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain and in environmental samples.

COMPOSITION - TYPICAL FORMULAS *

AGAR LISTERIA ACC. TO OTTAVIANI & AGOSTI (ALOA®)
DEHYDRATED MEDIUM AND READY-TO-USE MEDIUM IN FLASKS, AFTER RECONSTITUTION WITH 1 L OF WATER

Meat peptone	18.00 g
Tryptone	6.00 g
Yeast extract	10.00 g
Sodium pyruvate	2.00 g
Glucose	2.00 g
Magnesium glycerophosphate	1.00 g
Magnesium sulphate	0.50 g
Sodium chloride	5.00 g
Lithium chloride	10.00 g
Disodium hydrogen phosphate anhydrous	2.50 g
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.05 g
Agar	13.50 g

ALOA® SELECTIVE SUPPLEMENT	**(VIAL CONTENTS FOR 500 mL OF MEDIUM)**	**(VIAL CONTENTS FOR 200 mL OF MEDIUM)**
Nalidixic acid, sodium slat	0.010 g	0.004 g
Ceftazidime	0.010 g	0.004 g
Cycloheximide	0.025 g	0.01 g
Polymyxin B sulphate	38,350 UI	15,340 UI

ALOA® ENRICHMENT SUPPLEMENT	**(VIAL CONTENTS FOR 500 mL OF MEDIUM)**	**(VIAL CONTENTS FOR 200 mL OF MEDIUM)**
L-α-phosphatidylinositol	1.0 g	0.4 g

ALOA® - AGAR LISTERIA ACC. TO OTTAVIANI & AGOSTI, READY-TO-USE PLATES

Meat peptone	18.000 g
Tryptone	6.000 g
Yeast extract	10.000 g
Sodium pyruvate	2.000 g
Glucose	2.000 g
Magnesium glycerophosphate	1.000 g
Magnesium sulphate	0.500 g
Sodium chloride	5.000 g
Lithium chloride	10.000 g
Disodium hydrogen phosphate anhydrous	2.500 g
5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside	0.050 g
Agar	13.500 g
Nalidixic acid, sodium salt	0.020 g
Ceftazidime	0.020 g
Cycloheximide	0.050 g
L-α-phosphatidylinositol	2.00 g
Polymyxin B sulphate	76,700 IU
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Agar Listeria acc. to Ottaviani and Agosti (ALOA) is a chromogenic and selective medium for the detection and enumeration of *L. monocytogenes* and *Listeria* spp. in samples of the food chain and in environmental samples due to its ability to differentiate *L. monocytogenes* from other *Listeria* species, even in the presence of a mixed flora.
ALOA medium is recommended by ISO 11290-1[1] and ISO 11290-2[2] for detection and enumeration of *L. monocytogenes* and *Listeria* spp. and it is also cited by FDA-BAM[3], APHA[4] and other regulatory agencies[5,6].
ALOA medium was conceived by Franco Ottaviani and Marco Agosti[7] and industrialised by Biolife in the mid-1990s.
ALOA has been compared by several authors with PALCAM and Oxford media and some other chromogenic media: all results confirm the superiority of ALOA medium over conventional media and other chromogenic media.[8-13]
Lequerq[14] reported that ALOA was the best medium of the four tested and that its introduction into the laboratory methods to replace Oxford and PALCAM increases the isolation and counting of atypical *L. monocytogenes* strains.
Gracieux et al.[15] reported a higher recovery rate of virulent, hypovirulent and avirulent strains of *L. monocytogenes* with ALOA than with PALCAM medium and other chromogenic media.

Sacchetti et al.[16] reported that in an experiment with 132 food samples, ALOA and a second chromogenic medium allowed faster detection of *L. monocytogenes* with greater sensitivity and specificity than PALCAM medium.
According to Jadhav et al.[17], identification with MALDI-TOF was optimal with colonies grown on ALOA medium.
Peptones and yeast extract provide nitrogen, carbon, vitamins particularly of the B-group and trace elements for microbial growth. Glucose is a source of carbon and energy, sodium chloride maintains the osmotic balance, and sodium phosphate bibasic is included as a buffer system. Sodium pyruvate aids in resuscitation of stressed cells and magnesium salts stimulate the growth of *Listeria* spp. The selective action is due to the presence of lithium chloride in the basal medium and the addition of the antimicrobial mixture of the selective supplement containing ceftazidime, polymyxin B, nalidixic acid and cycloheximide. The medium markedly reduces the growth of the majority of concomitant Gram-positive and Gram-negative bacteria, as well as of yeasts and fungi.
The differential property of ALOA is due to the presence of the chromogenic compound 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside, a substrate for the detection of the enzyme ß-glucosidase, which is common to all *Listeria* species. Specific differential action is achieved with a substrate for phospholipase C (PI-PLC: phosphatidyl inositol phospholipase C). *Listeria monocytogenes* cleaves this specific substrate added to the medium base producing an opaque halo around the colonies. Most *Listeria ivanovii* also produce an opaque halo around the colonies after 48 h incubation. With the combined action of the two substrates, it is possible to differentiate the following colonies: *L. monocytogenes*: blue-green colonies surrounded by an opaque halo, *Listeria* other than-*monocytogenes* and *ivanovii*: blue-green colonies without the opaque halo.

Directions for medium preparation (dehydrated medium)
Suspend 35.3 g in 500 mL of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C add the contents of one vial of ALOA Enrichment Supplement pre-warmed to 48-50°C, and the contents of one vial of ALOA Selective Supplement, reconstituted with 5 mL of ethanol/sterile purified water (1:1). Mix well and distribute into sterile Petri dishes.

Directions for medium preparation (REF 511605K3 medium in flasks and supplements)
Dissolve the contents (200 mL) of one flask of ALOA medium in a water bath at 100°C. Cool to 47-50°C add the contents of one vial of ALOA Enrichment Supplement pre-warmed to 48-50°C, and the contents of one vial of ALOA Selective Supplement, reconstituted with 5 mL of ethanol/sterile purified water (1:1). Mix well and distribute into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	yellowish, opalescent
Prepared flasks appearance	yellowish, opalescent
Freeze-dried selective supplement	white, high, compact pellet; colourless and clear solution after reconstitution
Enrichment supplement appearance	cloudy, yellow suspension with a slight precipitate
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

Specimens
Foods, animal deeding stuffs, food chain and environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[1-6]

Test procedure
Detection of *Listeria monocytogenes* and *Listeria* spp. (ISO 11290-1)[1]
1. In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 g or 225 mL of Fraser Broth Half Concentration, to obtain a tenfold dilution, and homogenize.
2. Incubate the primary enrichment medium at 30 °C for 25 h ± 1 h.
3. Transfer 0.1 mL of the culture to a tube or bottle containing 10 mL of secondary enrichment medium (Fraser Broth) and incubate for 24 h ± 2 h at 37 °C. In the case of *Listeria* spp. other than *Listeria monocytogenes* detection, additional 24 h incubation can allow for recovery of more species.
4. From the primary enrichment culture inoculate, by means of a loop, the surface of the first selective plating medium, Agar Listeria according to Ottaviani and Agosti (ALOA), to obtain well-separated colonies. Proceed in the same way with the second selective plating-out medium of choice (e.g., PALCAM or Oxford Agar).
5. From the secondary enrichment medium, repeat the procedure with the two selective plating-out media.
6. Incubate ALOA plates at 37°C ± 1°C for 24 ± 2 hours; if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
7. Incubate the second plating out medium according to the Instructions for Use
8. Examine the dishes for the presence of presumptive colonies of *L. monocytogenes* or *Listeria* spp.

Notes
It is possible to store at 5 °C the pre-enriched sample after incubation before transfer to Fraser broth for a maximum of 72 h.
Half-Fraser broth and Fraser broth can be refrigerated at 5 °C before isolation on selective agar for a maximum of 72.
After incubation, ALOA plates can be refrigerated at 5 °C for a maximum of 48 h before reading.

Enumeration of *Listeria monocytogenes* and of *Listeria* spp. (ISO 11290-2)[2]
1. Prepare a sample suspension in Buffered Peptone Water or other suitable enrichment broth according to ISO 6887 (all parts); in case both determination and counting are performed according to parts 1 and 2 of ISO 11290, the sample suspension may be made in half-Fraser broth (with or without the addition of the selective supplement).
2. Inoculate 0.1 mL of the sample suspension and 0.1 mL of further decimal dilutions onto 90 mm plates of ALOA medium.
3. For samples with suspected low number of target-strains, inoculate 1 mL of the sample suspension and 1 mL of further decimal dilutions onto 140 mm plates of ALOA medium.
4. Examine after incubation at 37°C for 24 ± 2 hours and, if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
5. Count *L. monocytogenes* colonies and *Listeria* spp. colonies in plates with less than 150 colonies (90 mm diameter plates) or 360 colonies (140 mm plates) according to the section "reading and interpretation".

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies on plating out media.
Consider as presumptive *L. monocytogenes* the blue-green colonies surrounded by an opaque halo.
Consider as presumptive *Listeria* spp. the blue-green colonies with or without opaque halo.
Second plating-out medium: after incubation at the temperature described by the Instructions for Use, examine for the presence of typical colonies according to the characteristics of the chosen medium.
Confirm typical colonies by the methods and tests indicated in ISO 11290-1 or ISO 11290-2, after purification of the colonies in Tryptic Glucose Yeast Agar.

The mandatory confirmatory tests for *L. monocytogenes*, according to ISO 11290 and using ALOA medium, are the following: β-haemolysis (+), carbohydrate utilization (L-rhamnose +; D-xylose -). Optional confirmatory tests for *L. monocytogenes* are: catalase (+), mobility at 25°C (+). The mandatory confirmatory tests for *Listeria* spp. are: microscopic examination, catalase (+); optional tests are: VP (+), mobility at 25°C (+). Miniaturized galleries for the biochemical identification of *Listeria monocytogenes* may be used (Listeria Mono Confirm Test REF 193000)

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 13932	37°C /24-48h A	green-blue colonies surrounded by an opaque halo
L. monocytogenes NCTC 7973	37°C /24-48h A	green-blue colonies surrounded by an opaque halo
L. innocua ATCC 33090	37°C /24-48h A	green-blue colonies without opaque halo
L. ivanovii ATCC 19119	37°C /24-48h A	green-blue colonies with opaque halo
E. coli ATCC 25922	37°C / 48h A	inhibited
E. faecalis ATCC 19433	37°C / 48h A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The reading of plates with abundant growth can be facilitated by comparing the opacity of the medium at the edges where there may be no growth with that in the centre of the plate or by comparing with an uninoculated plate. Plates with a confluent and intense growth of *L. monocytogenes* will still appear intensely opaque; in the case of intense growth of *Listeria* sp. other than *monocytogenes* the plates will not be opaque. If there is any doubt, the colonies should be re-isolated.
- *L. ivanovii* at 24 hours and especially after 48 hours of incubation, presents blue-green colonies with an opaque halo. In these cases, confirmatory tests will allow correct identification.
- Some strains of *Bacillus cereus*, which are resistant to the selective agents, may produce flat, wrinkled, non-homogenous white to blue colonies with a large, intense halo.
- It has been reported[18] that some species of 6 genera of Gram-positive bacteria can grow on ALOA and sometimes generate blue or bluish colonies: *Bacillus* spp. (*B. circulans, B. clausii, B. licheniformis, B. oleronius*, *Cellulosimicrobium funkei*), *Enterococcus* spp. (*E. faecalis, E. faecium/durans*), *Kocuria kristinae, Marinilactibacillus psychrotolerans, Rothia terrae, Staphylococcus* spp. (*S. sciuri, S. saprophyticus subsp. saprophyticus/xylosus*), *Streptococcus*.
- Some strains of *L. monocytogenes* exposed to stress conditions, particularly acid stress, can show a very weak halo (or even no halo).[1,2]
- Some rare *L. monocytogenes* are characterized by a slow PIPLC activity. Such bacteria are detected when the total duration of incubation is more than, for example, four days. Some of these strains could be pathogenic. No *L. monocytogenes* strains have been described as PIPLC negative.[1,2]
- Rare strains of *L. monocytogenes* may not exhibit β-haemolysis.[1,2] If typical colonies on ALOA are β-haemolysis negative, additional confirmatory tests (Gram, catalase, mobility, CAMP test, PCR) are recommended.

STORAGE CONDITIONS

Dehydrated medium

Sore at +2°C /+8°C away from direct light in a dry place.

According to Corry *et al*.[19] the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up to four weeks.

Freeze-dried and liquid supplements, ready-to-use media

Store the products in the original package at +2°C /+8°C away from direct light.

REFERENCES

1. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
2. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.
3. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods, April 2022
4. APHA Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C. 2015.
5. Shaw S, Nundy D, Blais B. Performance of the ALOA medium in the detection of hemolytic Listeria species in food and environmental samples. Laboratory Services Division, Canadian Food Inspection Agency, Ottawa, Ontario, Canada K1A 0C6.
6. Loncarevic S, Økland M, Sehic E, Norli HS, Johansson T. Validation of NMKL method No. 136-Listeria monocytogenes, detection and enumeration in foods and feed. Int J Food Microbiol. 2008;124(2):154-63
7. Ottaviani F, Ottaviani M, Agosti M. Differential agar medium for Listeria monocytogenes. Quinper Froid Symposium Proceedings, P6 A.D.R.I.A. Quinper (F) 16-18 June, 1997
8. Ottaviani F, Ottaviani M, Agosti M. Esperienze su un agar selettivo e differenziale per Listeria monocytogens. Industrie Alimentari, XXXVI, 1997 luglio-agosto, 888.
9. Artault S, Bind JL, Delaval Y, Dureuil N, Gaillard N. AFNOR Validation of the ALOA method for the detection *of Listeria monocytogenes* in foodstuffs. Colloque de la Societé Francaise de Microbiologie, Paris, 19-20 Octobre, 2000.
10. Mioni R, Grimaldi M, Bordin P, Miglioranzi R, Ferrigno R. Ricerca di L.monocytogens negli alimenti. Valutazione di un nuovo terreno selettivo e differenziale specie-specifico e di un sistema rapido d'identificazione. Industrie Alimentari, XXXVII, giugno 1998, 732.
11. Vlaemynck G, Lafarge V, Scotter S. Improvement of the detection of *Listeria monocytogenes* by the application of ALOA, a diagnostic, chromogenic isolation medium. J Appl Microbiol 2000; 88:430.
12. Beumer LL. Horizontal method for the detection of *Listeria monocytogenes* ISO 11290-1. Change of Isolation Media. Wageningen University, The Nederlands, 2001.
13. Moroder L. Comparison of alternative methods for the enumeration of *Listeria monocytogenes* in food. FEMS-Symposium on the Versatility of Listeria species. Izmir, October 10-11, 2002
14. Leclerq A. Atypical colonial morpholgy and low recovery of L. monocytogenes strains on Oxford, PALCAM, Rapid'L.mon and ALOA solid media. J Microbiol Meth 2004; 57: 252-258.
15. Gracieux P, Roche SM, Pardon P, Velge P. Hypovirulent L.monocytogenes strains are less frequently recovered than virulent strains on PALCAM and Rapid'L.mono media. Int. J. Food Microbiol. 2003; 83:133-145.
16. Sacchetti R, Bianucci F, Ambrogiani E. Detection of *L.monocytogenes* in foodstuffs using chromogenic isolation media. New Microbiol 2003; 26:269-274.
17. Jadhav S, Gulati V, Fox EM, Karpe A , Beale DJ, Sevior D, Bhave M, Palombo EA. Rapid identification and source-tracking of Listeria monocytogenes using MALDI-TOF mass spectrometry. Int J Food Microbiol. 2015 Jun 2;202:1-9
18. Angelidis AS, Kalamaki MS, Georgiadou SS. Identification of non-Listeria spp. bacterial isolates yielding a β-D-glucosidase-positive phenotype on Agar Listeria according to Ottaviani and Agosti (ALOA). Int J Food Microbiol 2015; 193:114–129
19. Corry JEL, Curtis GDW, Baird RM. Handbook of Culture Media for Food and Water Microbiology, pp 658-662 Royal Society of Chemistry, Cambridge, UK. 2012

PACKAGING

Product	Type	REF	Pack
Agar Listeria acc. to Ottaviani & Agosti (ALOA®)	Dehydrated medium	4016052	500 g (7.1 L)
Agar Listeria acc. to Ottaviani & Agosti (ALOA®)	Dehydrated medium	4016054	5 kg (71 L)
ALOA® Enrichment Selective Supplements	Freeze-dried and liquid supplements	423501	4+4 vials, each for 500 mL of medium
ALOA® Enrichment Selective Supplements	Freeze-dried and liquid supplements	423505	5+5 vials, each for 200 mL of medium
ALOA® -Agar Listeria acc. to Ottaviani & Agosti	Ready-to-use plates	541605	2 x 10 plates ø 90 mm
ALOA® -Agar Listeria acc. to Ottaviani & Agosti	Ready-to-use plates	501605P	5 plates ø 150 mm
ALOA® Flasks Kit	Ready-to-use flasks and supplements	511605K3	4x200mL ALOA flasks + 4 vials of ALOA Enrichment Supplement and 4 vials of ALOA Selective Supplement, each for 200 mL of medium base

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ANTIBIOTIC MEDIA

ANTIBIOTIC SEED AGAR A1 - POLYMIXIN BASE AGAR A9 POLYMIXIN SEED AGAR A10 - NEOMYCIN ASSAY AGAR A11

Dehydrated culture media

INTENDED USE

Culture media for microbiological agar diffusion assay of antibiotics.

COMPOSITION - TYPICAL FORMULAS* (AFTER RECONSTITUTION WITH 1 L OF WATER)

TABLE 1

Medium	Antibiotic Seed Agar A1	Neomycin Assay Agar A11	Polymyxin Base Agar A9	Polymyxin Seed Agar A10
REF	4010752	4017752	4019202	4019252
Peptone	6.0	6.0		
Tryptone	4.0	4.0	17.0	17.0
Yeast extract	3.0	3.0		
Beef extract	1.5	1.5		
Soy peptone			3.0	3.0
Glucose	1.0	1.0	2.5	2.5
Sodium chloride			5.0	5.0
Dipotassium hydrogen phosphate			2.5	2.5
Agar	15.0	15.0	20.0	12.0
Quantity required (g/L)	30.5	30.5	50.0	42.0
Tween 80 (added to the base)				10

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Antibiotic media are prepared with formulations in compliance with the FDA publication 21 CFR.[1] The antibiotic media are identified numerically with names assigned by Grove and Randall.[2] Antibiotic media are intended for microbiological assay of antibiotics and for the quantitative determination of antibiotics in pharmaceutical preparations, foods, animal feed preparations, and other materials. The most common method for microbiological assay of antibiotics is the agar diffusion test performed by cylinder, perforated hole or paper disc tests. The culture medium is inoculated with the test strain and poured into dishes. Defined quantities of antibiotic under test and an antibiotic standard are applied as spots onto the plates. During incubation, inhibition zones of microbial growth develop around the application site, and their diameter is a measure of the activity of the antibiotic tested, compared with the inhibition zones given by standard solutions of the same antibiotic.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend the required quantity of culture medium (see Table 1) in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121° C for 15 minutes. Cool to 47-50°C and add the microbial test strain. Mix well and distribute in sterile Petri dishes. Polymyxin Seed Agar A10 requires the addition of 10 g/L of Tween 80 before autoclaving.

PHYSICAL CHARACTERISTICS

TABLE 2

Medium	Antibiotic Seed Agar A1	Neomycin Assay Agar A11	Polymyxin Base Agar A9	Polymyxin Seed Agar A10
REF	4010752	4017752	4019202	4019252
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder	beige, fine, homogeneous, free-flowing powder	beige, fine, homogeneous, free-flowing powder	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	yellow, limpid	yellow, limpid	yellow, limpid	yellow, limpid
Final pH at 20-25 °C	6.5 ± 0.1	7.8 ± 0.2	7.3 ± 0.2	7.3 ± 0.2

SPECIMENS
Pharmaceutical preparations, foods, animal feed preparations and other materials.

TEST PROCEDURE
Preparation of the microbial suspension[1]
Maintain organisms on agar slants containing 10 mL of the appropriate medium (generally Antibiotic Seed Agar A1). Incubate the slants at 32°C–35°C for 24 hours. Using 3 mL of sterile saline solution, wash the growth from the agar slant onto a large agar surface, such as a Roux bottle, containing 250 mL of the appropriate medium. Spread the suspension of organisms over the entire surface of the Roux bottle with the aid of sterile glass beads. Incubate the Roux bottle at 32°C–35°C. Wash the resulting growth from the agar surface with 50 mL of sterile saline solution. Determine the dilution factor that will give a 25% light transmission at a wavelength of 580 millimicrons using a suitable photoelectric colorimeter and a 13 mm diameter test tube as an absorption cell. It may be necessary to adjust the suspension. Determine the amount of suspension to be added to each 100 mL of agar by the use of test plates. Store the test organism suspension under refrigeration.
This general working scheme is valid for all test strains, with these exceptions:
1) For *Bacillus subtilis* centrifuge the growth from the Roux bottle, decant the supernatant, take up the microorganisms in 50-70 mL of saline and heat the resulting suspension at 70°C for 30 minutes.
2) For *Bacillus cereus* var. *mycoides*, use the general method with the modifications described for *Bacillus subtilis*, but heat the microbial suspension before centrifugation and wash the spore suspension three times with 25-30 mL of sterile distilled water. After the last wash, resuspend the spores in 50-70 mL of sterile distilled water.
3) For *Microsporum gypseum* incubate an Erlenmeyer flask containing 200 mL of Sabouraud Broth with 20% glucose added, inoculated with the microorganism, for 6-8 weeks at 25°C. Check the degree of sporification and when it exceeds 80%, collect the spores from the mycelial layer with a spatula: the spores are on the surface of the material floating in the broth. Suspend the collected spores in 50 mL of sterile saline and store in a refrigerator.
4) Keep *Enterococcus faecalis* in 10 mL of Antibiotic Broth A3. To carry out the assay, prepare a fresh subculture by transferring an aliquot of the stock cultures into 100 mL of Antibiotic Broth A3 and incubate 16-18 hours at 37°C.
5) For *Saccharomyces cerevisiae* incubate the slant of Antibiotic Seed Agar A1 at 30°C for 24 hours and the Roux bottle at 30°C for 48 hours.
Microbial suspensions prepared as described can be used without further dilution to inoculate the turbidimetric and diffusion dosing medium.
Microbiological agar diffusion assay
The activity of antibiotics is estimated by comparing the inhibition of growth of a sensitive microorganisms produced by 5 doses of reference substance and 1 dose of antibiotic to be examined.[3]
Prepare the inoculated suitable medium with a known quantity of a suspension of test microorganism sensitive to the antibiotic to be examined.
Mix the medium and the inoculum and pour into Petry dishes a quantity to form a layer 2-5 mm thick. Alternatively, the medium may consist of 2 layers, only the upper layer being inoculated. For each Petri dish, 21 mL of base layer and 4 mL of the seed layer may be generally suitable.
Prepare the solutions of the reference substance and of the antibiotic to be examined having known concentrations.
Pipette the antibiotic solutions into the cylinders or into the punched holes or on paper-discs with a diameter of 9 mm placed on the culture medium.
Incubate the inoculated plates for 16 to 18 hours at the appropriate incubation temperature for each antibiotic.

READING AND INTERPRETATION
After incubation, measure the diameters of the zones of inhibition using an appropriate measuring device such as a millimetre rule, callipers, or an optical projector. Draw a standard curve using the values of the standard solutions and read off the activities of the test solutions. Refer to appropriate procedures for results reading and interpretation.[1,3]

USER QUALITY CONTROL
All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains inoculated by poured plate method, useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Antibiotic Seed Agar A1		
Staphylococcus aureus ATCC 6538P	35-37°C/18-24 H/A	good growth
Neomycin Assay Agar A11		
Micrococcus luteus ATCC 9341	35-37°C/24-48 H/A	good growth
Staphylococcus epidermidis ATCC 12228	35-37°C/24-48 H/A	good growth
Polymyxin Base Agar A9, Polymyxin Seed Agar A10		
Bordetella bronchiseptica ATCC 4617	35-37°C/40-48 H/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS
Sore at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Food and Drug Administration. 21 CFR, Part 436 – Tests and Methods of Assay of Antibiotic and Antibiotic Containing Drugs. Subpart D—Microbiological Assay Methods. April 1, 1996
2. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopaedia, Inc. New York, N.Y.
3. USP 31 <81> Antibiotic, Microbial Assay

PACKAGING
Product	Type	REF	Pack
Antibiotic Seed Agar A1	Dehydrated medium	4010752	500 g (16.4 L)
Neomycin Assay Agar A11	Dehydrated medium	4017752	500 g (16.4 L)
Polymyxin Base Agar A9	Dehydrated medium	4019202	500 g (10 L)
Polymyxin Seed Agar A10	Dehydrated medium	4019252	500 g (11.9 L)

IFU rev 1, 2022/06

APT AGAR
APT BROTH
Dehydrated culture media

INTENDED USE
For the cultivation and enumeration of heterofermentative lactobacilli.

COMPOSITION - TYPICAL FORMULAS *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

APT Agar

Tryptone	12.50 g
Yeast Extract	7.50 g
Glucose	10.00 g
Sodium citrate	5.00 g
Sodium chloride	5.00 g
Dipotassium hydrogen phosphate	5.00 g
Manganous chloride	0.14 g
Magnesium sulphate	0.80 g
Ferrous sulphate	0.04 g
Sorbitan monooleate	0.20 g
Agar	15.00 g
Thiamine HCl	0.10 mg

APT Broth

Tryptone	12.50 g
Yeast Extract	7.50 g
Glucose	10.00 g
Sodium citrate	5.00 g
Sodium chloride	5.00 g
Dipotassium hydrogen phosphate	5.00 g
Manganous chloride	0.14 g
Magnesium sulphate	0.80 g
Ferrous sulphate	0.04 g
Sorbitan monooleate	0.20 g
Thiamine HCl	0.10 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
APT (All Purpose Tween) Agar was devised by Evans and Niven[1] and developed by Shipp[2] for the non-selective enumeration of heterofermentative lactobacilli capable of elaborating H_2O_2 during growth under aerobic condition. Such reaction on cooked meats results in a green discolouration.[3] APT Agar with added 20 g/L sucrose and bromocresol purple and with added 5 g/L glucose is effective in enumerating spoilage lactic acid producers in meat products and in seafoods respectively.[4] APT Agar can also be used to propagate pediococci.[4] APT Agar and APT Broth may be used for maintaining stock cultures and for culturing of *Weissella (Lactobacillus) viridescens* ATCC™ 12706 used in the assay of thiamine.[5] Tryptone provides nitrogen, carbon, minerals and amino acids for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, glucose is a source of carbon and energy, sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. The presence of manganese and citrate exhibits a synergic effect increasing the intensity of the growth of lactobacilli, whose lag phase is shortened by sorbitan monooleate.[6]

DIRECTIONS FOR MEDIA PREPARATION
APT Agar
Suspend 61.2 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and boil for 1 minute to completely dissolve the medium. Sterilise by autoclaving at 121°C for 15 minutes. Do not overheat.
APT Broth
Suspend 46.2 g in 1000 mL of cold purified water; heat slightly to dissolve the medium with frequent agitation. Distribute and sterilise by autoclaving at 121°C for 15 minutes. Do not overheat.
Double layer plates preparation[3]
Bottom layer: APT Agar (15 mL)
Top layer: APT Agar + MnO_2 (10 mL) prepared as follows.
To each 100 mL amount of APT Agar autoclaved and cooled to 45-47°C add 10 mL of a suspension of 20 g of MnO_2 in 200 mL of APT Broth, dispensed in 10 mL amounts and sterilized by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS
APT Agar and APT Broth

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	brown, limpid
Final pH at 20-25°C	6.7 ± 0.2

SPECIMENS
Food samples, cured meat products, tinned foods, fruit juices. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Prepare the sample dilutions in 0.1% peptone water diluent. Inoculate by surface spread or poured plate methods, by single- or double-layers techniques.
1. Using a sterile pipette, dispense 1 mL of the liquid test sample, or 1 mL of an initial suspension in the case of other products, into an empty Petri dish and mix with the molten medium pre-cooled to 44-46°C. Prepare the other plates under the same conditions using decimal dilutions of the test sample.
2. Alternatively, sample dilutions are surface plated to obtain discrete colonies.
3. Incubate the plates under aerobic conditions at 25 °C to 32°C for 72 hours to five days.

The inoculation method, temperature and incubation time must be chosen according to the type of sample to be examined and the research objectives. Consult the appropriate references for the recommended procedures for testing and interpretation.[3,4]

READING AND INTERPRETATION
H_2O_2 forms soluble compounds with suspended MnO_2. Colonies surrounded by a clear zone are regarded as potentially H_2O_2 producing lactic acid bacteria.
As these media are non-selective and permit the growth of contaminants, the presumptive diagnosis of the presence of lactobacilli should be confirmed by microscopic and biochemical examinations.

An artificial pollution test to confirm the diagnosis of bacterial greening of canned meats can be performed. Transfer a few colonies from the APT Agar plates to APT Broth tubes and incubate at 32°C for 24 hrs. Prepare a Petri dish with filter paper imbued with sterile water and put a slice of the test material under aseptic conditions. Inoculate the surface with a loopful of broth culture in APT Broth; incubate at 32°C for 24 hours and observe whether the meat has greened. If it occurs and if an un-inoculated control specimen is found to be unchanged, the diagnosis is confirmed. The presence of greening due to exceeding nitrites is to be distinguished from the bacterial greening by carrying out identification tests and assays of nitrites with the standard reagents.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of APT Agar supplemented with MnO_2.

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
L. brevis ATCC 14869	30°C / 5 days / A	good growth, colonies with transparent halo
L. sakei ATCC 15521	30°C / 5 days / A	good growth, colonies without transparent halo

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- APT Agar and APT Broth a non-selective media and coliforms and many other commensals grow well.[5]
- Avoid excessive heating as thiamine is a heat-labile factor.[5]
- Studies of MacLeaod and Snell showed that Mn^{++} inhibits growth of *L. arabinosus* and *L. pentosus*.[7]

STORAGE CONDITIONS

Sore at +10°C /+30°C away from direct light in a dry place.
According to Baird RM et al. prepared plates can be stored for at least 7 days at 4 ± 2°C.[3]

REFERENCES

1. Evans JB, Niven C. Nutrition of the heterofermentative Lactobacilli that cause greening of cured meat products. J Bacteriol 1951 Nov;62(5):599-603
2. Shipp HL. The green discolouration of cooked cured meats of bacterial origin. Technical circular n° 266, British Food Manufacturing Industries Research Association, Leatherhead, U.K.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 195-196.
4. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015
5. Deibel RH, Evans JB, Niven CF. Microbiological assay for he thiamine using lactobacillus viridescens. J Bacteriol 1951; 62:818-821
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Sharf JM. Recommended Methods for the Microbiological Examination of Foods. 2nd ed. APHA, Washington, D.C. 1966
8. MacLeod RA, Snell EE. Some mineral requirements of lactic acid bacteria. J Biol Chem 1947; 170:351

PACKAGING

Product	Type	REF	Pack
APT Agar	Dehydrated medium	4010852	500 g (8,1 L)
APT Broth	Dehydrated medium	4010902	500 g (10.8 L)

IFU ref 1, 2022/12

ASPARAGINE ENRICHMENT BROTH

Dehydrated culture medium

INTENDED USE

Liquid medium for the presumptive detection and enumeration of *Pseudomonas aeruginosa* in water samples.

COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

DL-asparagine	3.0 g
Dipotassium hydrogen phosphate	1.0 g
Magnesium sulphate	0.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

P. aeruginosa is an opportunistic environmental pathogen characterized by a high degree of adaptability, capable of growing in waters with very low nutrients concentrations and surviving in disinfected water.[1]
Asparagine Enrichment Broth is recommended by APHA[2] for the enumeration of *P. aeruginosa* in recreational waters by multiple tube technique. The medium is a strictly mineral base with asparagine as the sole source of carbon and nitrogen which is converted to aspartic acid by *P. aeruginosa*. Magnesium sulphates provide necessary cations for the activation and stimulation of fluorescein and pyocyanin production. Dipotassium hydrogen phosphate acts as a buffer system.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 4.5 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly to completely dissolve the powder if necessary. Distribute 10 mL in tubes and sterilise by autoclaving at 121ºC for 15 minutes. For 10 mL inocula, use tubes with 10 mL of double strength broth.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, limpid with a light precipitate
Final pH at 20-25 °C	7.0 ± 0.2

Specimens
Recreational waters. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

Test procedure
Perform a five tubes MPN test.
Use 10 mL single-strength broth for inocula of 1 mL or less. Use 10 mL double-strength broth for 10 mL inocula. Higher dilutions may be necessary for swimming pools.
Incubate at 35-37°C for 24-48 hours.

Reading and interpretation
After 24 hours and again after 48 hours of incubation, examine tubes under long-wave UV lamp (black light) in a darkened room.
Production of a green fluorescent pigment constitutes a positive presumptive test.
Confirm positive tubes by inoculating 0.1 mL of culture into Acetamide Broth and incubate at 36 ± 2 °C for 22 ± 2 h. After incubation, add 1 to 2 drops of Nessler reagent and examine the tubes for the production of ammonia. Development of a colour varying from yellow to brick red is a positive confirmed test for *P. aeruginosa*.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below is listed a test strain useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
P. aeruginosa ATCC 14207	35-37°C / 24 H/ A	growth with green fluorescent ring

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- Asparagine Enrichment Broth is a presumptive medium for *P. aeruginosa*, and further confirmatory tests are necessary for the identification.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

Storage conditions
Sore at +10°C /+30°C away from direct light in a dry place.

References

1. Briancesco R, Paduano S, Semproni M, Vitanza L, Bonadonna L. Behavior of Pseudomonas aeruginosa and Enterobacter aerogenes in Water from Filter Jugs. Int. J. Environ. Res. Public Health 2020; 17: 8263.
2. APHA Standards Methods for the Microbiological of Water and Wastewater. American Public Health Association, Washington D.C. 23rd, 2017.

Packaging
Product	Type	REF	Pack
Asparagine Enrichment Broth	Dehydrated medium	40109512	500 g (111 L)

IFU rev 1, 2022/12

AZIDE DEXTOSE BROTH
Dehydrated and ready-to-use culture medium

Azide Dextrose Broth; from left: uninoculated tube and tube with *Enterococcus faecium*.

Intended use
Selective liquid medium for the detection of faecal streptococci/enterococci in water, frozen food, milk and other samples.

Composition - typical formula * (after reconstitution with 1 L of water)
Peptocomplex	15.0 g
Beef extract	4.5 g
Glucose	7.5 g
Sodium chloride	7.5 g
Sodium azide	0.2 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
Enterococci are considered a better indicator of sewage contamination than *Escherichia coli* as they are more resistant to chlorine.
Azide Dextrose Broth is a medium initially formulated by Rothe[1] and tested by Mullmann and Seligmann[2] for the quantitative determination of streptococci in water, wastewater, shellfish and other materials with suspected faecal contamination. Azide Dextrose Broth has also been used for the isolation of streptococci in food, milk, water and other samples of sanitary interest as indicators of faecal contamination.[3,4]
Azide Dextrose Broth is used for the presumptive determination of enterococci in water and wastewater using the MPN technique followed by confirmation test in Bile Aesculin Azide Agar or Ethyl Violet Azide Broth.[5-8]
A similar procedure is indicated in the APAT, IRSA-CNR guidelines[9] for the detection of enterococci in water using the MPN method.

Peptocomplex and meat extract provide nitrogen, amino acids and trace elements for microbial growth; sodium azide limits the growth of Gram-negative bacteria by blocking the enzyme cytochrome oxidase; glucose is a fermentable carbohydrate; sodium chloride contributes to maintaining the osmotic balance of the medium

DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 34.7 g in 1000 mL of cold purified water. Heat gently to dissolve, distribute 10 mL into tubes and sterilise by autoclaving at 121°C for 15 minutes. For inocula of more than 1 mL per 10 mL medium prepare the liquid medium at double or multiple concentration.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	straw-coloured, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Drinking water, source water, fresh and marine recreational waters and food samples. Refer to applicable International Standards and regulations for the collection, transport, storage and preparation of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

1. Inoculate a series of tubes of Azide Dextrose Broth with appropriate graduated quantities of a 100 mL sample. Use sample volumes of 10 mL or less. The strength of the broth will be proportional to the sample size.
2. Incubate at 35 ± 0.5°C for 24 ± 2 hours and observe for microbial growth (turbidity of broth); if no turbidity is observed, continue incubation for a further 24 hours.
3. Streak a portion of growth from each positive tube on Bile Aesculin Azide Agar ISO Form. (REF 401018) and incubate at 35°C for 24 ± 2 hours.[6]
4. Alternatively, remove 1 mL of broth culture from the positive tubes and inoculate into the corresponding tubes containing Ethyl Violet Azide Broth (REF 401485) for confirmation testing. Incubate the tubes at 35 °C for 24+24 (±3) hours.

READING AND INTERPRETATION

Bacterial growth in Azide Dextrose Broth is evidenced by the development of turbidity.
After confirmation tests, apply MPN tables for estimating the number of faecal streptococci per volumetric unit of sample.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 19433	37°C /24H-A	good growth
E. faecium ATCC 19434	37°C /24H-A	good growth
E. coli ATCC 25922	37°C /24H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since some Gram-positive bacilli and cocci other than faecal streptococci grow in Azide Dextrose Broth, a confirmation test is required.

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place.
Ready-to-use medium in tubes
Sore tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Rothe (1948) Illinois State Health Department.
2. Mallmann WL, Seligmann A. comparative study of media for the detection of streptococci in water and sewage. Am J Public Health 1950; 40:286
3. Larkin, EP, Litsky, W, Fuller JE. Fecal streptococci in frozen foods I A bacteriological survey of some commercially frozen foods. Appl Microbiol 1955; 3:98-101
4. Splittstoesser DF, Wright R, Hucker GJ. Studies on Media for Enumerating Enterococci in Frozen Vegetables. Appl. Microbiol. 1962; 9:303.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
6. APHA Standard Methods for the Examination of Water and Wastewater 23rd ed. Washington, DC: American Public Health Association, 2017.
7. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D.C. 1976
8. WHO Examination of water for pollution control. Part III: Biological, Bacteriological and Virological Examination., ed. Oxford. Pergamon Press, World Health Organization.1982
9. APAT, IRSA-CNR Manuali e Linee Guida 29/2003 Metodi analitici per le acque. Cap 3, 7040.

PACKAGING

Product	Type	REF	Pack
Azide Dextrose Broth	Dehydrated medium	4011052	500 g (14.5 L)
Azide Dextrose Broth	Ready-to-use tubes	551105	20 x 10 mL

IFU rev 2, 2022/05

AZIDE DEXTOSE BROTH (ROTHE)

Dehydrated culture medium

INTENDED USE

Selective medium for the detection of enterococci in water and sewage.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20.0 g
Glucose	5.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.7 g
Potassium dihydrogen phosphate	2.7 g
Sodium azide	0.2 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Azide Dextrose Broth (Rothe) is a buffered version of the medium initially formulated by Rothe at the Illinois State Health Department.[1]
The presence of enterococci is an indicator of faecal contamination mainly in chlorinated water because they have a greater resistance to chlorine than *Escherichia coli*.
The peptone provides nitrogen, amino acids and trace elements for microbial growth, glucose is a fermentable carbohydrate; sodium azide limits the growth of Gram-negative bacteria by blocking the enzyme cytochrome oxidase; sodium chloride contributes to maintaining the osmotic balance of the medium; potassium phosphates buffer the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.6 g in 1000 mL of cold purified water. Heat gently to dissolve, distribute 10 mL into tubes and sterilise by autoclaving at 121°C for 15 minutes. For inocula of more than 1 mL per 10 mL medium prepare the liquid media at double or multiple concentration.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	pale yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Water and sewage and other samples. Refer to applicable International Standards and regulations for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

1. Shake the sample vigorously to ensure homogeneous distribution of the suspended microorganisms.
2. Perform counting in tubes, according to the most probable number method (MPN); vary the size of the inoculum (multiples or fractions of 1 mL) according to the type of sample, setting up at least five tubes for each dilution.
3. Incubate at 36 ±1°C for 24 hours and observe for microbial growth (turbidity of broth); if no turbidity is observed, continue incubation for a further 24 hours.
4. Remove 1 mL of broth culture from the positive tubes and inoculate into the corresponding tubes containing Ethyl Violet Azide Broth (REF 401484) for confirmation testing.
5. Incubate the tubes at 36 ±1°C for 24+24 (±3) hours. Consider tubes with turbidity accompanied by a violet-grey deposit at the bottom of the tube as positive.
6. Other media may be used for the confirmation test; consult the cited bibliography and other applicable literature.

READING AND INTERPRETATION

Bacterial growth in Azide Dextrose Broth (Rothe) is evidenced by the development of turbidity.
After confirmation tests, apply MPN tables for estimating the number of faecal streptococci per volumetric unit of sample.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 19433	35-37°C /24H-A	good growth
E. coli ATCC 25922	35-37°C /24H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since some Gram-positive bacilli and cocci other than faecal streptococci grow in Azide Dextrose Broth (Rothe), a confirmation test in Ethyl Violet Azide Broth or other suitable medium is required.

STORAGE CONDITIONS

Sore at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Rothe (1948) Illinois State Health Department.

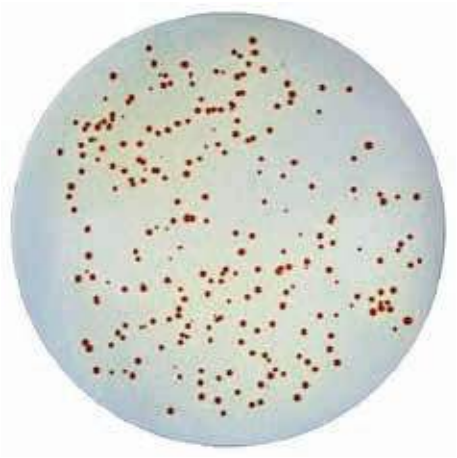
PACKAGING

Product	Type	REF	Pack
Azide Dextrose Broth (Rothe)	Dehydrated medium	4011062	500 g (14 L)

IFU rev 1, 2022/05

AZIDE MALTOSE AGAR KF

Dehydrated culture medium, ready-to-use plates



Azide Maltose Agar KF: *Enterococcus faecalis* colonies on a filter membrane

INTENDED USE

For the enumeration of enterococci in water and food samples.

COMPOSITION*

DEHYDRATED AZIDE MALTOSE AGAR KF

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10 g
Yeast extract	10 g
Sodium chloride	5 g
Sodium glycerophosphate	10 g
Maltose	20 g
Lactose	1 g
Agar	15 g
Sodium azide	400 mg
Bromocresol purple	15 mg

AZIDE MALTOSE AGAR KF, READY-TO-USE PLATES

Azide Maltose Agar KF	71.4 g
2,3,5-triphenyltetrazolium chloride	100 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Azide Maltose Agar KF, with added TTC is prepared according to the formula developed by Kenner, Clark and Kabler (Kenner-Faecal KF Medium)[1] for the selective isolation of faecal streptococci from surface water by direct inoculation or membrane filtration methods.
The medium is recommended by APHA[2] for the isolation and enumeration of enterococci in food samples by pour-plate technique.
The faecal streptococci group has long been considered an effective indicator of faecal contamination in aquatic ecosystems; although some authors consider the terms faecal streptococci, enterococci, intestinal enterococci and *Enterococcus* to be synonymous in the case of species detectable in the environment, the taxonomic ordering of the group has been the subject of numerous definitions.[3]
The term faecal streptococci has been used to denote a heterogeneous group of microorganisms both taxonomically and ecologically, grouped together on the basis of microscopic morphology, reactivity to Gram stain and the absence of catalase.
Studies in the 1980s subdivided the genus *Streptococcus*, on the basis of physiological characteristics and DNA hybridisation techniques, into three genetically different genera (*Enterococcus, Streptococcus, Lactococcus*), the first two of which include intestinal or faecal species.
The genus *Enterococcus* includes Lancefield group D streptococci, which share certain biochemical properties and have a wide range of tolerance to adverse conditions (ability to grow in 6.5% sodium chloride, pH 9.6 and 45°C); these phenotypic characteristics are, however, ascribable to most but not all species. The taxonomy of the genus is continually evolving: the genus, based on 16S rRNA sequence analysis, includes 79 species and 3 subspecies to date.[4]
Enterococci can be found in soil, water, dairy products, food and plants, and include species with a proven intestinal origin (*E. faecalis, E. faecium, E. durans/hirae, E. cecorum*) and others whose exclusively intestinal origin has not been fully proven (*E. raffinosus, E. dispar, E. flavescens, E. casseliflavus, E. gallinarum, E. mundtii, E. sulphureus*).[5-7]
Within the genus *Streptococcus*, only *S. bovis* and *S. equinus* are considered true faecal streptococci. These two streptococcus species are mainly found in animals.
The name Group D Streptococci, to denote faecal streptococci, is not to be trusted as the Lancefield Group D antigen is produced by *Enterococcus*, *Pediococcus* and some streptococci.
As a consequence of this taxonomic complexity and the multiple habitats of enterococci, more attention should be paid to their identification at the species level to discriminate those truly intestinal of animal, human and warm-blooded origin and their role as indicators of faecal pollution, for the assessment of the hygienic quality of water and food.
In Azide Maltose Agar KF, proteose peptone and yeast extract provide nitrogen, vitamins, amino acids and trace elements for microbial growth; lactose and maltose are fermentable carbohydrates: the production of acids induces the turning of the pH indicator bomocresol purple to yellow; sodium chloride contributes to the osmotic balance of the medium; sodium azide is a selective agent active in inhibiting the growth of Gram-negative bacteria; triphenyl tetrazolium chloride added to the base medium is reduced during bacterial growth to formazan, an insoluble pigment that colours the colonies pink-red.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 71.4 g in 1000 mL of cold purified water; heat to boiling, and boil for five minutes (or autoclave 10 min at 121°C, if total selectivity is required). Cool to 50°C and aseptically add 10 mL of TTC 1% Solution (REF 42111801). Mix well and pour into sterile 55- or 90-mm Petri dishes or hold at 45°C when using the pour-plate method.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pink, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Refer to applicable International Standards and regulations for the collection of water and food samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

Membrane filter technique

Filter an appropriate volume of water onto the membrane depending on the expected enterococci number. When the sample's bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate (20-60 UFC/dish).
Using aseptic technique, roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface. Incubate at 35-37°C for 48 hours.

Pour-plate method[2]
Place 1 mL of the decimal dilutions of the sample in 90 mm diameter plates, in duplicate;
Add approximately 15 mL of medium cooled to 45°C to each plate, carefully mix the inoculum with the agar and allow to solidify.
Incubate at 35-37°C for 48 hours.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies, if necessary, using a 15x stereoscopic microscope. Count and record pink to red colonies often surrounded by a yellow halo as enterococci.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. faecalis ATCC 19433	37°/ 48 H-A	Growth with pink-red colonies with yellow halo
E. faecium ATCC 19434	37°/ 48 H-A	Growth with pink-red colonies with yellow halo
S. aureus ATCC 25923	37°/ 48 H-A	Inhibited
E. coli ATCC 25922	37°/ 48 H-A	Inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- KF Agar was found to be unsuitable for marine water because *Vibrio alginolyticus* and other Gram-negative bacilli indigenous to this environment grew well on it and produced red colonies identical to those of faecal streptococci.[8]
- Many strains of *S. bovis* and *S. equinus* are inhibited by sodium azide.
- The intensity of TTC reduction varies depending of the isolated species.[2]
- Most but not all enterococci and streptococci ferment lactose and sucrose.[2]
- Some strains of *Pediococcus*, *Lactobacillus* and *Aerococcus* may grow on the medium, producing light pink colonies.[2]
- For the examination of dairy products, a more selective medium and a higher incubation temperature should be used to reduce the background growth of lactobacilli and lactic streptococci.[2]
- Over-heating of the medium lowers pH resulting in a decreased productivity.[9]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the medium base without TTC may be stored in screw capped flasks at +2°C /+8°C for approximately 6 months.[9]
Ready-to-use-plates
Sore plates in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Kenner, A, Clark HF, Kabler, PW. Fecal Streptococci I Cultivation and enumeration of Streptococci in surface waters. App Microbiol, 1961; 9:15
2. APHA Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington D.C., 2015
3. APAT-CNR-IRSA: Metodi per la determinazione di microrganismi indicatori d'inquinamento e di patogeni. 7040. Streptococchi fecali ed enterococchi.
4. Meier-Kolthoff, J.P., Sardà Carbasse, J., Peinado-Olarte, R.L. and Göker, M. (2022). TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res, 50, D801-D807; DOI: 10.1093/nar/gkab902
5. Hardie DJM, Whiley RA. Classification and overview of the genera Streptococcus and Enterococcus. J App Microbiol Symposium Supplement, 1997; 83:1S–11S
6. Lebreton F, Willems RJL, Gilmore MS. Enterococcus Diversity, Origins in Nature, and Gut Colonization. Gilmore MS, Clewell DB, Ike Y, et al., editors. 2014.
7. Pinto B, Pierotti R, Canale G. Characterization of 'faecal streptococci' as indicators of faecal pollution and distribution in the environment. Lett in App Microbiol 1999 ; 29 :258–263
8. Yoshpe-Purer Y. Evaluation of media for monitoring fecal streptococci in seawater. Appl Environ Microbiol. 1989 Aug; 55(8): 2041–2045
9. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD.

PACKAGING

Product	Type	REF	Pack
Azide Maltose Agar KF	Dehydrated medium	4011072	500 g (7 L)
Azide Maltose Agar KF	Ready-to-use plates	491107	3 x 10 plates ø 55 mm

IFU rev 1, 2023/01

BACILLUS CEREUS AGAR BASE (MYP)
BACILLUS CEREUS ANTIMICROBIC SUPPLEMENT
BACILLUS CEREUS SELECTIVE AGAR (MYP)
Dehydrated and ready-to-use culture medium and selective supplement

MYP: typical *Bacillus cereus* colonies

INTENDED USE
For the detection and enumeration of *B. cereus* group in foodstuffs and other samples.

COMPOSITION*
DEHYDRATED AND READY-TO-USEIN FLASKS BACILLUS CEREUS AGAR BASE (MYP) TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	1.00 g
Peptone	10.00 g
D-mannitol	10.00 g
Sodium chloride	10.00 g
Phenol red	0.025 g
Agar	12.00 g

BACILLUS CEREUS ANTIMICROBIC SUPPLEMENT (VIAL CONTENTS FOR 500 ML OF MEDIUM)

Polymyxin B sulphate	50,000 IU

BACILLUS CEREUS SELECTIVE AGAR (MYP) - READY-TO-USE PLATES

Bacillus Cereus Agar Base	43 g
Egg Yolk Emulsion 50%	100 mL
Polymyxin B sulphate	100,000 IU
Purified water	900 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Bacillus cereus is a group of ubiquitous facultative anaerobic sporeforming Gram-positive rods commonly found in soil. *Bacillus cereus* Group comprises six species: *B. cereus, B. thuringiensis, B. weihenstephanensis, B. mycoides, B. pseudo-mycoides, B. weihenstephanensis* and *B. anthracis*. *Bacillus cereus* is a foodborne pathogen that can produce two toxins, one heat stable and emetic and the other thermolabile the cause diarrhoea.[1] The infection is caused by ingestion of foods such as meat, rice and vegetables that are contaminated with *B. cereus*, and have been left at room temperature after cooking.
For the enumeration of vegetative cells and spores of *Bacillus cereus* in foods, a mannitol-egg yolk-phenol red (MYP) agar has been developed by Mossel in 1967[2] exploiting the failure of *B. cereus* to dissimilate mannitol, and the ability of most strains to produce phospholipase C. MYP agar is recommended by ISO 7932,[3] ISO 218714, FDA-BAM[5] and APHA[6] for detection and enumeration of *B. cereus* in foods.
Bacillus Cereus Selective Agar MYP contains beef extract and peptone that provide carbon, nitrogen, and minerals for microbial growth. This medium relies on the selective inhibitory component polymyxin B and two indicator systems: mannitol/phenol red and egg yolk.
The growth of many unwanted organisms is suppressed by polymyxin B, while target-organisms will not attack mannitol but dissimilate egg yolk and consequently give rise to typical bacilliform colonies with purple-red zones and halos.[7] Non-target organisms that ferment mannitol produce acid products and form yellow colonies.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)
Suspend 21.5 of Bacillus Cereus Agar Base MYP in 450 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minute and cool to 44-47 °C. Reconstitute under aseptic conditions the contents of one vial of Bacillus Cereus Antimicrobic Supplement (Ref. 4240001) with 5 mL of sterile purified water, add to the base medium and mix. Add 50 mL of Egg Yolk Emulsion, (Ref. 42111601), mix well and distribute into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)
Heat to boiling the medium in flasks and cool to 44-47°C. Reconstitute under aseptic conditions the contents of one vial of Bacillus Cereus Antimicrobic Supplement (Code 4240001) with 5 mL of sterile purified water, add 1 mL to the base medium and mix. Add 10 mL of Egg Yolk Emulsion (Code 42111601), mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS
Bacillus Cereus Selective Agar MYP

Dehydrated medium appearance	red-orange, fine, homogeneous, free-flowing powder
Solution and prepared medium in flask appearance	red, slightly opalescent
Prepared plates appearance	pink, opaque
Final pH at 20-25 °C	7.2 ± 0.2
Bacillus Cereus Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pastille
Solution appearance	colourless, limpid

SPECIMENS
Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[3-6]

TEST PROCEDURE
For the isolation and enumeration of *B. cereus* group strains in foodstuffs, according ISO 7932, the following method is recommended:
Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned.
Distribute 0.1 mL of test sample if the product is liquid, or of the initial suspension if solid onto the surface of two agar plates (90mm). Repeat the procedure using further decimal dilutions.

If low number of *B. cereus* is expected, distribute 1 mL of test sample if the product is liquid or 1 mL of the initial suspension if solid to each of two agar plates (140mm) or over the surface of three 90mm plates.
Incubate at 30°C in aerobic conditions for 18-24 hours. If colonies are not visible incubate the plates for further 24 hours before counting.

READING AND INTERPRETATION

Count the *B. cereus* group colonies in the plates with less than 150 colonies, that have the following characteristics: large, pink (indicating that mannitol fermentation has not occurred) and generally surrounded by a zone of precipitation (indicating the production of lecithinase).
If the plates have a high content of background flora which ferments mannitol, the characteristic coloration of the colonies and background may be reduced or no longer visible. In addition, some presumptive *Bacillus cereus* strains have only a slight egg yolk reaction or none at all. In such cases and in any other doubtful cases, these colonies should also be submitted to the confirmation.
Typical and atypical colonies on MYP Agar shall be confirmed by means of the haemolysis test on sheep blood agar.
Select five presumptive colonies from each plate and streak the selected colonies onto the surface of sheep blood agar in a manner which allows good interpretation of the haemolysis reaction. Incubate at 30 °C for 24 h ± 2 h and interpret the haemolysis reaction.
Results confirming presumptive *Bacillus cereus* strains:

- Formation of pink colonies surrounded by precipitate on MYP agar.
- Positive haemolysis reaction.

Optional tests intended for complementary investigations (i.e., epidemiological) on isolated *Bacillus cereus* group strains[3]: 1) detection of *cytK-1* or cttK2-gene variants of the gene encoding Cytotoxin K; 2) Detection of *Bacillus cereus* group strains able to produce cereulide; 3) Motility test for *B. antracis* screening; 4) Microscopic examination of the parasporal crystal from B*acillus thuringiensis*
Other tests useful for differentiating typical strains of *B. cereus* from other members of the *B. cereus* group:[5] 1) Microscopic observation (large Gram-positive rods in short-to-long chains; spores are ellipsoidal, central to sub-terminal, and do not swell the sporangium); 2 Glucose fermentation (+); 3) Voges Proskauer Reaction (+); 4) Nitrate reduction (+); 5) Motility test (+); 6) Decomposition of tyrosine (+); 7) Growth in presence of 0.001% lysozyme.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
B. cereus ATCC 11778	18-24h /30°C/A	growth, pink colonies with opaque halo
E. coli ATCC 11775	18-24h /30°C/A	no growth
B. subtilis ATCC 6633	18-24h /30°C/A	growth, yellow colonies without opaque halo

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- It appears that the spores of many, if not most, strains of *B. cereus* germinate readily on the surface of culture media used for enumeration. In most cases there does not seem to be a need for heat shock treatment to provoke germination. Sometimes a heat shock procedure is desirable, for example for spore counts or to inhibit growth of vegetative bacterial cells. In such cases, treatment for 15 min at 70 °C is recommended.[3]
- The confirmatory tests may in some instances be inadequate for distinguishing *B. cereus* from culturally similar organisms that could occasionally be encountered in foods. These organisms include 1) the insect pathogen *B. thuringiensis*, which produces protein toxin crystals; 2) *B. mycoides*, which characteristically produces rhizoid colonies on agar media; and 3) *B. anthracis*, which exhibits marked animal pathogenicity and is non-motile. With the exception of *B. thuringiensis*, which is currently being used for insect control on food and forage crops, these organisms are seldom encountered in the routine examination of foods.[5]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place.
According to ISO 7932, the plates may be stored prior to drying at between +2 °C and +8 °C for up to 4 days.[3]
Freeze-dried supplement, Ready-to-use plates and flasks
Sore in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Turenne C, Alexander DC Bacillus and other Aerobic Endospore-Forming Bacteria. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Mossel DAA, Koopman MJ, Jongerius E. Enumeration of Bacillus cereus in foods. Appl Microbiol 1967;15(3):650-3.
3. ISO 7932:2004/AMD 1:2020 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive Bacillus cereus — Colony-count technique at 30 degrees C — Amendment 1: Inclusion of optional tests
4. ISO 21871:2005 - Microbiology of food and animal feeding stuffs -- Horizontal method for the determination of low numbers of presumptive Bacillus cereus -- Most probable number technique and detection method
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 14: Bacillus cereus. Content current as of: 06/29/2021
6. APHA Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington D.C., 2015
7. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 233-234.

PACKAGING

Product	Type	REF	Pack
Bacillus Cereus Agar Base (MYP)	Dehydrated medium	4011112	500 g (11.6 L)
Bacillus Cereus Agar Base (MYP)	Ready-to-use medium in flask	5111112	6 x 90 mL
Bacillus Cereus Selective Agar (MYP)	Ready-to use-medium in plates	541112M	2 x 10 plates ø 90 mm
Bacillus Cereus Antimicrobic Supplement	Freeze-dried selective supplement	4240001	10 vials, each for 500 mL of medium
Egg Yolk 50% Solution	Liquid supplement	42111601	50 mL
Egg Yolk 50% Solution	Liquid supplement	42111605	100 mL
Egg Yolk 50% Solution	Liquid supplement	42111600	200 mL

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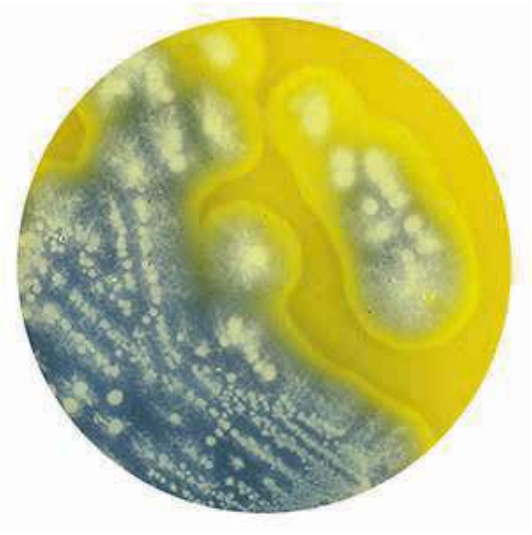
BACILLUS CEREUS AGAR BASE (PEMBA)
BACILLUS CEREUS ANTIMICROBIC SUPPLEMENT
BACILLUS CEREUS SELECTIVE AGAR (PEMBA)
Dehydrated and ready-to-use culture medium and selective supplement



PEMBA: typical *Bacillus cereus* colonies

INTENDED USE
For the detection and enumeration of *B. cereus* group in foodstuffs and other samples.

COMPOSITION*
DEHYDRATED BACILLUS CEREUS AGAR BASE (PEMBA)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

D-mannitol	10.00 g
Peptone	1.00 g
Sodium pyruvate	10.00 g
Sodium chloride	2.00 g
Magnesium sulphate	0.10 g
Potassium dihydrogen phosphate	0.25 g
Disodium hydrogen phosphate	2.50 g
Bromothymol blue	0.12 g
Agar	15.00 g

BACILLUS CEREUS ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Polymyxin B sulphate	50,000 IU

BACILLUS CEREUS SELECTIVE AGAR (PEMBA) - READY-TO-USE PLATES

Bacillus Cereus Agar Base (PEMBA)	41 g
Egg Yolk Emulsion 50%	50 mL
Polymyxin B sulphate	100,000 IU
Purified water	950 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Bacillus cereus is a group of ubiquitous facultative anaerobic sporeforming Gram-positive rods commonly found in soil. Group comprises six species: *B. cereus, B. thuringiensis, B. weihenstephanensis, B. mycoides, B. pseudo-mycoides, B. weihenstephanensis* and *B. anthracis*. *Bacillus cereus* is a foodborne pathogen that can produce two toxins, one heat stable and emetic and the other thermolabile the cause diarrhoea.[1] The infection is caused by ingestion of foods such as meat, rice and vegetables that are contaminated with *B. cereus*, and have been left at room temperature after cooking.
Hoolbrook and Anderson[2] in 1980 described the use and performance of an improved diagnostic and selective medium, polymyxin pyruvate egg yolk mannitol bromothymol blue agar-Pemba, for the detection of *Bacillus cereus* in foods.
PEMBA is recommended by ISO 21871[3] for detection and enumeration of low number of presumptive *B. cereus* in foods.
Bacillus Cereus Agar (PEMBA) contains a peptone that provides carbon, nitrogen, and minerals for microbial growth. This medium relies on the selective inhibitory component polymyxin B and two indicator systems: mannitol and bromothymol blue and egg yolk.
Selectivity is attained with polymyxin B and a critical concentration of nutrients. *B. cereus* will not attack mannitol but dissimilate egg yolk and consequently give rise to typical bacilliform turquoise to peacock blue colonies with halos.[4] Non-target organisms that ferment mannitol produce acid products and form yellow colonies.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 20.5 g in 470 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minute and cool to 44-47°C. Reconstitute under aseptic conditions the contents of one vial of Bacillus Cereus Antimicrobic Supplement (Ref. 4240001) with 5 mL of sterile purified water, add to the base medium and mix. Add 25 mL of Egg Yolk Emulsion, (Ref. 42111601), mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution appearance	blue-green, slightly opalescent
Prepared plates appearance	green-blue, opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS
Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards.[3]

TEST PROCEDURE
ISO 21871,[3] recommends the procedure summarised here.
Inoculate PEMBA agar plates after selective enrichment in tryptone soya polymyxin broth (TSPB). TSPB tubes can be prepared from Tryptic Soy Broth (REF 402155) with added Bacillus Cereus Antimicrobic Supplement (REF 4240001).
For enumeration method inoculate three tubes of double-strength TSPB with 10 mL of the primary dilution and inoculate single-strength TSPB with 1 mL of decimal dilutions of the primary dilution (initial suspension). For detection method inoculate 1 mL of the initial suspension to 9 mL of single-strength TSPB.
Incubate the inoculated TSPB tubes at 30°C for 48 h ± 4 h.
Mix well the TSPB tubes and streak an inoculation loop of culture from each of the tubes onto the surface of PEMBA.
Incubate the inoculated plates with the lid downwards at 37 °C for 18 h to 24 h.
If the colonies cannot be clearly assessed, continue incubating the plates for up to additional 24 h.

READING AND INTERPRETATION

After incubation is complete, examine the plates for the presence of typical or atypical colonies.[3]
On PEMBA, typical colonies of presumptive *Bacillus cereus* are about 2 mm to 5 mm in size, have an irregular edge which is between ragged and root-like with ground glass surface, are turquoise to peacock blue, possibly with a greyish white colony centre against a blue background, and have a precipitation halo (egg yolk reaction) up to 5 mm wide.
If the plates have a high content of background flora which ferments mannitol, the characteristic coloration of the colonies and background may be reduced or no longer visible. In addition, some presumptive *Bacillus cereus* strains have only a slight egg yolk reaction or none at all. In such cases and in any other doubtful cases, these colonies should also be submitted to the confirmation.
Typical and atypical colonies on PEMBA shall be confirmed by means of the haemolysis test on sheep blood agar and a microscopic examination.

- Streak the selected colonies from PEMBA onto the surface of sheep blood agar in a manner which allows well-separated colonies to develop. Incubate at 30 °C for 24 h and read haemolysis reaction. Each colony surrounded by a cleared zone is considered to be haemolysis-positive.
- Typical and atypical colonies on PEMBA may be confirmed by means of microscopic examination using malachite green solution for staining the spores and Sudan black B solution for staining the intracellular fat globules and re-stain with safranin solution.
- Examine the slide under a microscope using immersion oil. As a rule, the brick-shaped cells of presumptive *Bacillus cereus* are arranged in chains and are 4 µm to 5 µm long, 1 µm to 1,5 µm wide and contain fairly large amounts of intracellular fat which is stained black. The green stained spores may be central or subterminal, but they never distend the red stained sporangia.

Other tests useful for differentiating typical strains of *B. cereus* from other members of the *B. cereus* group:[5] 1) Microscopic observation (large Gram-positive rods in short-to-long chains; spores are ellipsoidal, central to sub-terminal, and do not swell the sporangium); 2 Glucose fermentation (+); 3) Voges Proskauer Reaction (+); 4) Nitrate reduction (+); 5) Motility test (+); 6) Decomposition of tyrosine (+); 7) Growth in presence of 0.001% lysozyme.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
B. cereus ATCC 11778	18-24h /30°C/A	growth, turquoise-blue colonies with opaque halo
E. coli ATCC 11775	18-24h /30°C/A	no growth
B. subtilis ATCC 6633	18-24h /30°C/A	growth, yellow colonies without opaque halo

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- For enumeration of presumptive *Bacillus cereus* spores only, the primary dilution can be heated at 80 °C for 10 min in a water bath.[3]
- The confirmatory tests may in some instances be inadequate for distinguishing *B. cereus* from culturally similar organisms that could occasionally be encountered in foods. These organisms include 1) the insect pathogen *B. thuringiensis*, which produces protein toxin crystals; 2) *B. mycoides*, which characteristically produces rhizoid colonies on agar media; and 3) *B. anthracis*, which exhibits marked animal pathogenicity and is non-motile. With the exception of *B. thuringiensis*, which is currently being used for insect control on food and forage crops, these organisms are seldom encountered in the routine examination of foods.[5]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place.
According to ISO 21871 the plates may be stored prior to drying at between 1 °C and 5 °C for up to 4 days.[3]
Ready-to-use plates and freeze-dried supplement
Sore in their original pack at +2°C /+-8°C away from direct light.

REFERENCES

1. Turenne C, Alexander DC Bacillus and other Aerobic Endospore-Forming Bacteria. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Holbrook R, Anderson JM. An improved selective and diagnostic medium for the isolation of Bacillus cereus in foods. Can J Microbiol 1980; 26: 753-759.
3. ISO 21871:2005 - Microbiology of food and animal feeding stuffs -- Horizontal method for the determination of low numbers of presumptive Bacillus cereus -- Most probable number technique and detection method
4. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 233-234.
5. US Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 14: Bacillus cereus. Content current as of: 06/29/2021

PACKAGING

Product	Type	REF	Pack
Bacillus Cereus Agar Base (PEMBA)	Dehydrated medium	4011122	500 g (12.2 L)
Bacillus Cereus Selective Agar (PEMBA)	Ready-to-use medium in plates	541112	2 x 10 plates ø 90 mm
Bacillus Cereus Antimicrobic Supplement	Freeze-dried selective supplement	4240001	10 vials, each for 500 mL of medium
Egg Yolk 50% Solution	Liquid supplement	42111601	50 mL
Egg Yolk 50% Solution	Liquid supplement	42111605	100 mL
Egg Yolk 50% Solution	Liquid supplement	42111600	200 mL

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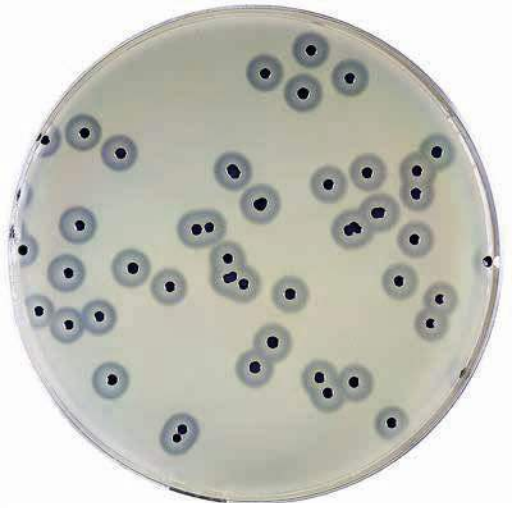
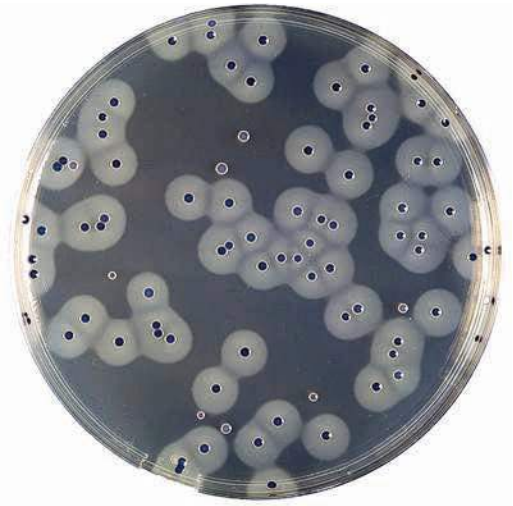
BAIRD PARKER AGAR BASE
BAIRD PARKER EGG YOLK TELLURITE AGAR
BAIRD PARKER RPF AGAR

Dehydrated and ready-to-use culture media



Baird Parker Egg Yolk Tellurite Agar: colonies of *S. aureus*



Baird Parker RPF Agar: colonies of *S. aureus* and *S. epidermidis*

INTENDED USE

Baird Parker Agar Base, supplemented with Egg Yolk Tellurite Emulsion or RPF Supplement, is used for the enumeration of coagulase-positive staphylococci in foods and in other samples.

COMPOSITION*

BAIRD PARKER AGAR BASE – DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	10.00 g
Meat extract	5.00 g
Yeast extract	1.00 g
Sodium pyruvate	10.00 g
Glycine	12.00 g
Lithium chloride	5.00 g
Agar	15.00 g

BAIRD PARKER EGG YOLK TELLURITE AGAR - READY-TO-USE PLATES

Enzymatic digest of casein	10.00 g
Meat extract	5.00 g
Yeast extract	1.00 g
Sodium pyruvate	10.00 g
Glycine	12.00 g
Lithium chloride	5.00 g
Agar	15.00 g
Egg yolk emulsion	50 mL
Potassium tellurite 1%	10 mL
Purified water	1000 mL

BAIRD PARKER RPF AGAR - READY-TO-USE PLATES

Enzymatic digest of casein	10.00 g
Meat extract	5.00 g
Yeast extract	1.00 g
Sodium pyruvate	10.00 g
Glycine	12.00 g
Lithium chloride	5.00 g
Agar	15.00 g
Fibrinogen	3.80 g
Trypsin inhibitor	25.00 mg
Rabbit plasma (EDTA)	25.00 mL
Potassium tellurite	25.00 mg
Purified water	975 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Staphylococci are Gram-positive, non-motile, non-sporeforming, facultative anaerobic, catalase-positive cocci, with spherical cells 0.5-1 µm in diameter, occurring as single cocci, in pairs, or tetrads, or as short chains.[1]. Staphylococci are part of the resident microbiota of mammals and birds, and their major habitats are the skin and mucous membranes.[1] Some species of staphylococci can cause a wide variety of infectious, usually pyogenic, processes in various parts of the body in animals and humans. Coagulase-positive staphylococci, especially *S. aureus*, through the production of enterotoxins, induce gastroenteritis following the consumption of contaminated food.
A selective and diagnostic medium for the enumeration of *S. aureus* in foods was first reported by Baird-Parker in 1962.[2] This medium is now widely recommended by international authorities for enumeration of coagulase positive staphylococci in foods and other materials of sanitary importance.[3-9]
In Baird Parker Agar, the enzymatic digest of casein, meat extract and yeast extract provide nitrogen, carbon, group B vitamins and minerals for microbial growth; sodium pyruvate is a critical component essential to both recovery of damaged *S. aureus* and their subsequent growth.[2,3] Selectivity is attained with lithium chloride, glycine and potassium tellurite which inhibit most bacteria present in the samples except coagulase-positive staphylococci. Selectivity can be improved by the addition of sulphamethazine for suppressing growth and swarming of *Proteus*.[6,10,11]
Egg yolk is the substrate to detect lecithinase and lipase activities: the egg yolk clearing reaction due to lecithinase is the diagnostic feature of characteristic colonies of *S. aureus*; an opaque zone of precipitation may form within the clear halo due to lipase activity. Rabbit plasma, fibrinogen, trypsin inhibitor are the substrates for detection of coagulase enzyme directly on the Baird Parker RPF plates.
Baird Parker Agar Base with Egg Yolk Tellurite Emulsion conforms to the formulation indicated by ISO 6888-1[6] and by FDA BAM[5], while with RPF Supplement corresponds to the medium recommended by ISO 6888-2[7] and by ISO 6888-1 too only as an alternative to the coagulase test for confirmation. Both media are recommended by ISO 6888-3[8] for the MPN-method determination of low number of coagulase-positive staphylococci after enrichment in Giolitti and Cantoni Broth.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Egg Yolk Tellurite Medium

Suspend 58 g in 1000 mL of cold purified water; heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and, using aseptic conditions, add 50 mL of Egg Yolk Tellurite Emulsion 20% (REF 423700); mix well and pour into sterile Petri dishes.

Rabbit Plasma Fibrinogen Medium
Suspend 5.8 g in 90 mL of cold purified water; heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and, using aseptic conditions, add the contents of one vial of RPF Supplement II (REF 423102) reconstituted with 10 mL of sterile purified water; mix well and pour into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS REF 5111162)
Dissolve the contents (100 mL) of one flask of Baird Parker Agar Base in a water bath at 100°C. Cool to 47-50°C and, add 5 mL of Egg Yolk Tellurite Emulsion 20%, using aseptic conditions. Mix well and pour into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS REF 5131022)
Dissolve the contents (90 mL) of one flask of Baird Parker Agar Base in a water bath at 100°C. Cool to 47-50°C and, add the contents of one vial of RPF Supplement II reconstituted with 10 mL of sterile purified water, using aseptic conditions. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS
Baird Parker Agar Base	
Dehydrated medium appearance	straw coloured, fine, homogeneous, free-flowing powder
Baird Parker Agar Base	
Prepared medium appearance	yellow, limpid
Baird Parker Egg Yolk Tellurite Agar	
Prepared plates appearance	yellow, uniformly opaque
Baird Parker RPF Agar	
Prepared plates appearance	beige, slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS
Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling, cosmetics, water. Consult the appropriate references for sample collection and preparation.[4-9]

TEST PROCEDURE
ISO 6888-1 recommends the procedure summarised below with Baird Parker Egg Yolk Tellurite Agar medium.
1. Transfer by means of a sterile pipette 0.1 mL of the test sample if liquid or 0.1 of the initial suspension in the case of other products, to each of two agar plates. Repeat the procedure for further decimal dilutions if necessary.
2. Carefully spread the inoculum as quickly as possible over the surface of the agar plate. Allow the plates to dry.
3. Invert the plates and incubate them for 24 ±2 hours at 34-38°C and re-incubate for a further 24 ± 2 hours.
4. After incubation for 24 hours mark on the bottom of the plates the positions of any typical colonies. Re-incubate, then mark any new typical colonies. Also mark any atypical colonies present.
5. For enumeration, only retain plates containing a maximum of 300 colonies in total (typical, atypical, background flora), and including a maximum of either 150 typical or atypical colonies, or both, at two successive dilutions.
6. Select for confirmation five characteristic colonies if there are only characteristics colonies, or five non-characteristic colonies if there are only non-characteristic colonies, or five characteristic and five non-characteristic colonies if both types are present, from each plate.

ISO 6888-2 recommends the procedure summarised below with Baird Parker RPF Agar medium:
1. Transfer, by means of a sterile pipette, 1 mL of the test sample if liquid, or 1 mL of the initial suspension in the case of other products, to a Petri dish. Repeat the procedure for further decimal dilutions if necessary.
2. Into each Petri dish, immediately pour 18 mL to 20 mL freshly prepared complete Baird Parker RPF Agar to obtain a depth of at least 3 mm.
3. Carefully mix the inoculum with the culture medium and leave to solidify by placing the Petri dishes on a horizontal surface.
4. After complete solidification, invert the dishes and incubate at 34 °C to 38 °C.
5. After incubation for 24 h ± 2 h, mark on the bottom of the plates the positions of any typical colonies present. If no colonies or no typical colonies are obtained at 24 h ± 2 h, re-incubate all plates at 34°C to 38 °C for a further 24 h ± 2 h (to a total of 48 h ± 4 h), and mark any typical colonies.
6. At the end of the incubation period, count the typical colonies in each dish. For enumeration, only retain plates containing a maximum of 300 colonies, with 100 typical colonies. One of the plates shall contain at least 10 colonies.

Following the procedure for enumeration and detection by MPN given by **ISO 6888-3**, inoculate Baird Parker Egg Yolk Tellurite Agar or Baird Parker RPF Agar plates by subculturing the selective enrichment in Giolitti & Cantoni Broth (REF 401516).
For spreading, preparation of the inoculated plates and incubation follow the instructions as given above.

READING AND INTERPRETATION
Baird Parker Egg Yolk Tellurite Agar
Typical colonies are black or grey, shining and convex (1 mm to 1.5 mm in diameter after incubation for 24 h ± 2 h, and 1.5 mm to 2.5 mm in diameter after incubation for 48 h ± 4 h) and are surrounded by a clear zone, which can be partially opaque. After incubation for at least 24 h, an opalescent ring immediately in contact with the colonies can appear in this clear zone.
Atypical colonies have the same size as typical colonies and can present one of the following morphologies:
- shining black colonies with or without a narrow white edge; the clear zone is absent or barely visible and the opalescent ring is absent or hardly visible;
-grey colonies free of clear zone.
The confirmation of coagulase positive staphylococci is undertaken by a coagulase tube test. Alternatively, it can be undertaken by a plate test using Baird Parker RPF Agar.
Baird Parker RPF Agar
Typical colonies are black or grey or even white, small and are surrounded by an opacity halo of precipitation, indicating coagulase activity. Proteus colonies can appear to look like those of coagulase-positive staphylococci early on in the incubation. However, they can be distinguished from staphylococci after 24 h ± 2 h and 48 h ± 4 h of incubation, as their colonies become more or less brownish and start to spread. As the Baird Parker RPF Agar medium is based on a coagulase reaction, it is not necessary to confirm this activity.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of Baird Parker Egg Yolk Tellurite Agar and Baird Parker Egg Yolk RPF Agar.[6,7]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Baird Parker Egg Yolk Tellurite Agar		
S. aureus ATCC 6538	36 ± 2 °C / 24 ± 2 h / A	growth, black or grey colonies, surrounded by a zone of clearing
S. saprophyticus ATCC 15305	36 ± 2 °C / 48 ± 4 h / A	growth, black or grey colonies, without the clearing zone
E. coli ATCC 25922	36 ± 2 °C / 48 ± 4 h / A	inhibited
Baird Parker RPF Agar		
S. aureus ATCC 6538	36 ± 2 °C / 24 ± 2 h / A	growth, black or grey colonies, surrounded by an opaque zone
S. saprophyticus ATCC 15305	36 ± 2 °C / 48 ± 4 h / A	growth, black or grey colonies, without the opaque zone
E. coli ATCC 25922	36 ± 2 °C / 48 ± 4 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- The methods described are intended to be applicable to the detection and enumeration of coagulase-positive staphylococci among which enterotoxinogenic strains are encountered. It is mainly concerned with *S. aureus*, but also with *S.intermedius* and certain strains of *S.hyicus*.[6-8]
- The confirmation of typical and atypical colonies is based on a positive coagulase reaction, but it is recognized that some strains of *Staphylococcus aureus* give weakly positive coagulase reactions. These latter strains can be confused with other bacteria but they can be distinguished by the use of additional tests.[6-8]
- Colonies with typical appearance after 24 h ± 2 h incubation on Baird Parker Egg Yolk Tellurite Agar can lose their typical appearance after 48 h ± 4 h incubation, due to overgrowth with enlargement of the clear zone during the second phase of incubation. Counting only at 48h ± 4h can lead to low counts or no counts.[6,8]
- Even if there is an inhibitor of trypsin in the Baird Parker RPF medium, colonies with typical appearance after 24 h ± 2 h incubation can lose typical appearance after 48 h ± 4 h incubation, due to enzymatic processes (trypsin) or due to overgrowth. Counting only at 48 h ± 4 h can lead to low counts or no counts.
- Bacteria belonging to genera other than staphylococci can give colonies with an appearance similar to staphylococci on Baird Parker Egg Yolk Tellurite Agar. Microscopic examination of Gram stain, before confirmation, will enable the distinction of other genera from staphylococci.[6-8]
- On Baird Parker RPF Agar, *Proteus* colonies can appear to look like those of coagulase-positive staphylococci early on in the incubation. However, they can be distinguished from staphylococci after 24 h ± 2 h and 48 h ± 4 h of incubation, as their colonies become more or less brownish and start to spread.[7]
- Occasionally other organisms exhibit growth without typical reactions on Baird-Parker Agar, e.g., some strains of streptococci, micrococci, corynebacteria and enterobacteria, some yeasts, fungi and bacilli that are easily distinguishable by the morphology and grey colour of the colonies.[11]

STORAGE CONDITIONS

Dehydrated medium

Sore at +10°C /+30°C away from direct light in a dry place.

According to ISO 6888-1, Baird Parker Agar Base may be store in flask for up to 6 months at 2-8°C, while the prepared Baird Parker Egg Yolk Agar and Baird Parker RPF Agar plates may be stored, prior to drying, at 2-8°C for up to 14 days.[6]

Ready-to-use plates and flasks

Sore in their original pack at 2-8°C away from direct light.

REFERENCES

1. Becker k, Dkow RL, vonEiff C. Staphylococcus, Micrococcus and other catalase positive Cocci. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Baird-Parker AC. An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J Appl Bact 1962; 25:12-19.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 197-199.
4. APHA Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C., 2015
5. FDA-BAM. Chapter No.12: Staphylococcus aureus. U.S. Food and Drug Administration - Bacteriological Analytical Manual. Content current as of 12/16/2019
6. ISO 6888-1:2021. Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)-Part 1: Method using Baird-Parker agar medium.
7. ISO 6888-2:2021. Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) -Part 2: Method using rabbit plasma fibrinogen agar medium.
8. ISO 6888-3:2003. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 3: Detection and MPN technique for low numbers.
9. ISO 22718 Second edition 2015-12-01 Cosmetics — Microbiology — Detection of Staphylococcus aureus
10. Smith BA, Baird-Parker AC. The use of sulphamezathine for inhibiting Proteus spp. on Baird-Parker's isolation medium for Staphylococcus aureus. J Appl Bact 1964; 27: 78-82.
11. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
12. ISO 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

PACKAGING

Product	Type	REF	Pack
Baird Parker Agar Base	Dehydrated medium	4011162 4011164	500 g (8.6 L) 5 Kg (86 L)
Baird Parker Agar Base	Ready-to-use flasks	5111162	6 x 100 mL
Baird Parker Agar Base+ RPF Supplement II	Ready-to-use flasks and supplements	5131022	4 x 90mL Baird Parker flasks + 4 vials of RPF Supplement II (each for 90 mL of medium base)
Baird Parker Egg Yolk Tellurite Agar	Ready-to-use plates	541116	2 x 10 plates ø 90 mm
Baird Parker Egg Yolk Tellurite Agar	Ready-to-use plates	491116	3 x 10 plates ø 55 mm
Baird Parker RPF Agar	Ready-to-use plates	543101	2 x 10 plates ø 90 mm
Baird Parker Egg Yolk Tellurite Agar	Ready-to-use plates	501116P	5 plates ø 150 mm

Note: Baird Barker Agar base is also available at pH 6.8 with the Reference Number 401116P2 (500 g)

The following products are available for the detection of coagulase-positive staphylococci, for which please refer to the specific monograph:

Egg Yolk Tellurite Emulsion 20%	Liquid supplement	423700 423701 423702	50 mL 100 mL 200 mL
RPF Supplement II	Freeze-dried supplement	423102 423102D	4 vials of 10 mL, each for 100 mL of medium 4 vials of 20 mL, each for 200 mL of medium

Coagulase Plasma EDTA	Identification reagent	429936	4 vials with 5 mL of rabbit plasma (4 x 15 mL: 120 tests)
Coagulase Plasma EDTA	Identification reagent	429937	4 vials with 2.5 mL of rabbit plasma (4 x 7,5 mL: 60 tests)
Coagulase Plasma EDTA	Identification reagent	429938	10 vials with 1 mL of rabbit plasma (10 x 3 mL: 60 tests)

IFU rev 2, 2022/07

BCSA BURKHOLDERIA CEPACIA SELECTIVE AGAR BASE
BCSA SELECTIVE SUPPLEMENT
BCSA BURKHOLDERIA CEPACIA SELECTIVE AGAR

Dehydrated medium, selective supplement and ready-to-use plates

BCSA: lactose/sucrose oxidizing and not oxidizing *B.cepacia* strains

INTENDED USE

In vitro diagnostics. Dehydrated medium and selective supplement for the determination of the absence of *Burkholderia cepacia* complex (Bcc) in non-sterile pharmaceutical products according to USP method and for the isolation of Bcc in clinical specimens mainly of respiratory origin.

COMPOSITION

BCSA BURKHOLDERIA CEPACIA SELECTIVE AGAR BASE

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Casein peptone	10 g
Yeast extract	1.5 g
Lactose	10 g
Sucrose	10 g
Sodium chloride	5 g
Phenol red	0.08 g
Crystal violet	0.002 g
Agar	11.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

BCSA SELECTIVE SUPPLEMENT

VIAL CONTENTS

Vancomycin	1.25 mg
Gentamicin	5 mg
Polymyxin B	300,000 UI

BCSA BURKHOLDERIA CEPACIA SELECTIVE AGAR (READY-TO-USE PLATES)

BCSA Selective Agar Base	48 g
Vancomycin	0.0025 g
Gentamicin	0.010 g
Polymyxin B	600,000 UI
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Burkholderia cepacia complex (Bcc) is a group of aerobic, Gram-negative, oxidase and catalase positive rods, actually comprising 20 species which are phenotypically nearly indistinguishable and can be sub grouped into nine genomovars[1].

Bcc has high metabolic versatility, wide environmental distribution, and variable virulence; furthermore, members of the *B. cepacia* complex have the ability to form biofilms in pharmaceutical water systems as well as the capability of overcoming antimicrobial preservative systems and being resistant to disinfectants.[2]

The species of Bcc group are opportunistic pathogens in mechanically ventilated patients, immunosuppressed, infants, the elderly and those with serious underlying disease.[2] Bcc causes serious infections in patients with cystic fibrosis and chronic granulomatous disease; two Bcc members, *B. cenocepacia* and B. *multivorans*, account for greater than 85% of the cystic fibrosis infections.[3]

A 2012 survey analysed the reported recalls from the U.S. market of non-sterile pharmaceutical products, cosmetics, medical devices and dietary supplements for microbiologically related issues for a 7-year period: the majority of these recalls (72%) were associated with objectionable microorganisms and the presence of *B. cepacia* represented the most frequent event (34%).[4]

Several recalls of non-sterile pharmaceutical products have also been reported in more recent years.[5,6]

The FDA was sufficiently concerned in 2017 to issue an advisory notice of the dangers of Bcc contamination of aqueous, non-sterile drug products[7].

In response to stakeholder requests, a test method for the determination of absence of *Burkholderia cepacia* complex was published in 2019 in the chapter <60> of USP, for defining test procedures and media formulations[8]. Among the various culture media described for the isolation of *B. cepacia*, namely MAST, BCA, OFPBL, BCSA, the choice fell on the latter because of its ability to support the faster growth of Bcc isolates and to suppress other respiratory organisms.[2,9,10,11]

BCSA Burkholderia Cepacia Selective Agar is prepared according to the formula described by Heny in 1997[9] and meets the USP <60> requirements[8].

BCSA contains peptones that supply nutrients for the growth of *Burkholderia cepacia* and other microorganisms; lactose and sucrose are oxidized by the majority of Bcc isolates and the acid end-products result in the medium changing from orange to yellow due to the presence of the pH indicator, phenol red. Crystal violet is added to inhibit growth of Gram-positive organisms; antimicrobials vancomycin, gentamicin and polymyxin B are incorporated to inhibit organisms other than Bcc. BCSA is intended for the determination of the absence of *Burkholderia cepacia* complex (Bcc), in non-sterile pharmaceutical products according to USP method[8] and for the isolation of Bcc in clinical specimens mainly of respiratory origin in patients with cystic fibrosis and other respiratory diseases.[12,13]

Directions for medium preparation

Suspend 24 g in 500 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and, under aseptic conditions, add the contents of one vial of BCSA Selective Supplement (4240073), reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

Physical characteristics

Dehydrated medium	
Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	limpid, red orange
Final pH at 25 °C	6.8 ± 0.3
Selective supplement	
Lyophilized pellet appearance	short, dense, white pastille
Solution appearance	limpid, colourless

Specimens

Pharmaceutical samples: non-sterile products for inhalation use or aqueous preparations for oral, oromucosal, cutaneous, or nasal use; follow the procedure described by USP for the sample preparation.[8]

Clinical specimens: BCSA agar is used to detect *B. cepacia* complex from expectorated sputum, deep pharyngeal swab and aspirates, bronchoalveolar lavages. Specimens should be submitted directly to the laboratory without delay. If there is to be a delay in processing, store the specimens for no more than 2 hours in the refrigerator.[9,10,12]

Good laboratory practices for collection, storage and transport to the laboratory should be applied.

Test procedure

Pharmaceutical samples

Before performing the test for the determination of the absence of *B. cepacia* complex (Bcc), the ability of the method to detect Bcc in the presence of the product to be tested must be established (Suitability of the Test Method). The details of the procedure are described in USP <60>.[8]

Prepare a 1:10 dilution of the product to be examined using no less than 1 g of product. Use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate a suitable amount (determined as described in Suitability of the Test Method) of Tryptic Soy Broth or an appropriate dilution of Tryptic Soy Broth as determined during method suitability (for example, a 1:10 dilution may be required when conducting optional testing of pharmaceutical waters). Mix and incubate at 30–35 °C for 48–72 h.

Subculture by streaking on a plate of BCSA, and incubate at 30–35°C for 48–72 h.

Clinical Specimens

Inoculate 100µL of the liquefied sputum or bronchoalveolar lavages onto a BCSA plate and spread inoculum over the entire surface of the agar plate. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate at 35-37°C for 48-72 hours.

AMCLI-SIFC[12] recommendation: incubation at 37°C for 3 days followed by an incubation at room temperature for one week and quantitative detection of CFUs. UK SMI B 57[13] recommendation: incubation at 35-37°C for 5 days with daily cultures reading.

Reading and interpretation

The possible presence of Bcc is indicated by the growth of greenish–brown colonies with yellow halos, or white colonies surrounded by a pink–red zone on BCSA. Any growth on BCSA, typical or atypical should be confirmed by identification tests with biochemical, immunological, molecular, mass spectrometry techniques after colonies purification on a suitable medium.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[8]

Control strains			Incubation T°/ t / atm	Expected results
B. cepacia	ATCC	25416	35°C / 48 h / A	good growth
B. cenocepacia ATCC BAA-485 or *B. multivorans* ATCC BAA-487			35°C / 48 h / A	good growth
P. aeruginosa	ATCC	9027	35°C / 72 h / A	growth inhibited
S. aureus	ATCC	6538	35°C / 72 h / A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- The yellow color change of the medium indicates the degradation of sucrose and/or lactose occurred producing acidification; this degradation may not be present in all Bcc strains. Therefore, it is recommended that any type of colony grown on BCSA be subjected to identification tests.
- There are reports that strains of *Burkholderia gladioli* and *Pseudomonas* spp. can be isolated on BCSA.[13]
- Although the superiority of the BCSA medium for the isolation of Bcc is recognized, Plonga[14] reports the failure to grow on a marketed BCSA of 7 strains out of 43 inoculated (sensitivity 86%). It is therefore possible that there are Bcc strains that may be sensitive to antibiotics present in the medium.
- Rapidly growing mycobacteria (RGM) could be recovered from routine cultures of samples from patients with cystic fibrosis by extending incubation of BCSA to 7 days.[15] However this strategy for the isolation of RGM still provides lower results than the use of more specific media.[14]
- The identification of Bcc members can be problematic since *B. cepacia* has a diverse genetic composition making accurate identification using phenotypic tests difficult. Many biochemical identification test systems have difficulty differentiating between the genera *Ralstonia, Burkholderia, Cupriavidus, Pandoraea, Achromobacter, Brevundimonas, Comamonas* and *Delftia*; this is compounded when attempting to differentiate within the *Burkhoderia* genera (the species members are phylogenetically very closely related with little differences in the way of phenotypic characteristics). For example, *B. cepacia* is closely related to the bacterial species *B. gladioli*.[1]
- The testing time of a pharmaceutical sample needs to be considered. The microbial growth kinetics of many Bcc organisms, due to their recovery from low-nutrient conditions, can often result in an extended lag phase; moreover, certain product ingredients can have an impact on microbial growth kinetics: by testing too early there may be insufficient bacterial cells for a Bcc contaminant to be detected.[1]
- The ability of the USP test to detect Bcc in the presence of the product to be tested must be established. The incubation time for the method suitability should not exceed the shortest incubation period specified.[8]

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place.
Selective supplement and ready-to-use plates
Sore the products in the original package at +2°C /+8°C away from direct light.

REFERENCES

1. Sandle T. Burkholderia cepacia complex: Review of origins, risks and methodologies. 2018. www.europeanpharmaceuticalreview.com/article/80557/burkholderia-cepaciacomplex-review-of-origins-risks-and-test-methodologies/
2. Cundell T. Excluding Burkholderia cepacia complex from Aqueous, Non-Sterile Drug Products. 2019 https://www.americanpharmaceuticalreview.com/Featured-Articles/358427-Excluding-i-Burkholderia-cepacia-i-complex-from-Aqueous-Non-Sterile-Drug-Products/
3. Reik R, Spilker T, LiPuma JJ. Distribution of Burkholderia cepacia complex species among isolates recovered from persons with or without cystic fibrosis. J Clin Microbiol 2005; 43:2926–2928.
4. Sutton S, Jimenez L. A Review of Reported Recalls Involving Microbiological Control 2004-2011 with Emphasis on FDA Considerations of "Objectionable Organisms" Posted January 1 2012 Amer. Pharm. Rev.
5. Abdallah M, Abdallah HA, Memish ZA. Burkholderia cepacia complex outbreaks amongst non-cystic fibrosis patients in the intensive care units: a review of adult and pediatric literature. Infez Med. 2018 Dec 1;26(4):299-307.
6. Marquez L, Jones KN, Whaley EM, et al. An outbreak of Burkholderia cepacia Complex infections associated with contaminated liquid Docusate. Infect. Control & Hosp. Epidemiol. 2017; 38(5): 567-573.
7. U.S. Food and Drug Administration, U.S. Department of Health and Human Services. FDA advises drug manufacturers that Burkholderia cepacia complex poses a contamination risk in non-sterile, water-based drug products, 2017. https://www.fda.gov/Drugs/DrugSafety/ucm559508.htm
8. USP <60> Microbiological Examination of Non-sterile Products: Tests for Burkholderia cepacia complex. December 1, 2019.
9. Henry DA, Campbell ME, JJ, Speert DP. Identification of Burkholderia cepacia isolates from patients with cystic fibrosis and use of a simple new selective medium. J Clin Microbiol 1997; 35:614–619.
10. Henry DA, Campbell ME, LiPuma JJ, McGimpsey C et al. Comparison of isolation media for recovery of Burkholderia cepacia complex from respiratory secretions of patients with cystic fibrosis. J Clin Microbiol 1999; 37(4):1004-1007.
11. Wright RM, Moore JE, Shaw A, et al. Improved cultural detection of Burkholderia cepacia from sputum in patients with cystic fibrosis. J Clin Pathol 2001;54:803–805.
12. AMCLI-SIFC Raccomandazioni per l'esecuzione delle indagini microbiologiche di campioni delle vie respiratorie di pazienti con fibrosi cistica. 2010.
13. Public Health England. (2019). Investigation of bronchoalveolar lavage, sputum and associated specimens. UK Standards for Microbiology Investigations. B 57 Issue 3.5. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-andconsistency-in-clinical-laboratories.
14. Plongla R, Preece CL, Perry JD, Gilligan P. Evaluation of RGM Medium for Isolation of Nontuberculous Mycobacteria from Respiratory Samples from Patients with Cystic Fibrosis in the United States. J Clin Microbiol 2017; 55(5):1469-1477.
15. Esther CR Jr, Hoberman S, Fine J, Allen S, et al. Detection of rapidly-growing mycobacteria in routine cultures of samples from patients with cystic fibrosis. J Clin Microbiol 2011; 49:1421–1425.

PACKAGING

Product	Type	REF	Pack
BCSA Burkholderia Cepacia Selective Agar Base	Dehydrated medium	4011532	500 g (10.4 L)
BCSA Burkholderia Cepacia Selective Agar Base	Dehydrated medium	4011534	5 kg (104 L)
BCSA Selective Supplement	Lyophilized supplement	4240073	10 vials, each for 500 mL of medium
BCSA Burkholderia Cepacia Selective Agar	Ready-to-use plates	541153	2 x 10 plates ø 90 mm

IFU rev 3, 2022/02.

BILE AESCULIN AGAR

Dehydrated culture medium

Bile Aesculin Agar: colonies of *Enterococcus faecalis*

INTENDED USE

Differential medium for the confirmation test of enterococci colonies isolated from food and water.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	3.0 g
Peptone	5.0 g
Oxgall	40.0 g
Ferric citrate	0.5 g
Aesculin	1.0 g
Agar	14.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In 1924, Rochaix[1] applied aesculin test for the differentiation of enterococci from other streptococci. Utilizing the esculin base, Meyer and Schonfeld[2], in 1926 then added bile: in their study they found that enterococci were able to grow and split esculin, whereas other streptococci could not. Swan[3] in his study used the esculin medium containing 4% bile salt.
Bile Aesculin Agar, prepared according to the formulation of Swan[3] and recommended by APHA[4] and MSDA[5], is used for the confirmation test of enterococci colonies isolated from water or food samples based on their ability to grow and hydrolyse the glycoside esculin in the presence of bile.[6] The use of this medium in conjunction with triple sugar iron agar and lysine iron agar has been proposed for differentiating the *Klebsiella-Enterobacter-Serratia* group from other *Enterobacteriaceae*.[7]

Peptone and beef extract provide nitrogen, carbon, amino acids and trace elements for microbial growth; bile salts are selective agents that limit the growth of Gram-positive bacteria other than enterococci; esculin is hydrolysed by this group of bacteria to glucose and aesculetin (6-7dihydroxycoumarin): aesculetin reacts with ferric citrate to form a dark brown or black complex.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 64 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation, cool to 47-50°C and distribute into sterile Petri dishes. Do not overheat.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	dark amber, slight opalescent
Final pH at 20-25 °C	6.4 ± 0.2

SPECIMENS

The sample consists of bacterial colonies isolated from food or water samples by the usual techniques, grown on selective media for enterococci.

TEST PROCEDURE, READING AND INTERPRETATION

Pick suspect colonies grown on enterococcal selective medium and perform a four-quadrant streak onto a Bile Aesculin Agar plate.
Incubate for 18-24 hours at 37°C and observe for the development of grey colonies surrounded by a brown halo (esculin hydrolysis positive). Re-incubate negative plates for additional 18-24 hours.
If the confirmation test is performed on colonies grown on filter membrane on enterococcal selective medium, transfer the membrane to Bile Aesculin Agar plate, incubate for 4 hours at 37°C and observe for the formation of a brown halo around the colonies.
The confirmation test can be completed by the catalase test by pouring a few drops of H_2O_2 over the colonies. Enterococci are aesculinase positive and catalase negative.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 29212	37°C /18-24H-A	good growth, grey colonies surrounded by a brown halo
E. faecium ATCC 19434	37°C /18-24H-A	good growth, grey colonies surrounded by a brown halo

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Because strains of *Lactococcus*, *Leuconostoc*, *Pediococcus* with phenotypic similarities have been isolated from human infections, the presumptive identification of enterococci based only on Bile Esculin test and growth in 6.5% NaCl broth can be erroneous.[8]
- Bile Esculin test was originally used to identify the enterococci. However, other Group D Streptococci and occasionally non-Group D streptococci and other genera, *Aerococcus* and *Listeria* can tolerate the bile concentration and split aesculin. [6]
- Some *viridans* species (*S. sanguis*, *S. mutans* and *S. anginosus)* are capable of splitting aesculin but cannot usually tolerate an increased concentration of 4% bile and hydrolyse aesculin in combination.[6]
- In the confirmation test performed with filter membrane transfer, an uneven distribution of bacterial colonies or the presence of high microbial loads may interfere with the differentiation of positive colonies due to the spread of colour to adjacent colonies.
- It is advisable not to extend the incubation beyond 24 hours because the extensive blackening of the medium hinders reading.

STORAGE CONDITIONS

Sore at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Rochaix, A. Milieux a l'esculine pour le diagnostic differential des bacteries du groups strepto-enteropneumocoque. C R Soc Biol. 1924; 90:771-772.
2. Meyer K, Schonfeld H. Uber die Untersheidung des Enterococcus vom Streptococcus viridans and die Beziehunger beider zum Streptococcus lactis. Zentralbl Bakteriol Parasitenkd Infectionskr Hyg Abt Orig. 1926; 99:402-416.
3. Swan A. The use of a bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J Clin Pathol 1954; 7:160-163.
4. APHA Standard Methods for the Examination of Water and Wastewater. 23th ed. American Public Health Association, Washington, D.C. 2017.
5. Manuel Suisse des Denrées Alimantaires (MSDA). Chapitre 56, Microbiologie. Juillet 2000
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Lindel SS, Quin P. Use of Bile-Esculin Agar for Rapid Differentiation of Enterobacteriaceae J Clin Microbiol.1975; 1(5): 440-443
8. Teixeira LM et al. *Enterococcus.* In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

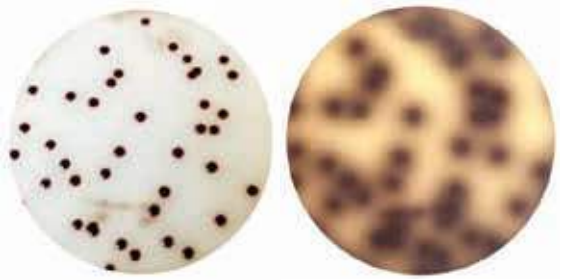
PACKAGING

Product	Type	REF	Pack
Bile Aesculin Agar	Dehydrated medium	4010172	500 g (7.8 L)

IFU rev. 2, 2022/07

BILE AESCULIN AZIDE AGAR ISO FORMULATION

Dehydrated and ready-to-use culture medium



E. faecalis grown on Slanetz Bartley Agar (at left), transferred to BEEA-ISO and incubated for 2 hours (at right).

INTENDED USE

Selective and differential medium for the confirmation test of enterococci colonies according to ISO 7899-2.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	17.00 g
Peptone	3.00 g
Yeast extract	5.00 g
Oxgall	10.00 g
Sodium chloride	5.00 g
Aesculin	1.00 g
Ferric ammonium citrate	0.50 g
Sodium azide	0.15 g
Agar	13.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In 1924, Rochaix[1] applied aesculin test for the differentiation of enterococci from other streptococci. Utilizing the esculin base, Meyer and Schonfeld[2], in 1926 then added bile: in their study they found that enterococci were able to grow and split esculin, whereas other streptococci could not. Isenberg, Goldberg and Sampson reduced the concentration of bile and added sodium azide.[3] Bile Aesculin Azide Agar ISO Formulation meets the requirements described by ISO 7899-2 for the confirmation test of enterococci colonies isolated from water, based on their ability to grow and hydrolyse the glycoside aesculin.[4] The method described in ISO 7899-2 involves enumeration of intestinal enterococci with membrane filters on Slanetz Bartley Agar medium, followed by confirmation on Bile Aesculin Azide Agar. The method described by APHA for water samples requires Azide Dextrose Broth for MPN technique followed by a confirmatory streaking on Bile Aesculin Azide Agar.[5]
Tryptone, peptone and yeast extract provide nitrogen, carbon, amino acids, group B vitamins and trace elements for microbial growth; bile salts are selective agents that limit the growth of Gram-positive bacteria other than enterococci while sodium azide inhibits the growth of Gram-negative bacteria; esculin is hydrolysed by enterococci to glucose and aesculetin (6-7dihydroxycoumarin): aesculetin reacts with ferric ammonium citrate to form a dark brown or black complex.[6] Compared to Isenberg's classic formulation, the ISO medium contains less sodium azide and no sodium citrate.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 54.7 g of in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121° C for 15 minutes. Do not exceed sterilisation time and temperature. Cool to approximately 47-50 °C and transfer into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	tan with trace blue cast, limpid
Final pH at 20-25 °C	7.1 ± 0.1

SPECIMENS

The methods of analysis described here is suitable for the examination of drinking water, water from swimming pools and other clean or disinfected water. MF method can be applied to all types of water with the exception of water with a high amount of suspended matter or considerable load of interfering microorganisms.[4] MPN method is recommended for drinking water, source water, and both fresh and marine recreational waters.[5] The application of the MF method appears particularly appropriate for the analysis of large quantities of water containing low number of intestinal enterococci. Refer to the cited Standards[4,5] for operational sampling details.

TEST PROCEDURE

Membrane filter method[4]

1. Filter a suitable volume of sample through a 0.45 µm membrane filter.
2. Place the membrane on a Slanetz Bartley Agar plate (REF 402046) and incubate at 36 ± 2°C for 44 ± 4 hours.
3. After incubation, consider as typical all colonies that show red, brown or pink colour.
4. If typical colonies are observed, transfer the membrane to the surface of a Bile Aesculin Azide Agar ISO Formulation plate and incubate at 44 ± 0.5°C for 2 hours.

MPN technique[5]

1. Inoculate a series of tubes of Azide Dextrose Broth (REF 401105) with appropriate graduated quantities of a 100 mL sample. Use sample volumes of 10 mL or less. The strength of the broth will be proportional to the sample size.
2. Incubate at 35 ± 0.5°C for 24 ± 2 hours and observe for microbial growth (turbidity of broth); if no turbidity is observed, continue incubation for a further 24 hours.
3. Streak a portion of growth from each positive tube on Bile Aesculin Azide Agar ISO Form. and incubate at 35°C°C for 24 ± 2 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth, recording each specific morphological and colour characteristic of the colonies.
Count as intestinal enterococci all colonies with a brown to black halo that had previously grown red-brown or pink on Slanetz Bartley Agar or showing turbidity in Azide Dextrose Broth.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 29212	44°C /2 H-A	good growth, colonies surrounded by a black halo
E. faecium ATCC 27270	44°C /2 H-A	good growth, colonies surrounded by a black halo

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- ISO 7899 describes a method for the isolation and enumeration of intestinal enterococci, mainly belonging to the species *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae*. In addition, other species referable to the genus *Enterococcus* and some species referable to the genus *Streptococcus* (i.e., *S. bovis* and *S. equinus*) may occasionally be detected. These *Streptococcus* species do not survive long in water and it is likely that a quantitative assessment is not possible. For the purposes of water testing, enterococci may be considered as indicators of faecal pollution. However, it should be noted that some enterococci found in water may also occasionally originate from different habitats.[4]
- *S. bovis* and *S. equinus* can be differentiated by further tests: growth at 45°C in BHI Broth, absence of growth in BHI Broth with 6.5% NaCl.[5]
- In the confirmation test performed with filter membrane transfer, an uneven distribution of bacterial colonies or the presence of high microbial loads may interfere with the differentiation of positive colonies due to the spread of colour to adjacent colonies.

STORAGE CONDITIONS

Dehydrated medium

Sore at +10°C /+30°C away from direct light in a dry place.

According to ISO 7889-2, prepared plates can be stored in the dark and protected against evaporation for up to 2 weeks at 5 °C ± 3 °C.

Ready-to-use plates

Sore plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Rochaix, A. Milieux a l'esculine pour le diagnostic differential des bacteries du groups strepto-enteropneumocoque. C R Soc Biol. 1924; 90:771-772.
2. Meyer K, Schonfeld H. Uber die Untersheidung des Enterococcus vom Streptococcus viridans and die Beziehunger beider zum Streptococcus lactis. Zentralbl Bakteriol Parasitenkd Infectionskr Hyg Abt Orig. 1926; 99:402-416.
3. Isenberg HFD, Goldberg D, Sampson J. Laboratory studies with a selective Enterococcus medium. Appl Microbiol.1970 Sep;20(3):433-6.
4. ISO 7899-2:2000 Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method
5. APHA Standard Methods for the Examination of Water and Wastewater 23rd ed. Washington, DC: American Public Health Association, 2017.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Bile Aesculin Azide Agar ISO Formulation	Dehydrated medium	4010182	500 g (9 L)
Bile Aesculin Azide Agar ISO Formulation	Ready-to-use plates	541018	2 x 10 plates ø 90 mm
Bile Aesculin Azide Agar ISO Formulation	Ready-to-use plates	491018	3 x 10 plates ø 55 mm

IFU rev 1, 2022/05

BISMUTH SULPHITE AGAR

Dehydrated culture medium

INTENDED USE

In vitro diagnostic. Highly selective medium for the isolation of micro-organisms belonging to the genus *Salmonella*, especially *Salmonella enterica* subsp. *enterica* serovar Typhi, from clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef Extract	5.000 g
Tryptone	5.000 g
Peptone	5.000 g
D-glucose	5.000 g
Disodium hydrogen phosphate	4.000 g
Bismuth sulphite indicator	8.000 g
Ferrous sulphate	0.300 g
Brilliant green	0.025 g
Agar	20.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Bismuth Sulphite Agar is a modification of the original formulation devised by Wilson and Blair.[1,2] It is intended for the detection and isolation of *Salmonella enterica* serovar Typhi and other salmonellae from clinical specimens,[3-4] foods,[5,6] water[7] and other samples suspected of containing these pathogens. The medium has a strong inhibitory action and is suitable for heavily contaminated samples. The use of this medium may be particularly useful when lactose fermenting strains of salmonellae are sought.

The freshly precipitated bismuth sulphite in the medium by the heat action, is responsible of the inhibitory properties against coliform bacteria, in the presence of glucose and of a certain excess of sodium sulphite[2]. The selective action of the medium toward Gram-positive organisms is enhanced by the presence of brilliant green.[2] Ferrous sulphate is an indicator of hydrogen sulphide production. The reduction of bismuth sulphite, in the acidic environment created by the fermentation of glucose, results in the production of hydrogen sulphide which, on reacting with the iron salt, precipitates as iron sulphide. This reaction causes a black colony and a brown or black precipitate, while the reduction of bismuth ions to metallic bismuth produces a metallic sheen around the colonies. Blackening of the colony and the formation of the metallic sheen do not occur if the colonies are too small (in the area of the plate where growth is very compact) and if the medium becomes too acidic.[3] Peptones are a source of nitrogen, carbon, vitamins and minerals for bacterial growth; glucose is a source of energy; disodium hydrogen phosphate limits the excess acidity formed during colony development; agar is the solidifying agent.

Directions for medium preparation

Suspend 52 g in 1000 mL of cold purified water and mix thoroughly. Heat with frequent agitation until boiling and continue to boil for 30 to 60 seconds to dissolve the agar and obtain a uniform suspension (precipitate will not dissolve). Cool to 50-55°C, mix gently to disperse the precipitate evenly and pour into sterile Petri dishes (25 mL medium per plate). Allow the medium to solidify with the dish uncovered. Do not overheat, do not autoclave, do not re-dissolve the medium after preparation. Use the plates no later than 48 hours after preparation, storing them in the dark at room temperature and avoiding excessive drying.

Physical characteristics

Dehydrated medium appearance	pale green, fine, homogeneous, free-flowing powder
Prepared plates appearance	pale green to pale straw, with a smooth cream-like opacity.
Final pH at 20-25 °C	7.7 ± 0.2

Specimens

Bismuth Sulphite Agar is intended for the bacteriological processing of clinical specimens such as faeces, rectal swabs, urines and non-clinical samples such as foods and water. The medium can be inoculated with faeces suspended in saline or other liquid transport medium or with the faecal sample enriched in an appropriate selective broth. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[8] For the preparation of non-clinical samples consult the appropriate references.[5,6,7]

Test procedure

Clinical specimens

Inoculate the sample on the surface of the medium. Streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic condition at 35-37°C for 48 hours at 35-37°C. Examine after 24 hours for typical colonies. If plates show little or no growth after 24 hours, incubate an additional 18-24 hours.

Summary of method reported for foods by ISO 6579-1, Annex D

Non-selective pre-enrichment in Buffered Peptone Water, incubation at 34-38 °C for 18 hours.

Additional selective enrichment, in addition to MKTTn and RVS, in Selenite Cystine Broth with incubation at 37°C for 24 and 48h.

Sub-culture on Bismuth Sulphite Agar and XDL Agar plates, incubated at 37°C for 24-48 hours.

Observation of the plates at 24 and 48 hours to detect the presence of typical colonies for confirmation testing.

For a detailed discussion of the method please refer to the current standard.[5]

Reading and interpretation

After incubation, observe the bacterial growth and record each specific morphological and chromatic characteristic of the colonies.

The main characteristics of the colonies on Bismuth Sulphite Agar are given below.

Salmonella Typhi: "rabbit-eye" colonies, round, flat, black, at 18 hours surrounded by a brownish or black zone with or without metallic sheen; after 48 hours the colonies are uniformly black with a marked brown-black halo.

Salmonella Paratyphi A and other salmonellae: variable colony morphology after 18 hours: they may be black, green or clear and mucoid. Uniformly black colonies are observed after 48 hours, often with diffuse staining of the medium and a pronounced metallic sheen.

Shigella spp.: inhibited but some strains (*S. flexneri* and *S. sonnei*) may grow as brownish-green colonies.

Other organisms such as coliforms, *Serratia*, *Proteus*: usually inhibited, but occasional strains give green or brown opaque colonies without metallic sheen or staining of the surrounding medium.

Although *S*. Typhi can grow within 24 hours, the final reading of the colony characteristics must be made after 48 hours of incubation.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
S. Typhimurium ATCC 14028	35-37°C / 24-48 H/ A	brown/ grey/ black colonies, with or without metallic reflections at 24 hours
S. Enteritidis ATCC13076	35-37°C / 24-48 H/ A	brown/ green/ black colonies, with or without metallic reflections at 24 hours
E. coli ATCC 25922	35-37°C / 48 H/ A	partially inhibited, green and brown colonies without metallic sheen
E. faecalis ATCC 29212	35-37°C / 48 H/ A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Keep the bottle of powdered medium tightly closed in the dark as the powder tends to deteriorate rapidly when exposed to the atmosphere; deterioration is indicated by the formation of non-friable aggregates and a brownish colour of the powder; once dissolved in water the deteriorated powder forms a brown solution instead of pale green and loses its differential and selectivity characteristics.
- Prolonged heating of the medium during preparation decreases its selectivity.
- Bismuth Sulphite Agar in plates should not be stored for longer than 2 days. After 3 days of storage the medium changes to a green colour with reduction of selectivity, resulting in smaller number of *Salmonella* recovered. Preferably, medium should be used on day prepared and not stored.[3,5-7]
- It is imperative to streak for well isolated colonies; in heavy growth areas *S*. Typhi appears light green and hence would be interpreted as negative for *S*. Typhi growth.[3]
- Atypical colonies may develop if medium is heavy inoculated with organic materials; to prevent this, suspend faecal specimen in sterile saline, centrifuge and use supernatant for inoculation.[3]
- Colonies on Bismuth Sulphite Agar may be contaminated with other viable organisms, and isolated colonies should be subcultured to less selective medium (e.g., MacConkey agar).[3]
- Do not autoclave; heating for period longer than necessary just to dissolve ingredients destroys its selectivity.[3]
- Bismuth Sulphite Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms but should be used in conjunction with other less selective enteric agars (XLD Agar, Brilliant Green Agar, SS Agar, Hektoen Enteric Agar).
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Sore at +2°C /+8°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The medium should be prepared fresh, just prior to use or stored at room temperature for not more than 2 days. The user is responsible for the manufacturing and quality control processes.

REFERENCES

1. Wilson JW, Blair EM. Use of a glucose bismuth sulphite iron medium for the isolation of B.typhosus and B.proteus.1927; J Hyg 26:374
2. Wilson JW, Blair EM. Further experience of the bismuth sulphite media in the Isolation of Bacillus typhosus and B. paratyphosus B from faeces, sewage and water.1931; J Hyg 31:138
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
5. ISO 6579:2017/ ISO ISO 6579:2017 Amd1:2020. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Content current as of: 03/18/2022
7. ISO 19250:2010 Water quality — Detection of Salmonella spp
8. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

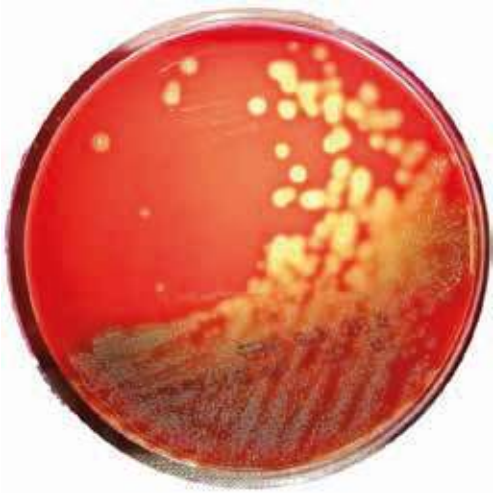
PACKAGING

Product	Type	REF	Pack
Bismuth Sulphite Agar	Dehydrated medium	40121022	500 g (9.6 L)

IFU rev 1, 2022/04

BLOOD AGAR BASE

Dehydrated culture medium



Group A β-haemolytic Streptococcus on Blood Agar Base supplemented with sheep blood.

INTENDED USE

In vitro diagnostic. Non selective, general-purpose medium to be used non-supplemented or supplemented with defibrinated animal blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of their haemolytic properties.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	10 g
Tryptose	10 g
Sodium chloride	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Blood Agar Base is a general-purpose medium that can be used non-supplemented or with the addition of various enrichments such as blood, serum, carbohydrates.
Beef extract and tryptose are a source of carbon, nitrogen and trace elements are necessary for microbial growth; sodium chloride contributes to the osmotic balance of the medium.
Without additions, the medium can be used as a rich nutrient agar or as a medium for short-term maintenance of microbial cultures.
With the addition of serum or other enrichments, Blood Agar base becomes suitable for the cultivation of fastidious microorganisms such as streptococci, pneumococci, meningococci, *Haemophilus*.
Supplemented with sterile defibrinated sheep or horse blood, the medium is intended for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of haemolytic properties of streptococci, staphylococci and other microorganisms. With the addition of sheep blood, the medium is particularly suitable for the isolation of *Streptococcus pyogenes*.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and aseptically add 5-7% of sterile defibrinated sheep or horse blood. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Blood agar plates appearance	deep red, opaque
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Blood Agar Base supplemented with sheep blood and poured in plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimen types, related to specific infections.[1-3] Blood Agar plates are not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible.
Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[1]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5-10% CO_2, and record the results after 18-24, 48 and, if necessary, 72 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
By cultivation on sheep blood agar plates prepared with Blood Agar Base, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α-prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

Here below are summarized the colonies characteristics of some microorganisms which can be isolated on blood agar sheep plates.[4]

- The colonies of Group A streptococci are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of group B streptococci are surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or sub-surface β-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of group A colonies to be of any value in identification.
- Group D streptococcal colonies are non-haemolytic.
- Pneumococcal colonies, when the culture has been incubated in CO_2 incubators, are surrounded by a fairly large zone of α-haemolysis.
- The viridans streptococcal colonies may be surrounded by a small zone of α-haemolysis or have no zone of haemolysis; rarely they show an α-prime haemolysis.
- Staphylococci colonies are yellow or white with or without the β-haemolysis zone.
- *Listeria monocytogenes* colonies are surrounded by a small β-haemolytic zone.

Once colonies have grown on blood agar plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	good growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	good growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A or CO_2	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Depending on the specimens analysed and the microorganisms being tested for, it is recommended for the examination of clinical specimens to use also additional media such us selective media and Chocolate Agar.
- The growth and type of haemolysis depend on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood[5]; *Neisseria, Mycobacterium, Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- The haemolytic reactions of some strains of group D streptococci are influenced by the type of blood used: they are beta-hemolytic with horse, human and rabbit blood and alpha-haemolytic with sheep blood.
- The incubation atmosphere influences the haemolytic reactions of beta-haemolytic streptococci: for optimal performance, incubate the plates in aerobic conditions with 5-10% CO_2 or in anaerobic conditions.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Sore at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
2. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
3. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
4. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington, DC: American Society for Microbiology; 1991.

5. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

PACKAGING

Product	Type	REF	Pack
Blood Agar Base	Dehydrated medium	4011552 4011554	500 g (12.5 L) 5 kg (125 L)

IFU rev 2, 2022/03

BLOOD AGAR BASE N° 2

Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. Improved blood agar base to be used with defibrinated animal blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of their haemolytic properties.

COMPOSITION *

BLOOD AGAR BASE N° 2, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	15.0 g
Liver extract	2.5 g
Yeast extract	5.0 g
Sodium chloride	5.0 g
Agar	13.0 g

BLOOD AGAR SHEEP ° 2, READY-TO-USE PLATES
TYPICAL FORMULA

Blood Agar Base n° 2	40.5 g
Defibrinated sheep blood	50 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Blood Agar Base N° 2 is a general-purpose medium with richer nutritive properties than other blood agar base media and with special capacity to promote the pigment production by bacteria; it can be used with the addition of various enrichments such as blood, serum, carbohydrates for the cultivation of fastidious microorganisms.
Peptone, liver extract and yeast extract are sources of carbon, nitrogen, vitamins and trace elements for microbial growth; sodium chloride contributes to the osmotic balance of the medium.
With the addition of serum or other enrichments, Blood Agar Base n° 2 becomes suitable for the cultivation of fastidious microorganisms such as streptococci, pneumococci, meningococci, *Haemophilus*.
Supplemented with sterile defibrinated sheep or horse blood, the medium is intended for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of haemolytic properties of streptococci, staphylococci and other microorganisms.
Blood Agar Base n° 2 conforms to the formulation reported by FDA-BAM.[1] The formulations according to ISO standard 7932[2] and 11290[3] differ for their final pH values (ISO 7032: 7.0 ± 0.2; ISO 11290: 7.2 ± 0.2).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C, and add 5-7% of sterile defibrinated sheep or horse blood. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Blood agar plates appearance	deep red, opaque
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Blood Agar Base n° 2 supplemented with sheep blood and poured in plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimen types, related to specific infections.[4-6] Blood Agar plates are not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible.
Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[4]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5-10% CO_2, and record the results after 18-24, 48 and, if necessary, 72 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
By cultivation on sheep blood agar plates prepared with Blood Agar Base N°2, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α-prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

Here below are summarized the colonies characteristics of some microorganisms which can be isolated on blood agar sheep plates.[7]

- The colonies of Group A streptococci are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of group B streptococci are surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or subsurface β-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of group A colonies to be of any value in identification.
- Group D streptococcal colonies are non-haemolytic.
- Pneumococcal colonies, when the culture has been incubated in CO_2 incubators, are surrounded by a fairly large zone of α-haemolysis.
- The viridans streptococcal colonies may be surrounded by a small zone of α-haemolysis or have no zone of haemolysis; rarely they show an α-prime haemolysis.
- Staphylococci colonies are yellow or white with or without the β-haemolysis zone.
- *Listeria monocytogenes* colonies are surrounded by a small β-haemolytic zone.

Once colonies have grown on blood agar plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	good growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	good growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A or CO_2	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Depending on the specimens analysed and the microorganisms being tested for, it is recommended for the examination of clinical specimens to use also additional media such us selective media and Chocolate Agar.
- The growth and type of haemolysis depend on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood[8]; *Neisseria, Mycobacterium, Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- The haemolytic reactions of some strains of group D streptococci are influenced by the type of blood used: they are beta-hemolytic with horse, human and rabbit blood and alpha-haemolytic with sheep blood.
- The incubation atmosphere influences the haemolytic reactions of beta-haemolytic streptococci: for optimal performance, incubate the plates in aerobic conditions with 5-10% CO_2 or in anaerobic conditions.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Sore at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), M22 Blood Agar Base #2
2. ISO 7932:2004. Microbiology of food and animal feeding stuffs. Horizontal Methods for the enumeration of presumptive *Bacillus cereus.* Colony count technique at 30°C.
3. ISO 11290:2107. Microbiology of food and animal feeding stuffs. Horizontal Methods for the detection and enumeration of Listeria monocytogenes and of Listeria spp
4. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
5. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
6. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
7. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington,DC: American Society for Microbiology; 1991.
8. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

PACKAGING

Product	Type	REF	Pack
Blood Agar Base N° 2	Dehydrated medium	4011562 4011564	500 g (12,3 L) 5 kg (123 L)
Blood Agar Sheep N° 2	Ready-to-use plates	541156	2 x 10 plates ø 90 mm

IFU rev 2, 2022/02

BLOOD AGAR BASE N° 2 pH 7.2

Dehydrated and ready-to-use culture medium

INTENDED USE

Improved blood agar base to be used with defibrinated animal blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms and for the determination of their haemolytic properties.

COMPOSITION *

BLOOD AGAR BASE N° 2 PH 7.2, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of animal tissues	15.0 g
Liver extract	2.5 g
Yeast extract	5.0 g
Sodium chloride	5.0 g
Agar	13.0 g

BLOOD AGAR SHEEP ISO FORMULATION
READY-TO-USE PLATES, TYPICAL FORMULA

Blood Agar Base n° 2	40.5 g
Defibrinated sheep blood	50 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Blood Agar Base N° 2 pH 7.2 is a general-purpose medium with richer nutritive properties than other blood agar base media and with special capacity to promote the pigment production by bacteria; it can be used with the addition of various enrichments such as blood, serum, carbohydrates for the cultivation of fastidious microorganisms.
Blood Agar Base N° 2 pH 7.2 conforms to the formulation reported by ISO standard 7932[1] and 11290[2] and differs from the standard formulation (REF 401156) in the final pH value (7.2 ± 0.2).
Enzymatic digest of animal tissues, liver extract and yeast extract are sources of carbon, nitrogen, vitamins and trace elements for microbial growth; sodium chloride contributes to the osmotic balance of the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C, and add 5-7% of sterile defibrinated sheep or horse blood. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Blood agar plates appearance	deep red, opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

For confirmatory tests according to ISO Standards, Blood Agar Base n° 2 pH 7.2 may be inoculated with pure culture of strains isolated on selective media.[1,2] For general use consult the appropriate references.[3]

TEST PROCEDURE

General use

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5-10% CO_2, and record the results after 18-24, 48 and, if necessary, 72 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

Confirmatory tests for *Bacillus cereus* group [1]

Streak, stab or spot the selected colonies onto the surface of Blood Agar n° 2 pH 7.2 supplemented with sheep blood.
Incubate at 30°C for 24 ± 2 hours and interpret the haemolysis reaction.

Confirmatory tests for *Listeria monocytogenes* [2]

Stab the selected colonies onto the surface of pre-dried plates of a Blood Agar n° 2 pH 7.2 supplemented with sheep blood.
Incubate at 37°C for 18 h to 24 h or until growth is satisfactory and interpret the haemolysis reaction.
If the result of the haemolysis test is difficult to interpret, the CAMP test on the same medium is recommended to demonstrate clearly that haemolysis is due to listeriolysin activity.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
By cultivation on sheep blood agar plates prepared with Blood Agar Base N°2 pH 7.2, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α-prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 13932	35-37°C / 18-24H / A	good growth, beta haemolysis
L. innocua ATCC 33090	35-37°C / 18-24H / A	good growth, beta haemolysis
B. cereus ATCC11778	35-37°C / 18-24H / A	good growth, beta haemolysis

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- The growth and type of haemolysis depend on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood.[4] *Neisseria, Mycobacterium, Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- The haemolytic reactions of some strains of group D streptococci are influenced by the type of blood used: they are beta-haemolytic with horse, human and rabbit blood and alpha-haemolytic with sheep blood.
- The incubation atmosphere influences the haemolytic reactions of beta-haemolytic streptococci: for optimal performance, incubate the plates in aerobic conditions with 5-10% CO_2 or in anaerobic conditions.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

Storage conditions

Sore at +10°C /+30°C away from direct light in a dry place.

References

1. ISO 7932:2004. Microbiology of food and animal feeding stuffs. Horizontal Methods for the enumeration of presumptive *Bacillus cereus.* Colony count technique at 30°C.
2. ISO 11290:2107. Microbiology of food and animal feeding stuffs. Horizontal Methods for the detection and enumeration of Listeria monocytogenes and of Listeria spp
3. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
4. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

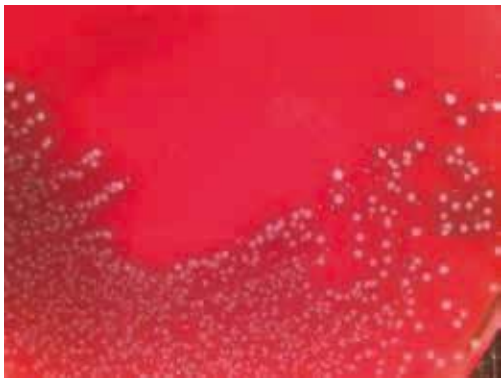
Packaging

Product	Type	REF	Pack
Blood Agar Base N° 2 pH 7.2	Dehydrated medium	401156P2	500 g (12,3 L)
Blood Agar Sheep ISO formulation	Ready-to-use plates	541156P	2 x 10 plates ø 90 mm

IFU rev 0, 2023/02

BLOOD AGAR HORSE

Ready-to-use plates



Blood Agar Horse:
Group A β-haemolytic *Streptococcus*

Intended use

In vitro diagnostic device. General purpose medium with defibrinated horse blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for determination of haemolytic properties.

Composition - typical formula *

Pancreatic digest of casein	15.0 g
Soy peptone	5.0 g
Sodium chloride	5.0 g
Agar	13.5 g
Growth factors	1.5 g
Defibrinated horse blood	50.0 mL
Purified water	1000.0 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

The history of blood agar is uncertain. The inclusion of blood as a nutritive supplement in culture media may pre-date the use of agar[1]; in their 1903 Manual of Bacteriology, Muir and Ritchie[2] list its inclusion before they discuss “agar-agar” as a replacement for gelatine as a solidifying agent.[2]
The term "blood agar", as we know it today, generally refers to an enriched base medium to which defibrinated mammalian blood has been added.
Biolife Blood Agar Horse is prepared from Tryptic Soy Blood Agar Base with 5% defibrinated horse blood.
Blood Agar Horse is a general purpose, enriched medium used to grow fastidious and non-fastidious organisms and to differentiate bacteria based on their haemolytic properties.
Horse blood provides X (hemin) and V (NAD) factors required for the growth of some fastidious microorganisms including *Haemophilus influenzae.* Selected casein and soy peptones improve the bacterial haemolytic reactions and provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The inclusion of a mixture of growth factors enhances the growth of fastidious organisms. The presence of horse blood enables the determination of bacterial haemolytic properties, as a useful tool for the orientation of bacterial identification.

Physical characteristics

Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

Specimens

Blood Agar Horse plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens’ types, related to specific infections.[3-5] Blood Agar Horse is not suitable for direct inoculation of

blood samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[3]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5-10% CO_2, and record the results after 18-24, 48 and if necessary 72 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
By cultivation on Blood Agar Horse, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.

Haemolytic properties referred to blood agar with sheep blood might be different with Blood Agar Horse plates.
Blood Agar Horse provides clearer β-haemolysis of streptococci than Blood Agar Sheep.
- Group A streptococci: colonies surrounded by a well-defined zone of complete haemolysis
- Group B and C haemolytic streptococci: larger colonies (2-4 mm) surrounded by a zone of transparency (β-haemolysis)

H. haemolyticus colonies produce β-haemolysis and mimic *Streptococcus pyogenes.*
Enterococci produce β-haemolysis on horse blood and are not normally haemolytic with sheep blood.
S. aureus which is usually β-haemolytic on sheep blood, will often be non-haemolytic on horse blood.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	good growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	good growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A or CO_2	good growth
H. influenzae	ATCC	10211	35-37°C / 18-24H / A or CO_2	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional media such us selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected. *Neisseria*, *Mycobacterium*, *Bordetella, Legionella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- Even if the microbial colonies present on the plates are differentiated on the basis of their morphological, chromatic and haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Sore plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Buxton T. Blood agar plates and hemolysis protocols. ASM Science, 2005
2. Robert M, Ritchie J. 1903. Manual of Bacteriology. The MacMillan Company, London, 1903.
3. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
4. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
5. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019

PACKAGING

Product	Type	REF	Pack
Blood Agar Horse	Ready-to-use plates	541180	2 x 10 plates ø 90 mm

IFU rev 1, 2020/08

BLOOD AGAR SHEEP

Ready-to-use plates

INTENDED USE

In vitro diagnostic device. Non selective, general-purpose medium with defibrinated sheep blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for determination of haemolytic properties.

COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	15.0 g
Soy peptone	5.0 g
Sodium chloride	5.0 g
Agar	15 g
Defibrinated sheep blood	50.0 mL
Purified water	1000.0 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The history of blood agar is uncertain. The inclusion of blood as a nutritive supplement in culture media may pre-date the use of agar[1]; in their 1903 Manual of Bacteriology, Muir and Ritchie[2] list its inclusion before they discuss “agar-agar” as a replacement for gelatine as a solidifying agent.[2] The term "blood agar", as we know it today, generally refers to an enriched base medium to which defibrinated mammalian blood has been added. Biolife Blood Agar Sheep is prepared from Tryptic Soy Agar with 5% defibrinated sheep blood. Blood Agar Sheep is a general purpose, enriched medium used to grow fastidious and non-fastidious organisms and to differentiate bacteria based on their haemolytic properties. Blood Agar Sheep is prepared with selected casein and soy peptones for improving the haemolytic reactions: they provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The presence of sheep blood enables the determination of bacterial haemolytic properties, as a useful tool for the orientation of bacterial identification.
Blood Agar Sheep is useful for performing the CAMP (Christie Atkins Munch-Petersen) test for presumptive identification of *Streptococcus agalactiae* and for use with optochin and bacitracin discs for presumptive identification of group A streptococci.

PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Blood Agar Sheep plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens types, related to specific infections.[3-5] Blood Agar Sheep is not suitable for direct inoculation of blood samples Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[3]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5 -10% CO_2, and record the results after 18-24, 48 and if necessary, 72 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.
CAMP test: a known haemolytic strain of *S. aureus* (ATCC 33862) is streaked in a straight line across the centre of the plate. Test inoculum is streaked in a straight line (2-3 cm in length) perpendicular to *S. aureus* streak but without touching it. A known Group B *Streptococcus* may also be streaked similarly as a positive control. Four-five test organisms may be tested per plate. The plate is incubated at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
By cultivation on Blood Agar Sheep, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α-prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

Here below are summarized the colony characteristics of some microorganisms which can be isolated on Blood Agar Sheep plates.[6]

- The colonies of Group A streptococci are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of Group B streptococci are surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or subsurface β-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of Group A colonies to be of any value in identification.
- Group D streptococcal colonies are non-haemolytic.
- Pneumococcal colonies, when the culture has been incubated in CO_2 incubators, are surrounded by a fairly large zone of α-haemolysis.
- *Viridans* streptococcal colonies may be surrounded by a small zone of α-haemolysis or have no zone of haemolysis; rarely they show an α-prime haemolysis.
- Staphylococci colonies are yellow or white with or without the β-haemolysis zone.
- *Listeria monocytogenes* colonies are surrounded by a small β-haemolytic zone.

Once colonies have grown on Blood Agar Sheep plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.
CAMP: a positive test for CAMP factor appears as “arrowhead” haemolysis between the junction of growth of *S. aureus* and Group B *Streptococcus*. There is no enhanced or “arrowhead” haemolysis if the test isolate is not Group B Streptococcus.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[7]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	good growth, β-haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	good growth, α-haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	good growth, diffuse β-haemolysis
E. coli	ATCC	25922	35-37°C / 18-24H / A or CO_2	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional media such as selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic models other than expected. *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium[8]; *Neisseria*, *Mycobacterium*, *Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- Even if the microbial colonies present on the plates are differentiated on the basis of their morphological, chromatic and haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Sore plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Buxton T. Blood agar plates and hemolysis protocols. ASM Science, 2005
2. Robert M, Ritchie J. 1903. Manual of Bacteriology. The MacMillan Company, London, 1903.
3. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
4. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
5. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
6. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington,DC: American Society for Microbiology; 1991.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
8. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

PACKAGING

Product	Type	REF	Pack
Blood Agar Sheep	Ready-to-use plates	541151	2 x 10 plates ø 90 mm

IFU rev 1 2020/05

BOLTON BROTH: see CAMPYLOBACTER BOLTON ENRICHMENT BROTH BASE

BORDETELLA SELECTIVE AGAR

Ready-to-use plates

Bordetella pertussis on Bordetella Selective Agar

INTENDED USE

In vitro diagnostic device. Selective medium for the detection of *Bordetella pertussis* and *Bordetella parapertussis* in clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Beef extract	10.000 g
Peptone	10.000 g
Starch	10.000 g
Charcoal	4.000 g
Sodium chloride	5.000 g
Nicotinic acid	0.001 g
Agar	12.000 g
Cephalexin	0.040 g
Defibrinated horse blood	100 mL
Purified water	900 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Pertussis, commonly known as whooping cough, is a highly contagious disease that is caused by the fastidious Gram-negative coccobacillus *B. pertussis* that colonises the respiratory tract; *B. parapertussis* causes a pertussis-like infection that is less severe than that caused by *B. pertussis*. *B. pertussis* is the most fastidious and slowest growing of the *Bordetella* species; its growth is inhibited by fatty acids, metal ions, sulphides, and peroxides. Isolation of *B. pertussis* requires a medium containing charcoal that binds the toxic compounds.

Bordetella Selective Agar, known also as Regan Lowe Agar, is prepared according to the formulation described by Sutcliffe and Abbott[1] and consists of Charcoal Agar Base supplemented with defibrinated horse blood and cephalexin.

Beef extract and peptone provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance. Charcoal, along with starch, neutralizes fatty acids and peroxides, which are toxic to *Bordetella*. Horse blood and nicotinic acid are added for growth promotion. Cephalexin partially suppresses growth of normal nasopharyngeal flora, including penicillin-resistant *Haemophilus influenzae* strains, *Staphylococcus* and *Neisseria* spp.[2]

Physical characteristics

Medium appearance	black, opaque
Final pH at 20-25°C	7.4 ± 0.2

Specimens

Bordetella Selective Agar plates can be directly inoculated with perinasal swab, nasopharyngeal aspirate, nasopharyngeal swab.[3]

The swab should be inserted well into the nasopharynx, rotated several times, and left in place for 30–60 seconds.[3] Optimal recovery is achieved by obtaining samples with calcium alginate or synthetic-polyester swabs on a flexible wire or by aspiration; cotton swabs should not be used because they contain toxic substances such as fatty acids on the cotton fibers.[4] Upon collection, the specimen should be immediately inoculated on Bordetella Selective Agar at the patient's location or placed directly into suitable transport medium, such as Regan & Lowe transport medium.[5] General non-nutritive bacteriological transport media, such as Amies medium, should be used only if they contain charcoal and the storage time does not exceed 24 h.[6] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Collect specimens before antimicrobial therapy where possible. Consult appropriate references for further information.[3,4,6]

Test procedure

Allow plates to come to room temperature. The agar surface should be smooth and moist. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate at 35-37°C in a humidified environment, in aerobic conditions, for 7days, and examine daily for the appearance of colony growth, morphologically consistent with *Bordetella* spp.

Katzko et al.,[7] reported enhanced recovery of *Bordetella* spp. from nasopharyngeal swabs by extending the incubation of plated primary cultures beyond the usual seven days to a total of twelve days.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

B. pertussis grows with small, white to grey, drop-like glistening colonies.

Colonies of B. pertussis may not be visible without the aid of a microscope for 2–4 days.

To prevent overgrowth by spreading colonies or moulds, use a sterile scalpel or needle to remove the portions of the agar that contain these contaminants. Plates may be discarded as negative after 7 days of incubation.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
B. pertussis ATCC 9787	35-37°C / 44-48 H / A	good growth
S. aureus ATCC 25923	35-37°C / 44-48 H / A	inhibited
E. coli ATCC 25922	35-37°C / 44-48 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Because of the possibility of inhibition of the growth of some strains of *B. pertussis* by cephalexin, it is recommended that a plate lacking cephalexin or containing different antimicrobial agent(s) be inoculated along with the cephalexin-containing plate.[4]
- Cephalexin inhibits most nasopharyngeal normal flora; exceptions are *P. aeruginosa* and fungi.[2]
- It is best to obtain a culture from nasopharyngeal specimens collected during the first 2 weeks of cough. This is when viable bacteria are still present in the nasopharynx. After the first 2 weeks, sensitivity decreases and the risk of false-negatives increases.[3]
- The rate of culture positivity among patients with pertussis has been shown to vary markedly (e.g. 20%–83%). This wide variation undoubtedly is due to differences in patient ages, vaccination status, length of illness, antibiotic administration, organism load at the time specimens were collected, adequacy of the specimens, transport medium and culture medium used, incubation conditions and the expertise and familiarity of laboratory personnel with *B. pertussis* cultures. Notwithstanding these considerations, culture remains an important vehicle for establishing an etiologic diagnosis of pertussis.[4]
- Cultures are unlikely to be positive in adolescents and adults with more than 3 weeks of coughing.[3]
- A negative culture does not exclude pertussis.[3]
- Diagnostic sensitivity can be maximised by supplementing culture with polymerase chain reaction (PCR) methods and serology. PCR is more sensitive than culture as it does not require organisms to be viable. Serology is particularly useful in diagnosing infection in patients who have been coughing for four weeks, when both culture and PCR would be anticipated to be unhelpful.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions

Sore plates in their original pack at 2-8°C away from direct light.

References

1. Sutcliffe EM, Abbott JD. Selective medium for the isolation of Bordetella pertussis and parapertussis. BMJ 1971; ii:732-733.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

3. Public Health England- UK Standards for microbiology investigations (UK SMI): Investigation of whooping cough. B6, n° 9, 2018
4. Gary V. Doern. Detection of Selected Fastidious Bacteria. Clinical Infectious Diseases 2000; 30:166–73
5. Regan, J., and F. Lowe. Enrichment medium for the isolation of Bordetella. J. Clin. Microbiol. 1977; 6:303-309.
6. Müller FM, Hoppe JE, Wirsing von König CH. Laboratory diagnosis of pertussis: state of the art in 1997. J Clin Microbiol 1997;35(10):2435-43
7. Katzko G, Hofmeister M, Church D. Extended Incubation of Culture Plates Improves Recovery of Bordetella spp. J Clin Microbiol *1996*; 34:1563-1564

PACKAGING

Product	Type	REF	Pack
Bordetella Selective Agar	Ready-to-use plates	549905	2 x 10 plates ø 90 mm

IFU rev 1, 2020/08

BRAIN HEART INFUSION AGAR

Dehydrated and ready-to-use culture medium

S.aureus on Brain Heart Infusion Agar

INTENDED USE

In vitro diagnostics. General purposes medium for the cultivation and maintenance of fastidious and non-fastidious microorganisms, from a variety of clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Brain heart infusion and peptones	27.5 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	2.5 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Brain Heart Infusion (BHI) Agar is based on the formula proposed in 1919 by Edward Rosenow[1] and later modified in 1923 by Russell Haden[2]. Modern BHI Agar typically uses a dried infusion from porcine brain and heart, rather than calf brain tissue, and uses disodium phosphate as a buffer, rather than the calcium carbonate used by Rosenow and Haden.
BHI Agar is a general purpose, nutritionally rich medium for the cultivation and maintenance of a variety of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria and fungi from clinical and non-clinical specimens[3]. With the addition of antimicrobials such as penicillin and streptomycin[4] or cycloheximide and chloramphenicol[5], the medium may be used for selective isolation of pathogenic fungi as the growth of bacteria and many saprophytic fungi is inhibited or delayed. Supplemented with antimicrobials and blood, it supports the growth of the tissue phase of *Histoplasma capsulatum* and other pathogenic fungi such as *Coccidioides immitis*.[6,7]
Brain heart infusion and peptones are sources of nitrogen, carbon, vitamins and minerals for microbial growth; glucose provides an energy source, sodium chloride maintains osmotic balance, dibasic sodium phosphate is included as a buffer system. Because BHI Agar contains glucose at a concentration of 0.2%, it is not useful for bacterial haemolysis detection.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 52 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes. BHI Agar can also be distributed in tubes before sterilization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Brain Heart Infusion Agar can be used in plate for the sub-culture of colonies grown on primary isolation media, for the purification of the colonies or in tubes for the maintenance of the cultures. It can also be inoculated with a variety of clinical and non-clinical samples following the procedures described in the literature.[8,9] Good laboratory practices for collection, transport and storage of clinical specimens should be applied. Collect specimens before antimicrobial therapy where possible.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate in aerobic or anaerobic atmosphere at 35-37°C for at least 48 hours or in duplicate in air at 25 ± 2°C and 35 ± 2°C for 48 hours or more. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols. Consult the procedures outlined in the references for further information.[8,9]

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of varying morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	37°C / 24H / A	good growth
C. albicans ATCC 18805	25°C / 72H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- If BHI Agar is used for the inoculation of non-sterile clinical specimens, a selective medium should also be streaked to avoid overgrowth by contaminating organisms.
- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Sore at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the tubed and bottled medium prepared by the user can be stored at 2-8°C for 6 months.[4]

Ready-to-use plates

Sore plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Rosenow EC. Studies on elective localization. J Dent Research 1919; 1:205-49.
2. Hayden RL. Elective localization in the eye of bacteria from infected teeth. Arch Int Med1923; 32:828-49.
3. Atlas R, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.
6. Howell A. Public Health Reports 1948; 63:173-178.
7. Creitz JR, Puckett TF. A Method for Cultural Identification of Coccidioides Immitis. Amer J Clin Path 1954; 24:1318-1323.
8. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270
9. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Content current as of: 02/21/2020

PACKAGING

Product	Type	REF	Pack
Brain Heart Infusion Agar	Dehydrated medium	4012352 4012354	500 g (9,6L) 5 kg (96 L)
Brain Heart Infusion Agar	Ready-to-use plates	541235	2 x 10 plates ø 90 mm

IFU rev 4, 2022/01

BRAIN HEART INFUSION BROTH

Dehydrated and ready-to-use culture medium

Brain Heart Infusion Broth:
from left: un-inoculated tube, growth of S. *aureus*

INTENDED USE

In vitro diagnostics. General purpose liquid medium for the cultivation of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria and fungi from a variety of clinical specimens.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Dehydrated brain infusion	12.5 g
Dehydrated heart infusion	5.0 g
Enzymatic digest of animal tissues	10.0 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	2.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Brain Heart Infusion (BHI) Broth is based on the formula proposed in 1919 by Edward Rosenow[1] and later modified in 1923 by Russell Haden[2]. Modern BHI Broth typically uses a dried infusions from brain and heart, rather than calf brain tissue, and uses disodium phosphate as a buffer, rather than the calcium carbonate used by Rosenow and Haden.

BHI Broth is a general purpose, nutritionally rich medium for the cultivation and maintenance of a variety of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria, yeasts and moulds, from clinical and non-clinical specimens using suitable incubation temperatures and time[3]. BHI Broth is used for the preparation of staphylococcal broth culture for performing coagulase test.[4] BHI Broth can be used to start the culture process for urease test of *H. pylori*.[5,6]

Brain and heart infusions and peptone are sources of nitrogen, carbon, vitamins and minerals for microbial growth; glucose provides an energy source, sodium chloride maintains osmotic balance, dibasic sodium phosphate is included as a buffer system.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37 g in 1000 mL of cold purified water; heat to dissolve, distribute and sterilize by autoclaving at 121°C for 15 minutes

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	gold-yellow, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Brain Heart Infusion Broth can be used for the sub-culture of colonies grown on primary isolation media. It can also be inoculated with a variety of clinical and non-clinical samples following the procedures described in the literature.[6] Good laboratory practices for collection, transport and storage of clinical specimens should be applied. Collect specimens before antimicrobial therapy where possible.

TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube or bottle with a colony grown on a plating medium or with one or two drops of the specimen, if liquid, using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of growth is closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[7]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24H / A	good growth
E. coli ATCC 25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Sore at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the tubed and bottled medium prepared by the user can be stored at 2-8°C for 6 months.[8]

Ready-to-use medium in tubes

Sore tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Rosenow EC. Studies on elective localization. J Dent Research 1919; 1:205-49.
2. Hayden RL. Elective localization in the eye of bacteria from infected teeth. Arch Int Med1923; 32:828-49.
3. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
4. ISO 6888-1:1999 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1: Technique using Baird-Parker agar medium.
5. Public Health England- UK Standards for microbiology investigations (UK SMI) B55. Investigation of infectious causes of dyspepsia. Issue no: 7; 03.10.2019
6. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology . In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
8. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Brain Heart Infusion Broth	Dehydrated medium	4012302 4012304	500 g (13.5) 5 kg (135 L)
Brain Heart Infusion Broth	Ready-to-use tubes	551230	20 x 9 mL glass

IFU rev 5, 2022/01

BRILLIANT GREEN AGAR

Dehydrated culture medium

Brilliant Green Agar: *Salmonella* sp. colonies (red) and *E. coli* colonies (yellow)

INTENDED USE

Selective medium for the isolation and differentiation of *Salmonella* spp. other than *Salmonella* Typhi.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Agar	20.0 g
Phenol red	80.0 mg
Brilliant green	12.5 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Originally described by Kristensen *et al.*[1], Brilliant Green Agar was subsequently modified by Kauffmann[2] to obtain a highly selective plating medium for the isolation and identification of salmonellae from faeces and other pathological material, and from food and dairy products.
The presence of brilliant green may inhibit the growth of non-pathogenic enteric bacteria and *Shigella* spp., by selectively favouring *Salmonella*, except *S*. Typhi and *S*. Paratyphi.[3] For this reason, Brilliant Green Agar should be used in parallel with other enteric plating media such as MacConkey Agar, and XLD Agar.
Peptocomplex and yeast extract provide nitrogen, carbon, vitamins and minerals for microbial growth; sodium chloride maintains the osmotic equilibrium; lactose and sucrose are fermentable carbohydrates; phenol red serves as an acid-base indicator giving a yellow colour to lactose and/or sucrose fermenting bacteria while lactose non-fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation. This medium also contains brilliant green, which inhibits the growth of the majority of Gram-positive and Gram-negative bacteria, including *Salmonella* Typhi and *Shigella* species.
A modification of Brilliant Green Agar which reduces growth of contaminants such as *Citrobacter* spp., *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas* and *Proteus mirabilis* has been described by Watson[4] and Walker[5] by the addition of sulfacetamide sodium salt (1.0 mg/mL) and mandelic acid sodium salt (0.25 mg/mL).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 58 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-pink, fine, homogeneous, free-flowing powder
Prepared plates appearance	reddish-brown, limpid
Final pH at 20-25 °C	6.9 ± 0.2

SPECIMENS

Refer to applicable International Standards and regulations for the collection of food and water samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

Heavily inoculate a Brilliant Green Agar plate directly with the specimen and/or with the enriched cultures in a pre-enrichment medium such as Buffered Peptone Water, and in selective enrichment broths such as Selenite Broth, Selenite Cystine Broth, Tetrathionate Broth.
Incubate the Brilliant Green Agar plate at 35-37°C and examine for suspected colonies after 18 to 24 hours and after 42 to 48 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Salmonella spp. other than *S*. Typhi and *S*. Paratyphi A form red-pink-white opaque colonies, surrounded by a diffused red halo.
E. coli/Klebsiella/Enterobacter grow less luxuriantly and form yellow-green colonies, surrounded by a halo of the same colour.
Proteus does not swarm if dry plates are inoculated and produces yellow-pink mucoid colonies (sucrose fermentation, variable).
Shigella, is completely inhibited by brilliant green.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Enteritidis ATCC 13076	35-37°C/18-24 H/A	good growth, red colonies with red halo
S. Typhimurium ATCC 14028	35-37°C/18-24 H/A	good growth, red colonies with red halo
E. coli ATCC 25922	35-37°C/18-24 H/A	scanty growth, yellow colonies
E. faecalis ATCC 19433	35-37°C/18-24 H/A	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Colonies of *Salmonella* spp. vary from red-pink-white depending on length of incubation and strain type; however, any of these colours indicate non-lactose fermenter strain.[3]
- S. Typhi, S. Paratyphi and *Shigella* do not grow adequately on this medium.[3]

- Slow lactose fermenters, *Proteus, Citrobacter* and *Pseudomonas* may grow on Brilliant Green Agar with red colonies mimicking enteric pathogens.[3] It is advised to screen the colonies by flooding the plate with one drop of MUCAP Test reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C8 esterase enzyme, typical of *Salmonella* spp.[6]
- Since the medium is highly selective it is recommended the simultaneous inoculation of less selective media such as MacConkey Agar, and XLD Agar along with an enrichment broth.[3]
- The medium is normally reddish brown in colour; after incubation turns bright red but returns to normal colour at room temperature.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS
Sore at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin[3] the self-prepared plates can be stored at +2°C / +8°C in the dark and protected against evaporation for up to 6-8 weeks.

REFERENCES

1. Kristensen M, Lester V, Jurgens A. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br J Exp Pathol 1925; 5:291
2. Kauffmann F. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z. Hyg.Infektionskr. 1935; 117: 26.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Watson C, Walker AP. A modification of brilliant green agar for improved isolation of Salmonella J Appl Bacteriol. 1978 Oct;45(2):195-204.
5. Walker AP. A note of the inhibition of Pseudomonas aeruginosa by a modification of brilliant green agar for improved salmonella isolation J Appl Bacteriol. 1981 Dec;51(3):405-8.
6. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526

PACKAGING
Product	Type	REF	Pack
Brilliant Green Agar	Dehydrated medium	4012552	500 g (8.6 L)

IFU rev 1, 2022/06

BRILLIANT GREEN AGAR MODIFIED
Dehydrated and ready-to-use culture medium

INTENDED USE
Selective medium for the isolation and differentiation of *Salmonella* spp. other than *Salmonella* Typhi.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Beef extract	5.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Disodium hydrogen phosphate	1.0 g
Sodium dihydrogen phosphate	0.6 g
Lactose	10.0 g
Sucrose	10.0 g
Agar	13.0 g
Phenol red	90.0 mg
Brilliant green	4.7 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Originally described by Kristensen *et al*.[1], Brilliant Green Agar was modified by Kampelmacher[2,3] at National Institute of Public Health of Utrecht, to obtain a highly selective plating medium for the isolation of salmonellae from pig faces and minced meat.
Brilliant Green Agar Modified has been recommended for the isolation of *Salmonella*, other than *S*. Typhi, from water and associated materials[4], poultry and poultry products[5]. It is included in ISO 6579 as one of the second selective media for detection of *Salmonella* in foods.[6]
The presence of brilliant green may inhibit the growth of non-pathogenic enteric bacteria and *Shigella* spp., by selectively favouring *Salmonella*, except S. Typhi and S. Paratyphi.[7] For this reason, Brilliant Green Agar should be used in parallel with other enteric plating media such as XLD Agar.
Beef extract, peptone and yeast extract provide nitrogen, carbon, vitamins and minerals for microbial growth; phosphates act as buffer system; lactose and sucrose are fermentable carbohydrates; phenol red serves as an acid-base indicator giving a yellow colour to lactose and/or sucrose fermenting bacteria while lactose non-fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation. This medium also contains brilliant green, which inhibits the growth of the majority of Gram-positive and Gram-negative bacteria, including *S*. Typhi and *Shigella* species.

DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION
Suspend 52.7 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation. Cool to 47-50°C and distribute into sterile Petri dishes. Do not sterilize by autoclaving.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	red-orange, fine, homogeneous, free-flowing powder
Prepared plates appearance	red-orange, limpid
Final pH at 20-25 °C	6.9 ± 0.1

SPECIMENS
Food and water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

The detection of *Salmonella* in foodstuffs requires four successive stages:[6]

1- Pre-enrichment in Buffered Peptone Water inoculated with the test portion, then incubated between 34°C and 38 °C for 18 h.
2- Enrichment in selective broth. Rappaport-Vassiliadis Soy Broth (RVS broth) or Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar and Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn broth) are inoculated with the culture obtained in Buffered Peptone Water. The RVS broth or the MSRV agar is incubated at 41.5 °C for 24 h and the MKTTn broth at 37°C for 24 h.
3- Plating out on selective solid media. From the cultures obtained in the enrichment selective broth, the following two selective solid media are inoculated:
   - Xylose Lysine Deoxycholate agar (XLD agar);
   - any other solid selective medium complementary to XLD agar (for examples, Brilliant Green Agar Modified).

The XLD agar and Brilliant Green Agar Modified plates are incubated at 37 °C and examined after 24 h.

4- Confirmation. Colonies of presumptive *Salmonella* are sub-cultured and their identity is confirmed by means of appropriate biochemical and serological tests.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Salmonella spp. other than *S*. Typhi and *S*. Paratyphi A form red-pink-white opaque colonies, surrounded by a diffused red halo.
E. coli/Klebsiella/Enterobacter grow less luxuriantly and form yellow-green colonies, surrounded by a halo of the same colour.
Proteus does not swarm if dry plates are inoculated and produces yellow-pink mucoid colonies (sucrose fermentation, variable).
Shigella, is completely inhibited by brilliant green.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Enteritidis ATCC 13076	35-37°C/18-24 H/A	good growth, red colonies with red halo
S. Typhimurium ATCC 14028	35-37°C/18-24 H/A	good growth, red colonies with red halo
E. coli ATCC 25922	35-37°C/18-24 H/A	scanty growth, yellow colonies
E. faecalis ATCC 19433	35-37°C/18-24 H/A	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Colonies of *Salmonella* spp. vary from red-pink-white depending on length of incubation and strain type; however, any of these colours indicate non lactose fermenter strain.[7]
- S. Typhi, S. Paratyphi and *Shigella* do not grow adequately on this medium.[7]
- Slow lactose fermenters, *Proteus*, *Citrobacter* and *Pseudomonas* may grow on Brilliant Green Agar with red colonies mimicking enteric pathogens.[7] It is advised to screen the colonies by flooding the plate with one drop of MUCAP Test reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C8 esterase enzyme, typical of *Salmonella* spp.[8]
- Since the medium is highly selective it is recommended the simultaneous inoculation of less selective media such as MacConkey Agar, and XLD Agar along with an enrichment broth.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 6-8 weeks.[7]
Ready-to-use plates
Sore plates in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Kristensen M, Lester V, Jurgens A. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br J Exp Pathol 1925; 5:291
2. Edel W, Kampelmacher EH. Comparative studies on Salmonella isolation in eight European Laboratories. Bull Wld Hlth Org 1968; 39, 487-491.
3. Edel W. Kampelmacher EH. Salmonella isolation in nine European laboratories using a standardized technique. Bull Wld Hlth Org 1969; 41, 297 - 306.
4. H. M. S. O. Methods for the isolation and identification of salmonellae (other than Salmonella typhi) from water and associated materials. 1982
5. British Poultry Meat Society. A manual of recommended methods for the microbiological. 1982
6. ISO 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
8. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection. J Clin Microbiol 1992; 30:525-526.

PACKAGING

Product	Type	REF	Pack
Brilliant Green Agar Modified	Dehydrated medium	4012562	500 g (9.5 L)
Brilliant Green Agar Modified	Ready-to-use medium	541256	2 x 10 plates ø 90 mm

IFU rev 3, 2022/12

BRILLIANT GREEN BILE BROTH 2%

Dehydrated and ready-to-use culture medium

Brilliant Green Bile Broth 2% - from the left: *E. coli*, *K. pneumoniae*, *Salmonella* sp.

INTENDED USE

Selective medium for the confirmatory test of coliforms in foodstuffs.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Oxgall	20.0 g
Lactose	10.0 g
Peptone	10.0 g
Brilliant green	13.3 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Brilliant Green Bile Broth 2% is a modification of MacConkey's liquid medium, formulated by Dunham and Schoenlein[1] in 1926 to attain the maximum recovery of bacteria of the coli-aerogenes group, while inhibiting most Gram-positive organisms.[2]
Brilliant Green Bile Broth 2% is recommended by ISO 4831, ISO 4832, FDA-BAM for the confirmatory test of coliform bacteria in foods and by APHA for the confirmatory test of coliforms in waters.[3-6]
Peptone provides nitrogen, carbon and minerals for microbial growth; lactose is a fermentable carbohydrate. Brilliant green inhibits Gram-positive organisms and some Gram-negative bacteria other than coliforms, while oxgall, which is a mixture of bile salts, provides an added suppressive effect on Gram-positive organisms; brilliant green and oxgall also suppress the growth of the anaerobic lactose-fermenting bacteria, which could give false positive reactions. Coliforms, which are resistant to the inhibitory effects of both brilliant green and oxgall, at 30 °C or 37 °C cause fermentation of lactose with the production of gas.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water; heat slightly to completely dissolve the powder, mix well and distribute 10 mL into test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. Cool the broth as quickly as possible. With inocula greater than 1 mL for 10 mL of medium, use multiple strength medium and sterilise at 100°C for 30 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-green, fine, homogeneous, free-flowing powder
Prepared tubes appearance	blue-green, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Food and water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

For the confirmation of coliform bacteria enumerated with the most probable number (MPN) technique[3] proceed as following:
From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth (REF 401580) inoculate with a loop a tube of Brilliant Green Bile Broth and incubate at 30°C or 37°C for 24 h ± 2 h or, if gas formation is not observed, for 48 ± 2 h.
For the confirmation of coliform bacteria enumerated with the colony-count technique[4] proceed as following:
Inoculate five colonies of each doubtful type cultivated on Violet Red Bile Lactose Agar (REF 402185), into tubes of Brilliant Green Bile Broth 2%. Incubate the tubes at 30°C or 37°C for 24 h ± 2 h.

READING AND INTERPRETATION

The medium becomes turbid and yellowish-green in colour when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.
Consider as coliforms the colonies or the growth in liquid media that show gas formation in the Durham tube of Brilliant Green Bile Broth test tubes incubated at 30 or 37°C for 24-48 hours.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	30°C/24 H/A	growth, with gas production
E. coli ATCC 8739	30°C/24 H/A	growth, with gas production
C. freundii ATCC 43864	30°C/24 H/A	growth, with gas production
E. faecalis ATCC 19433	30°C/24 H/A	growth partially inhibited without gas

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Dehydrated medium
Sore at +10°C /+30°C away from direct light in a dry place.
According to Baird RM et al. the self-prepared tubes can be stored at 4 ± 2°C in screw capped containers for up to four weeks.[2]
Ready-to-use tubes
Sore tubes in their original pack at +2°C/ +8°C away from direct light.

References

1. Dunham HG, Schoenlein HW. Brilliant Green bile media. Stain Technol. 1926; 1:129-134
2. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 206-207.
3. ISO 4831:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique
4. ISO 4832:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique
5. FDA-BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Content current as of:10/09/2020.
6. APHA Standard Methods for the Examination of Water and Wastewater, 23rd ed. 2017.

Packaging

Product	Type	REF	Pack
Brilliant Green Bile Broth 2%	Dehydrated medium	4012652 4012654	500 g (12.5 L) 5 kg (125 L)
Brilliant Green Bile Broth 2%	Ready-to-use tubes	551265	20 x 10 mL with Durham tubes

IFU rev 2, 2022/06

BROMOCRESOL PURPLE GLUCOSE AGAR
(DEXTROSE TRYPTONE AGAR)
Dehydrated culture medium

Intended use
For the enumeration of spores of mesophilic and thermophilic *Bacillus.*

Composition - typical formula * (after reconstitution with 1 L of water)

Tryptone	10.00 g
Glucose	5.00 g
Soluble starch	2.00 g
Bromocresol purple	0.04 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
Dextrose Tryptone Agar has been originally devised by Williamas[1] during the studies on the cultivation and enumeration of thermophilic bacteria. In the 1930's, the National Canners Association specified the use of Dextrose Tryptone Agar for isolating "flat sour" organisms from food products. Canned food spoilage, referred to as 'flat-sour' spoilage, is caused by the outgrowth of facultative anaerobic *Geobacillus stearothermophilus, Bacillus coagulans, Bacillus thermoacidurans*. The microbial spoilage of canned food is caused by three reasons: 1) Survival of spores of thermophilic bacteria; 2) Growth of survived thermophilic bacteria due to inadequate cooling, inadequate heat treatment, and improper storage temperature; 3) Recontamination of microorganisms due to can leakage.
In flat-sour spoilage, the foods become sour due to the production of acid from carbohydrates with no can swelling.
Bromocresol Purple Glucose Agar (Dextrose Tryptone Agar) can be used to isolate *Bacillus coagulans* and other mesophilic or thermophilic microbes responsible for food spoilage.
Tryptone provides nitrogen and minerals for microbial growth; glucose is a fermentable carbohydrate and a source of carbon and energy for microbial growth; soluble starch is a protective agent and promotes spore germination; bromocresol purple serves as an acid-base indicator giving a yellow colour to glucose fermenting bacteria while glucose non-fermenting bacteria develop blue colonies.

Directions for medium preparation
Suspend 32 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C mix well and distribute into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	purple-grey, fine, homogeneous, free-flowing powder
Prepared plates appearance	purple, limpid
Final pH at 20-25 °C	7.0 ± 0.2

Specimens
Refer to applicable international standards and regulations for the collection of food samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

Test procedure
The instructions below are included only as guideline of the use of the medium and will vary depending on the origin of the sample and the exact purpose of the test. For more precise details, consult the cited references.[2-6]
Destroy the vegetative cells by heating the sample.
- Inoculate the plates with 1 ml of the sample or of its tenfold dilutions and pour 15 ml of medium into Petri dishes
- Cover and mix the inoculum with the medium.
- Incubate at 30°C for 5 days to enumerate *Bacillus* spores.
- Incubate at 55°C for 5 days to enumerate thermophilic *Bacillus* spores. Pour several drops of sterile paraffin oil in the lid of the plate as a tight seal.

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Count the total number of colonies, the total number of acid-producing (yellow halo) colonies and the total number of non-acid producing colonies (blue halo).

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
B. stearothermophilus ATCC 10149	55°C /72H/A	good growth, yellow colonies
B. subtilis ATCC 6633	35-37°C/18-24 H/A	good growth, yellow colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS
Sore at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Williams OB. Tryptone medium for the detection of flat-sour spores. Food Res 1936; 1: 217-221.
2. National Canners Association (1993) Bacterial Standards for Sugar.
3. National Canners Association (1968) Laboratory Manual for Food Canners and Processors. Vol.1. p13.
4. National Canners Association (1954) A Laboratory Manual for the Canning Industry' 1st ed., National Canners Association, Washington.
5. Salfinger Y, Tortorello ML (2015) Compendium of Methods for the Microbiological Examination of Foods, 5th EdAmerican Public Health Association, Washington, D.C.
6. Wehr HM, Frank JH (2004), Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

PACKAGING
Product	Type	REF	Pack
Bromocresol Purple Glucose Agar (Dextrose Tryptone Agar)	Dehydrated medium	4012732	500 g (15.6 L)

IFU rev 2, 2022/06

BRUCELLA BROTH (ALBIMI)
Dehydrated culture medium

INTENDED USE
General purpose medium for the cultivation of fastidious and non-fastidious microorganisms.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)
Tryptone	10.0 g
Peptone	10.0 g
Yeast extract	2.0 g
Glucose	1.0 g
Sodium chloride	5.0 g
Sodium bisulphite	0.1 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Brucella Broth (Albimi) is prepared according to the formulation given by APHA for Albimi broth.[1,2] It may be used as general purpose medium capable of supporting the growth of a wide variety of microorganisms, including the more nutritionally fastidious species.[2]
This medium is recommended for the cultivation of *Brucella* species and was recommended for use as the liquid medium component of biphasic blood culture bottles and as a medium base for the enrichment of *Campylobacter*.[2]
Brucella Broth (Albimi) contains tryptone, peptone and yeast extract which are sources of nitrogen, carbon, vitamins and minerals for microbial growth. Sodium chloride maintains the osmotic equilibrium, sodium bisulfite is a reducing agent and glucose is a source of carbon and energy.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 28.1 g in 1000 mL of cold purified water. Heat to dissolve with frequent agitation. Distribute into tubes or bottles and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS
This culture medium is not intended for direct use with samples, except as a 'back-up' enrichment medium in addition to the primary culture medium. For detailed information on sample collection and handling procedures, consult appropriate texts.[3]

TEST PROCEDURE
For *Brucella* cultivation, specimens may be inoculated into the broth after the inoculation of plated media.
Incubate tubes for up to 7 days at 35 -37°C in an aerobic atmosphere supplemented with carbon dioxide.
For the cultivation of other fastidious organisms incubate at the temperature and for the time required by laboratory procedures.

READING AND INTERPRETATION
After incubation observe the presence of growth (turbidity of the medium).

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of control strains depends on the intended use of the medium.

Limitations of the method
- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- The preparation of selective diagnostic media with the addition of specific compounds must be validated by the user.

Storage conditions
Sore at +10°C /+30°C away from direct light in a dry place.

References

1. Hausler WJ (ed). Standards Methods for the Examination of Dairy Products. 14th ed. Washington DC, American Public Health Association; 1976.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Araj GE. Brucella. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

Packaging
Product	Type	REF	Pack
Brucella Broth (Albimi)	Dehydrated medium	4012742	500 g (17.8 L)

IFU rev 2, 2022/05

BRUCELLA MEDIUM BASE
Dehydrated culture medium

Intended use
General purpose basal medium for the cultivation of *Brucella*.

Composition - typical formula * (after reconstitution with 1 L of water)
Peptone	10 g
Beef extract	5 g
Glucose	10 g
Sodium chloride	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
Brucellosis is a widespread zoonotic disease, transmitted mainly from ruminants to humans. It is a disease of major public health importance, animal welfare and economic significance worldwide.[1]
Brucella Medium Base can be used to prepare the glucose serum antibiotics medium described by Jones and Brinley Morgan, and recommended by the WHO for the selective isolation of *Brucella*, including fastidious strains, and *Brucella abortus* type II, which is very difficult to grow on common media.[2] Brucella Medium Base may be used for the preparation of Farrell's medium (FM)[3] for the isolation of *B.abortus* from contaminated samples by supplementation with foetal bovine serum 5%, polymyxin B 5 mg/L, bacitracin 25 mg/L, natamycin 50 mg/L, nalidixic acid 5 mg/L, vancomycin 20 mg/L and nystatin 17,7 mg/L.
Since nalidixic acid and bacitracin contained in various selective media can inhibit the growth of some *Brucella* strains[4] a medium with foetal bovine serum 5%, natamycin 20 mg/L, amphotericin B 4 mg/L, vancomycin 20 mg/L, nystatin 17.7 mg/L, colistin 7,5 mg/L and nitrofurantoin 10 mg/L has been proposed.[5]
Peptone and beef extract provide nitrogen, vitamins, minerals and amino acids for microbial growth. Glucose is the fermentable carbohydrate providing carbon and energy. Sodium chloride maintains the osmotic balance, agar is the solidifying agent. The addition of foetal calf serum enhances the productivity properties of the medium while the addition of antibiotics makes the medium inhibitory for yeasts, moulds and common Gram-positive and Gram-negative bacteria.

Directions for medium preparation
Suspend 45 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 5% of inactivated horse serum (horse serum held at 56°C for 30 minutes) or 5% of foetal bovine serum. For specific uses, add the required antimicrobials mix.

Physical characteristics
Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25°C	7.5 ± 0.2

Specimens
Brucella are excreted in large numbers by animals at parturition and can be cultured from a range of material including vaginal mucus, placenta, foetal stomach contents, and milk.[1] Brucella Medium Base may be inoculated with a variety of specimens for the isolation/cultivation of *Brucella* spp. Good laboratory practices for collection, transport and storage of the samples should be applied.[1]

Test procedure
Milk samples should be allowed to stand overnight at 4°C before lightly centrifuging.
The cream and the deposit are spread on to the surface of at least three plates of solid selective medium.
Inoculate the surface of the plated medium with 10 µl of the initial suspension and/or diluted sample.
Streak the inoculum with a loop or with a bent sterile glass rod over the four quadrants of the plate to obtain well isolated colonies
Incubate the plates in an inverted position at a temperature of 35 ± 2ºC in an atmosphere of 10% CO_2 and examine every two days for ten days.

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Examined in indirect sunlight, *Brucella* colonies appear translucent, with a slightly amber tinge. Bacterial colonies may be provisionally identified as Brucella on the basis of their cultural properties and appearance, Gram staining, and agglutination with positive antiserum.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
B. bronchispetica ATCC 4617	35-37°C / 18-24H / C	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- The preparation of selective diagnostic media with the addition of specific compounds must be validated by the user.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Corbel MJ, Elberg SS, Cosivi O. Brucellosis in Humans and Animals. World Health Organization Press; Geneva: 2006.]
2. Jones LM, Brinley Morgan WJ. A preliminary report on a selective medium for the culture of Brucella, including fastidious types. Bull Wld Hlth Org 1958; 19:200.
3. Farrell ID. The development of a new selective medium for the isolation of Brucella abortus from contaminated sources', Research in Veterinary Science 1974; 16(3): 280–286
4. Marin CM, Alabart JL, Blasco JM. Effect of antibiotics contained in two Brucella selective media', J Clin Microbiol 1966; 34(2), 426–428
5. Ledwaba MB et al. Investigating selective media for optimal isolation of Brucella spp. in South Africa. Onderstepoort J Vet Res v.87(1); 2020.

PACKAGING
Product	Type	REF	Pack
Brucella Medium Base	Dehydrated medium	4012752	500 g (11 L)

IFU rev 1, 2022/05

BRYANT BURKEY BROTH BASE WITH RESAZURIN
Dehydrated culture medium

INTENDED USE
Medium for the detection and enumeration of spores of lactate fermenting clostridia.

COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)
Tryptone	15.0 g
Yeast extract	5.0 g
Beef extract	7.5 g
Sodium acetate	5.0 g
Cysteine HCl	0.5 g
Resazurin	2.5 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Bryant and Burkey Medium is based on the lactate fermentation media described by Rosenberger[1] and Bryant and Burkey[2], as modified by Bergère[3]. Bryant Burkey Broth Base with Resazurin is prepared according to the formulation recommended by CNERNA[4].
It is suitable for the enumeration of spores of lactate fermenting *Clostridia* spp. in silage, milk and dairy products and in particular for the detection of *Clostridium tyrobutyricum* responsible for the "late blowing" in brine salted semi-hard and hard cheese. The gas produced by the growth of clostridia swells the cheese and is responsible for defect known as butyric swelling, resulting in bad taste.
Tryptone, yeast extract and beef extract provide nitrogen, carbon, vitamins, minerals and amino acids for microbial growth. L-cysteine is the reducing agent and resazurin is a redox indicator and monitors the oxygen level. Sodium acetate promotes the spore germination, which is activated by the heat treatment of the sample and improves the selectivity of the medium. Sodium lactate is not included in the medium so it must be added; sodium lactate, in the presence of sodium acetate, is fermented under anaerobic conditions by *C. tyrobutyricum* and other lactate-fermenting clostridia into butyric acid, acetic acid and gases (CO_2 and H_2). Gas production is demonstrated by an upward movement of a paraffin plug which is overlaid on the medium.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 33 g in 1000 mL of cold purified water and add 10 g of 50% sodium lactate solution. Heat to dissolve stirring constantly. Distribute 10 mL in 16x160 mm tubes. Sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	light brown to reddish, limpid
Final pH at 20-25 °C	5.9 ± 0.1

SPECIMENS
Dairy samples decontaminated by heating for 10 minutes at 75°C in order to destroy all the vegetative forms. For detailed information on sample collection and handling procedures, consult appropriate texts.[4]

Test procedure
1. Cool the tubes to 25°C after autoclaving or regenerate the anaerobic conditions by heating the tubes to 100°C for 10 minutes if the medium is pink for more than 1/3 of its height. Do not repeat the operation more than once.
2. Inoculate colourless tubes with 1 mL of sample and 1 mL of its tenfold dilutions. using MPN method with five tubes.
3. Overlay the medium with 2 mL (1.5-2 cm) of paraffin autoclaved at 121°C for 15 minutes and cooled to 58-60°C.
4. Heat the tubes at 75°C for 10 minutes to destroy vegetative cells and activate the germination of spores.
5. Cool the tubes rapidly in an ice-water bath to solidify the paraffin.
6. Incubate the inoculated tubes at 37°C for up to 7 days. The tubes are evaluated every 48 hours.

Reading and interpretation
After incubation observe the presence of growth (turbidity of the medium) and gas formation.
Tubes with growth and gas formation indicated by a 1 cm raise of the paraffin plug are considered positive.[4]
Calculate the number of *Clostridium* spp by MPN tables/software.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
C. tyrobutyricum ATCC 25755	35-37° / 44-48H /AN	growth with gas

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place.

References

1. Rosenberger KF. The development of methods for the study of obligate anaerobes in silage. Proc Soc Appl Bacteriol 1951; 14:161-164
2. Bryant MP, Burkey LA. The characteristics of lactate-fermenting sporeforming anaerobes from silage. J Bacteriol 1956; 71: 43-46
3. Bergère JL, Gouet P, Hermier J, Mocquot G. Les Clostridium du groupe butyrique dans les produits laitiers. Ann Inst Pasteur 1968;19: 41-54
4. CNERNA (Commission « Qualité Bactériologique du lait » du Centre National de Coordination des Etudes et Recherches sur la Nutrition et l'Alimentation): Recommandations pour l'estimation de la contamination du lait en spores de Clostridia par la méthode de culture en milieu liquide. Revue Laitière Française 1986; 451:39-45.

Packaging
Product	Type	REF	Pack
Bryant Burkey Broth Base with Resazurin	Dehydrated medium	4012692	500 g (15.1 L)

IFU rev 1, 2022/05

BUFFERED PEPTONE WATER
Dehydrated and ready-to-use culture medium

Intended use
Buffered Peptone Water is used as non-selective pre-enrichment medium and diluent in procedures for the detection and enumeration of bacteria, and pathogens such as *Salmonella, Cronobacter, Listeria monocytogenes, Listeria* spp. *Enterobacteriaceae*, in foods, animal feeding stuffs, water and other materials.
The medium complies with the specifications given by ISO 6579, ISO 11290-2, ISO 22964, ISO 21528-1, ISO 6887, ISO 19250.

Composition - typical formula * (after reconstitution with 1 L of water)
Dehydrated medium and ready-to-use tubes and flasks

Peptone°	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate anhydrous	3.5 g ^
Monopotassium phosphate	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
°Includes enzymatic digest of casein
^ equivalent to disodium hydrogen phosphate dodecahydrate 9 g/L

Principle of the method and explanation of the procedure
Buffered Peptone Water is the historical formulation included in the Biolife catalogue since the late 1960s, prepared with a universal peptone which includes enzymatic digest of casein, particularly rich in nutrients and with a buffer system that allows optimal recovery of microorganisms even when present in the sample in a very low number or sub-lethally injured.
Buffered Peptone Water can be used as:
- a non-selective pre-enrichment medium for the detection of *Salmonella* according to ISO 6579,[1,2] in samples of the food chain and according to ISO 19250 in water samples;[3]
- a non-selective pre-enrichment medium for the detection of *Cronobacter* according to ISO 22964 in samples of the food chain;[4]
- a non-selective enrichment medium for the detection of *Enterobacteriaceae* according to ISO 21528 in samples of the food chain;[5]
- a diluent for the enumeration of *Listeria monocytogenes* and of *Listeria* spp. according to ISO 11290-2;[6]
- a diluent for the enumeration of microorganisms according to ISO 6887.[7]

Peptone provides carbon, nitrogen, vitamins and minerals for microbial growth, sodium chloride maintains the osmotic balance, while phosphates buffer the medium at pH 7.0. The pH 7.2 medium variant according to FDA-BAM is available under code 401278S.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 20 g in 1000 mL of cold purified water. Mix thoroughly and warm gently to completely dissolve the powder, if necessary. Distribute into flasks or tubes of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Waters, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable International Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice. [1-7]

TEST PROCEDURE

For details of sample preparation and enrichment, refer to the Standards cited according to the intended use.[1-7]
Pre-enrichment for *Salmonella* detection: in general, 225 mL of Buffered Peptone Water are inoculated with 25 g of the test portion, then incubated between 34°C and 38°C for 18 h ± 2 h. It is permissible to store the pre-enriched sample after incubation at 2-8°C for a maximum of 72 h.

READING AND INTERPRETATION

Microbial growth in Buffered Peptone Water is evidenced by the development of turbidity in the broth.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of strains depends on the intended use. Consult the suitable ISO Standard.[8]

LIMITATIONS OF THE METHOD

- Buffered Peptone Water is a general-purpose medium without selective properties. Suitable selective liquid and solid media must be inoculated with the growth obtained in Buffered Peptone Water.
- The test sample may increase the turbidity of the medium although bacterial growth is not present. Subculture to appropriate media is necessary to verify growth of organisms.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579-1 autoclaved Buffered Peptone Water may be stored in closed containers at 2-8 °C for up to six months.[1]
Ready-to-use medium in tubes and bottles
Store tubes and flasks in their original pack at 2-8°C away from direct light.

REFERENCES

1. ISO 6579-1:2017/AMD 1:2020 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC
2. ISO/TS 6579-2:2012 Microbiology of food and animal feed - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 2: Enumeration by a miniaturized most probable number technique.
3. ISO 19250:2010 Water quality - Detection of Salmonella spp.
4. ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of Cronobacter spp.
5. ISO 21528-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae -Part 1: Detection of Enterobacteriaceae
6. ISO 11290-2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -Part 2: Enumeration method
7. ISO 6887-1:2017 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 1: General rules for the preparation of the initial suspension and decimal dilutions
8. ISO 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media

PACKAGING

Product	Type	REF	Pack
Buffered Peptone Water	Dehydrated medium	4012782 4012784	500 g (25 L) 5 kg (250 L)
Buffered Peptone Water	Ready-to-use medium in tubes	551278	20 x 9 mL
Buffered Peptone Water	Ready-to-use medium in flasks	5112782 5112783	6 x 90 mL 6 x 225 mL

IFU rev 2, 2022/06

BUFFERED PEPTONE WATER (CASEIN)

Dehydrated culture medium

INTENDED USE

Buffered Peptone Water (Casein) is used as non-selective pre-enrichment medium and diluent in procedures for the detection and enumeration of bacteria, and pathogens such as *Salmonella, Cronobacter, Listeria monocytogenes* in food and animal feeding stuffs, water and other materials. The medium complies with the specifications given by ISO 6579, ISO 11290-2, ISO 22964, ISO 21528-1, ISO 6887, ISO 19250.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate anhydrous	3.5 g ^
Potassium dihydrogen phosphate	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
^ equivalent to disodium hydrogen phosphate dodecahydrate 9 g/L

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Most of the International Standards, recommending the use of Buffered Peptone Water, indicate as the ingredient of animal origin to be included in the formula, a material with the generic term "Peptone" and as an example cite enzymatic digest of casein.[1-6]
The ISO 19250 Standard for the detection of *Salmonella* in water, recommending the use of Buffered Peptone Water, explicitly mentions enzymatic digest of casein as the ingredient of animal origin to be included in the formula.[3]
Buffered Peptone Water (Casein) is manufactured with a selected enzymatic digest of casein, particularly rich in nutrients and with a buffer system that allows optimal recovery of microorganisms even when present in the sample in a very low number or sub-lethally injured.
Buffered Peptone Water (Casein) can be used as:

- a non-selective pre-enrichment medium for the detection of *Salmonella* according to ISO 6579,[1,2] in samples of the food chain and according to ISO 19250 in water samples;[3]
- a non-selective pre-enrichment medium for the detection of *Cronobacter* according to ISO 22964;[7]
- a non-selective enrichment medium for the detection of *Enterobacteriaceae* according to ISO 21528;[4]
- a diluent for the enumeration of *Listeria monocytogenes* and of *Listeria* spp. according to ISO 11290-2;[5]
- a diluent for the enumeration of microorganisms according to ISO 6887.[6]

The enzymatic digest of casein provides carbon, nitrogen, vitamins and minerals for microbial growth, sodium chloride maintains the osmotic balance, while phosphates buffer the medium at pH 7.0.

DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 20 g in 1000 mL of cold purified water. Mix thoroughly and warm gently to completely dissolve the powder, if necessary. Distribute into flasks or tubes of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Waters, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable international standards[1-7] for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

For details of sample preparation and enrichment, please refer to the standards cited according to the intended use.[1-8]
For the pre-enrichment of *Salmonella* in water Buffered Peptone Water (Casein) is inoculated at ambient temperature with a known volume of the sample or its dilutions, then incubated at 36 ± 2 °C for 18 h ± 2 h. Larger volumes can be concentrated using membrane filtration and the membrane filter is then added to Buffered Peptone Water.

READING AND INTERPRETATION

Microbial growth in Buffered Peptone Water (Casein) is evidenced by the development of turbidity in the broth.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of strains depends on the intended use. Consult the Standard cited.[8]

LIMITATIONS OF THE METHOD

- Buffered Peptone Water (Casein) is a general-purpose medium without selective properties. Suitable selective liquid and solid media must be inoculated with the growth obtained in Buffered Peptone Water (Casein).
- The test sample may increase the turbidity of the medium although bacterial growth is not present. Subculture to appropriate media is necessary to verify growth of organisms.

STORAGE CONDITIONS

Upon receipt, store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579-1, autoclaved Buffered Peptone Water may be stored in closed containers at 2-8 °C for up to six months.[1]

REFERENCES

1. ISO 6579-1:2017/AMD 1:2020 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC
2. ISO/TS 6579-2:2012 Microbiology of food and animal feed - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 2: Enumeration by a miniaturized most probable number technique.
3. ISO 19250:2010 Water quality - Detection of Salmonella spp.
4. ISO 21528-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae -Part 1: Detection of Enterobacteriaceae
5. ISO 11290-2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -Part 2: Enumeration method
6. ISO 6887-1:2017 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 1: General rules for the preparation of the initial suspension and decimal dilutions
7. ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of Cronobacter spp.
8. ISO 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media

PACKAGING

Product	Type	REF	Pack
Buffered Peptone Water (Casein)	Dehydrated medium	401278C2 401278C4	500 g (25 L) 5 kg (250 L)

IFU rev 2, 2022/06

BUFFERED PEPTONE WATER (MEAT)

Dehydrated culture medium

INTENDED USE

Buffered Peptone Water (Meat) is used as non-selective pre-enrichment medium or as a diluent in several microbiological procedures.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of animal tissue	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate anhydrous	3.5 g ^
Monopotassium phosphate	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
^ equivalent to disodium hydrogen phosphate dodecahydrate 9 g/L

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The ISO Standards in which Buffered Peptone Water is mentioned, specify the generic term 'Peptone' in the medium formulation and cite the enzymatic digest of casein as an example.[1-5] According to ISO 11133[6], annex A, the term “peptone” is used to identify enzymatic digests of different biological materials (animal tissue, casein, soy, heart, gelatine, animal and plant tissue).
European Pharmacopoeia, recommending the use of Buffered Peptone Medium, indicates a material with the generic term "Peptone" as the ingredient of animal origin to be included in the formula without further specifying the origin of the animal tissue.[7]
Buffered Peptone Water (Meat) is manufactured with a selected enzymatic digest of animal tissue (meat), very rich in nutrients and with a buffer system that allows optimal recovery of microorganisms even when present in the sample in a very low number or sub-lethally injured.
Buffered Peptone Water (Meat) can be used as a non-selective pre-enrichment medium or as a diluent in several in procedures such as

- detection of *Salmonella* according to ISO 6579[1] in samples of the food chain and according to EP[7] in in herbal medicinal products for oral use and extracts used for their preparation
- detection of *Cronobacter* according to ISO 22964;[2]
- detection of *Enterobacteriaceae* according to ISO 21528;[3]
- enumeration of *Listeria monocytogenes* and of *Listeria* spp. according to ISO 11290-2;[4]
- preparation of the sample according to ISO 6887-1.[5]

The enzymatic digest of animal tissue provides carbon, nitrogen, vitamins and minerals for microbial growth, sodium chloride maintains the osmotic balance, while phosphates buffer the medium at pH 7.0.

DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 20 g in 1000 mL of cold purified water. Mix thoroughly and warm gently to completely dissolve the powder, if necessary. Distribute into flasks or tubes of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Food samples, herbal medicinal products and extracts used for their preparation. Refer to EP and Iso Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice.[1-7]

TEST PROCEDURE

For details of sample preparation and enrichment, refer to the Standards cited according to the intended use.[1-5,7]
Pre-enrichment for *Salmonella* detection: in general, 225 mL of Buffered Peptone Water are inoculated with 25 g of the test portion, then incubated between 34°C and 38°C for 18 h ± 2 h.

READING AND INTERPRETATION

Microbial growth in Buffered Peptone Water (Meat) is evidenced by the development of turbidity in the broth.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of strains depends on the intended use. Consult the cited references.[1-7]

LIMITATIONS OF THE METHOD

- Buffered Peptone Water (Meat) is a general-purpose medium without selective properties. Suitable selective liquid and solid media must be inoculated with the growth obtained in Buffered Peptone Water (Meat).
- The test sample may increase the turbidity of the medium although bacterial growth is not present. Subculture to appropriate media is necessary to verify growth of organisms.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579-1 autoclaved Buffered Peptone Water may be stored in closed containers at 2-8 °C for up to six months.[1]

References

1. ISO 6579-1:2017/AMD 1:2020 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the
2. ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of Cronobacter spp.
3. ISO 21528-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae -Part 1: Detection of Enterobacteriaceae
4. ISO 11290-2:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. -Part 2: Enumeration method
5. ISO 6887-1:2017 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 1: General rules for the preparation of the initial suspension and decimal dilutions
6. ISO 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media
7. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.31 Microbiological Examination of herbal medicinal products for oral use and extracts: 01/2014:20631.

Packaging

Product	Type	REF	Pack
Buffered Peptone Water (Meat)	Dehydrated medium	401278B2 401278B4	500 g (25 L) 5 kg (250 L)

IFU rev 2, 2022/11

BUFFERED PEPTONE WATER pH 7.2

Dehydrated culture medium

Intended use

Buffered Peptone Water pH 7.2 is used as non-selective pre-enrichment medium for the procedure of detection and enumeration of *Salmonella,* according to FDA-BAM in foods.

Composition - typical formula * (after reconstitution with 1 L of water)

Peptone	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate anhydrous	3.5 g ^
Monopotassium phosphate	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

^ equivalent to disodium hydrogen phosphate dodecahydrate 9 g/L

Principle of the method and explanation of the procedure

Buffered Peptone Water pH 7.2 is manufactured with a selected peptone, particularly rich in nutrients and with a buffer system that allows optimal recovery of microorganisms even when present in the sample in a very low number or sub-lethally injured.
The medium is prepared according to FDA-BAM medium 192 specification whose pH is set at 7.2 ± 0.2,[1] while ISO Standards (e.g ISO 6579) require a medium with pH 7.0 ± 0.2
Buffered Peptone Water pH 7.2 can be used as a non-selective pre-enrichment medium according to FDA-BAM chapter 5 for the detection of *Salmonella* in some foods and animal feeding stuffs.[2]
The peptone provides carbon, nitrogen, vitamins and minerals for microbial growth, sodium chloride maintains the osmotic balance, while phosphates buffer the medium at pH 7.2.

Directions for dehydrated medium preparation

Suspend 20 g in 1000 ml of cold purified water. Stir and heat if necessary to completely dissolve the powder. Distribute into flasks or tubes of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.

Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	pale yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

Specimens

Foods, animal feeding stuffs. Refer to applicable international standard[2] for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

Test procedure

For details of sample preparation and enrichment, please refer to the Standard cited according to the intended use.[2]
For the pre-enrichment for *Salmonella*, in general, 225 mL of Buffered Peptone Water pH 7.2 are inoculated with 25 g of the sample, then incubated for 24 ± 2 h at 35°C.

Reading and interpretation

Microbial growth in Buffered Peptone Water pH 7.2 is evidenced by the development of turbidity in the broth.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of strains depends on the intended use. Consult the Standard cited.[1]

Limitations of the method

- Buffered Peptone Water pH 7.2 is a general-purpose medium without selective properties. Suitable selective liquid and solid media must be inoculated with the growth obtained in Buffered Peptone Water pH 7.2.
- Th test sample may increase the turbidity of the medium although bacterial growth is not present. Subculture to appropriate media is necessary to verify growth of organisms.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579-1[2] autoclaved Buffered Peptone Water may be stored in closed containers at 2-8 °C for up to six months.

REFERENCES

1. FDA BAM Media M192: Buffered Peptone Water (BPW) Content current as of: 03/27/2018
2. FDA-BAM. Chapter 5: Salmonella. Content current as of: 03/18/2022
3. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp.

PACKAGING
Product	Type	REF	Pack
Buffered Peptone Water pH 7.2	Dehydrated medium	401278S2 401278S4	500 g (25 L) 5 kg (250 L)

IFU rev 2, 2022/06

BUFFERED SODIUM CHLORIDE-PEPTONE SOLUTION: see PHARMACOPOEIA DILUENT

CAMPYLOBACTER AGAR BLASER WANG

Ready-to-use plates

Campylobacter jejuni on Campylobacter Agar Blaser Wang

INTENDED USE
In vitro diagnostic device. Selective medium for the isolation of *Campylobacter* spp. from faecal specimens.

COMPOSITION - TYPICAL FORMULA*
Peptocomplex	10.0 g
Tryptose	10.0 g
Peptone	3.0 g
Maize starch	1.0 g
Sodium chloride	5.0 g
Agar	12.0 g
Amphotericin B	2.0 mg
Cephalothin	15.0 mg
Trimethoprim	5.0 mg
Vancomycin	10.0 mg
Polymyxin B	2500 IU
Defibrinated sheep blood	50 mL
Purified water	950 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Campylobacter spp. are Gram-negative, oxidase-positive, non-sporeforming, curved, spiral, or S-shaped rods, 0.2–0.9 µm wide and 0.5–5 µm long. Organisms are usually motile by means of a single polar unsheathed flagellum at one or both ends, that gives them a very characteristic "corkscrew" motility.[1] They are nutritionally fastidious and grow under strictly anaerobic or microaerobic (containing approximately 5-10% O_2 and 5-10% CO_2 for recovery) conditions, but a number of *Campylobacter* species, including *C. concisus, C. curvus, C. gracilis, C. mucosalis, C. rectus, C. showae* and some strains of *C. hyointestinalis,* require a hydrogen-enriched atmosphere (3-7% H_2 is required) for growth, a condition not routinely used in the diagnostic laboratories.[2]
Gastrointestinal *Campylobacter* infections are acquired by ingestion of undercooked poultry, seafood, meat and produce, by the contact with animals and by drinking untreated water or milk. In some instances, infection can progress to life-threatening extra-gastrointestinal diseases.[2]
C.jejuni accounts for about 90% of reported infections and most of the remainder are caused by *C.coli* and *C.lari*; other *Campylobacter* species have also been isolated from cases of diarrhoea (*C. helveticus, C. upsaliensis, C. hominis, C. gracilis, C. lanienae, C. peloridis, C. concisus, C. mucosalis, C. fetus, C. hyointestinalis, C. sputorum , C.i nsulaenigrae*).[2] The species most commonly associated with disease in humans are thermophilic, i.e. they will grow at 42-43°C and 37°C, but not at 25°C; *C.jejuni* subspecies *doyley*, *C. fetus*, and *C. fetus* subspecies *venerealis* do not grow at 42°C.[3]
Since the early 1970s, when *C. jejuni* and *C. coli* have been recognised as agents of gastrointestinal infections associated with food poisoning, several liquid and plated culture media have been developed, originally designed for the examination of faeces and then extended to the detection of *Campylobacter* in food and water.[3] The selective media for isolation of *Campylobacter* consist of a non-selective base to be used with or without animal blood and of a mixture of antimicrobial compounds; among the isolation media proposed in the literature, the review by Corry and Atabay[3] mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.
Campylobacter Agar Blaser Wang (known also as Campy-BAP) is prepared according to the formulation devised by Blaser and Wang,[4] who modified the formulation of Skirrow by adding cephalothin and amphotericin B and substituting laked horse blood with defibrinated sheep blood. The peptones supply nitrogen, carbon, and trace elements for microbial growth. Yeast extract is a source of the group B vitamins. Sheep blood supplies additional nutrients. The selective agents of the medium are vancomycin, with a strong inhibitory activity against Gram-positive bacteria, polymyxin B, cephalothin and trimethoprim, which mainly suppress the growth of Gram-negative bacteria and amphotericin B, included as an antifungal compound.

PHYSICAL CHARACTERISTICS
Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Faecal specimens are preferred for isolating *Campylobacter* spp. from patients with gastrointestinal infections; however, rectal swabs are acceptable for culture.[5] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Solid faeces: faeces may be diluted 1:4 in sterile saline solution or 0.1% peptone water. It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens.[3] Inoculate 3-5 drops on the medium surface.
Liquid stool: inoculate 3 drops on the medium surface.
Rectal swabs: roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
For all type of specimens, streak with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Incubate in a microaerobic atmosphere consisting approximately of 5% O_2, 10% CO_2, and 85% N_2, at 39-42°C for 40-48 hours.[3,]
If non-thermophilic species should be isolated, incubate inoculated plates at 37 ± 2°C in a microaerobic atmosphere.

READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Campylobacter colonies are usually grey/white or creamy grey in colour, swarming and moist in appearance. They may appear as a layer of growth over the surface of the agar. Colonies are usually non-pigmented.
Campylobacter species are oxidase positive. If a colony phenotypically resembling *Campylobacte*r species is oxidase negative, subculture to blood agar and retest after 24hr incubation.[2]
The presumptive identification of thermophilic and enteropathogenic *Campylobacte*r can be done on the basis oxidase test (+) and the characteristic motility. For a complete explanation of the identification criteria and methods, refer to the quoted references.[2,5]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[6]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. jejuni	ATCC 33291	41-42°C / 40-48h / M	good growth
E. coli	ATCC 25922	41-42°C / 40-48h / M	partially or totally inhibited

M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Cephalothin and polymyxin B can be inhibitory to some strains of *C. jejuni* and *C. coli* and also to many of the other less commonly encountered *Campylobacter* species, such as *C. upsaliensis, C. hyointestinalis*, and *C. fetus*.[2]
- To achieve the highest yield of *Campylobacter* from stool samples, a combination of media based on different selective systems appears to be the optimal method (e.g., Blaser Wang medium and a less selective Campylobacter blood free selective medium, such as Karmali or CCDA).[2]
- Some species of *Campylobacter*, such as *C. concisus, C. rectus, C. curvus, C. gracilis* and *C. showae* require increased hydrogen for primary isolation and growth. These species are usually not recovered under the conventional microaerobic conditions with the hydrogen concentration lower than 2%.[2,5]
- *Campylobacter* species have different optimal temperature for growth. The choice of incubation temperature for routine cultures of stool is critical in determining the spectrum of species that will be isolated.[5] *C. jejuni* subspecies *doylei*, *C. fetus* and *C. fetus* subspecies *venerealis* do not grow at 42°C.[3]
- Blood free formulations (e.g., Karmali, CCDA) appear to have better performances than blood containing media.[5]
- The clinical advantage of enrichment broths formulated to enhance the recovery of *Campylobacter* has not been studied adequately.[5] Enrichment seems not to be necessary for samples collected in the acute campylobacteriosis phase, while *Campylobacter* recovery increases in asymptomatic patients, in studies involving low numbers of the target organism, in samples not readily sent to the laboratory and in samples taken in the convalescence phase after an episode of diarrhoea.[7,8]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Corry JEL, Atabay HI. Culture Media for the Isolation of Campylobacters, Helicobacters and Arcobacters. *In* Handbook of Culture Media for Food and Water Microbiology, edited by Corry JEL, Curtis GDW, Baird RM. Published by the Royal Society of Chemistry, 3rd Edition 2012.
2. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018.
3. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014.
4. Blaser MJ, Berkowicz ID, Laforce FM, Cravens J, Reller LB, Wang WL. Campylobacter enteritis: clinical and epidemiological features. Ann Intern Med 1979; 91:179–185.
5. Nachamkin I. Campylobacter and Arcobacter. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
6. CLSI (formerly NCCLS). Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
7. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982; 35:462.
8. Hutchinson DN, Bolton FJ. Is enrichment culture necessary for the isolation of Campylobacter jejuni from faeces? J Clin Pathol 1983; 36:1350-1352.

PACKAGING

Product	Type	REF	Pack
Campylobacter Agar Blaser Wang	Ready-to-use plates	541111	2 x 10 plates ø 90 mm

IFU rev 1, 2020/09

CAMPYLOBACTER BLOOD AGAR BASE
PRESTON ANTIMICROBIC SUPPLEMENT
(PRESTON MEDIUM)
Dehydrated culture medium and selective supplement

Campylobacter jejuni
on Preston medium

INTENDED USE
Basal medium and selective supplement for the isolation of thermotolerant *Campylobacter* spp.

COMPOSITION *
CAMPYLOBACTER BLOOD AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Beef Extract	10 g
Peptone	10 g
Sodium Chloride	5 g
Agar	15 g

PRESTON ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 mL OF MEDIUM)

Cycloheximide	50 mg
Rifampicin	5 mg
Trimethoprim	5 mg
Polymyxin B	2.500 U.I.

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Campylobacter spp. are Gram-negative, oxidase-positive, non-sporeforming, S-shaped, or spiral rods, 0.2–0.9 µm wide and 0.5–5 µm long. Organisms are usually motile by means of a single polar unsheathed flagellum at one or both ends, that gives them a very characteristic "corkscrew" motility.[1] An atmosphere containing reduced oxygen (5 to 6%) is required for microaerobic growth. The species most commonly associated with disease in humans are thermotolerant: they will grow at 42°C - 43°C and 37°C, but not at 25°C. *Campylobacter jejuni* subspecies *doylei*, *Campylobacter fetus* and *C. fetus* subspecies *venerealis* do not grow at 42°C.[2]
In *Campylobacter* infection (campylobacteriosis), the symptoms usually range from none to severe, including fever, abdominal cramping, and diarrhoea (with or without blood/faecal white cells); nausea and vomiting may accompany the diarrhoea. Extraintestinal infections have been reported following *Campylobacter* enteritis in less than 0.15% of patients, usually in very old or very young subjects, and include bacteraemia, hepatitis, pancreatitis, meningitis, endocarditis, septic arthritis, abortion, neonatal sepsis; *C. jejuni* is the most often recognized infection preceding the development of Guillain-Barré syndrome.[1]
Campylobacter infections are acquired by ingestion of undercooked poultry, seafood, meat and produce, by the contact with animals and by drinking untreated water or milk. Most infections are caused by *C. jejuni* subsp. *jejuni* and *C. coli*: other species which sometimes cause diarrhoea are *C. lari*, *C. fetus subsp. fetus*, *C. jejuni subsp. doylei* and *C. upsaliensis*.
Since the early 1970s, when *C. jejuni* and *C. coli* have been recognised as agents of gastrointestinal infections associated with food poisoning, several liquid and plated culture media have been developed, originally designed for the examination of faeces and then extended to the detection of *Campylobacter* in food and water.[3] The selective media for isolation of *Campylobacter* consist of a non-selective base to be used with or without animal blood and of a mixtures of antimicrobial compounds; among the isolation media proposed in the literature, the review by Corry and Atabay[3] mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.
Preston medium, is prepared according to the formulation described by Bolton and Robertson[2] and consists of Campylobacter Blood Agar Base with the addition of Preston Antimicrobic Supplement and lysed horse blood. Campylobacter Blood Agar Base is essentially a Nutrient Broth n ° 2 with the addition of agar and was chosen by Bolton and Robertson for its low content in trimethoprim inhibitors.[2]
A comparison of Skirrow's, Butzler's, Blaser's, Campy-BAP and Preston media for Campylobacter spp. was made by Bolton *et al.*[3] using human, animal and environmental specimens. Butzler's medium gave the lowest isolation rate and Preston medium, which was the most selective, the highest isolation rate. Enrichment culture using Preston enrichment broth gave a higher isolation rate than direct plating onto Preston medium. Therefore Preston medium can be used for the isolation of *C. jejuni* and *C. coli*, from specimens of human origin, environmental samples, animals and poultry.
Enrichment seems not to be necessary for samples collected in the acute campylobacteriosis phase, while *Campylobacter* recovery increases in asymptomatic patients, in studies involving low numbers of the target organism, in samples not readily sent to the laboratory and in samples taken in the convalescence phase after an episode of diarrhea.[2,4]
Preston medium may be used as a second enrichment medium in the procedure recommended by ISO 10272-1[5] for the detection of *Campylobacter spp.* in food, especially in the presence of background flora resistant to 3rd generation ß-lactams like cefoperazone not inhibited by the first choice medium mCCD agar.
Meat extract and peptone provide nitrogen, carbon and trace elements for microbial growth; sodium chloride maintains the osmotic balance; lysed horse blood neutralizes the trimethoprim antagonists which may be present as residues in the peptones[1]. The antimicrobials of the selective supplement have the following properties: polymyxin is an antibiotic active against Gram-positive bacteria, trimethoprim mainly inhibits the growth and swarming of *Proteus* spp., rifampicin has an inhibitory activity on Gram-positive and Gram-negative bacteria other than *Campylobacter*, cycloheximide is included in the formulation as an antifungal.[1]

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 20 g in 475 mL of cold purified water. Heat to boiling, stirring constantly, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 25 mL of lysed horse blood (REF 90HLX100) and the content of one vial of Preston Antimicrobic Supplement reconstituted with 2 mL of acetone/sterile purified water (1:1) under aseptic conditions. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS
Campylobacter Blood Agar Base

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.5 ± 0.2

Preston Antimicrobic Supplement

Freeze-dried supplement appearance	short, red-brown pastille
Reconstituted supplement appearance	red, limpid

SPECIMENS

Good laboratory practices for collection, transport and storage of the samples should be applied. For non-clinical samples, refer to the applicable international standard.[5]

TEST PROCEDURE

Allow plates to come to room temperature.

Food chain samples[5]: detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters, e.g., cooked or frozen products.

1. Combine a quantity of 10 g or 10 mL of the test portion with 90 mL of the enrichment medium Bolton broth* so to obtain a 1 in 10 dilution, and homogenize.
2. Incubate the initial suspension in a microaerobic atmosphere at 37 ± 1°C for 4 h to 6 h, then at 41,5 ± 1°C for 44 h ± 4 h.
3. Using the culture obtained in the enrichment medium inoculate with a sterile 10 µL loop the surface of the first selective isolation medium, mCCD agar**. Proceed in the same manner with the second *Campylobacter* selective isolation medium chosen by the user (e.g., Preston Medium) ***
4. Incubate the plates at 41,5 ± 1°C in a microaerobic atmosphere for 44 h ± 4 h.
5. After 44 h ± 4 h of incubation, examine the plates for typical and/or suspect colonies of *Campylobacter.*

Notes

* Bolton Broth: Campylobacter Bolton Enrichment Broth Base, REF 401286B, added with Bolton Broth Selective Supplement REF 4240025 and Lysed Horse Blood REF 90HLX100.

** mCCD Agar: Campylobacter Free Medium Base (mCCDA), REF 4012822, with the addition of CCDA Antimicrobic Supplement (REF 4240020).

*** Using a second plating medium with selective agents different from those in mCCD agar could improve *Campylobacter* detection, especially in the presence of background flora resistant to 3rd generation ß-lactams like cefoperazone.

READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Campylobacter colonies usually are grey/white or creamy grey in colour, swarming and moist in appearance. They may appear as a layer of growth over the surface of the agar. Colonies are usually non-pigmented.

The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat, not only from strain to strain, but also from batch to batch of the selective culture medium used.[5]

Campylobacter species are oxidase positive. If a colony phenotypically resembling *Campylobacte*r species is oxidase negative, subculture to blood agar and retest after 24h incubation.[6]

The presumptive identification of thermophilic and enteropathogenic *Campylobacter* can be done on the basis oxidase test (+), the absence of growth with an incubation at 25°C and the characteristic microscopic morphology/motility (curved bacilli with a spiralling “corkscrew” motility).

For a complete explanation of the identification criteria and methods, refer to the quoted references.[5,6]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[10]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. jejuni	ATCC 33291	39-42°C / 40-48h / M	good growth
C. coli	ATCC 43478	39-42°C / 40-48h / M	good growth
E. coli	ATCC 25922	39-42°C / 40-48h / M	partially or totally inhibited
S. aureus	ATCC 25923	39-42°C / 40-48h / M	inhibited

A: aerobic incubation; M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- With incubation at 42-43°C some strains of *C. fetus, C. upsaliensis C. jejuni* subsp. *doylei* may not grow on the medium.[1]
- Campylobacter is sensitive to the moisture level on plating media: dry plates yield smaller colonies, the more humid the medium the more colonies develop with typical morphology.[1]
- If possible, use the plates on the day of their preparation. If necessary, store laboratory prepared plates for no more than 5 days in the dark at 2-8 °C. Excessive drying of the plates can lead to diagnostic errors.[1]
- Prolonged incubations beyond 48 hours may result in the development of contaminants that mask the growth of Campylobacter.[3]
- To achieve the highest yield of *Campylobacter* from stool samples or food, a combination of media that includes Preston medium and a second selective medium, based on a different selective system, appears to be the optimal method (e.g., mCCDA).[5,10]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Selective supplement

Store the product in the original package at +2°C /+8°C away from direct light.

According to Baird RM et al. the self-prepared plates can be stored at +2/+8°C for 10 days.[11]

REFERENCES

1. Corry JEL, Atabay HI. Culture Media for the Isolation of Campylobacters, Helicobacters and Arcobacters. *in* Handbook of Culture Media for Food and Water Microbiology, edited by Corry JEL, Curtis GDW, Baird RM. Published by the Royal Society of Chemistry, 3rd Edition 2012.
2. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982; 35:462
3. Bolton FJ, Coates D, Hinchliffe PM, Robertson L. Comparison of selective media for isolation of Campylobacter jejuni/coli. J Clin Pathol 1983; 36:78-83.
4. Hutchinson DN, Bolton FJ. Is enrichment culture necessary for the isolation of Campylobacter jejuni from faeces? J Clin Pathol 1983; 36:1350-1352
5. ISO 10272-1:2017: Microbiology of the food chain — Horizontal method for detection of Campylobacter spp.
6. Fitzgerard C, Nachamamkin I. Campylobacter and Arcobacter. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.998.
7. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014
8. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018

9. Hunt JM, Abeyta C, Tran T. *In* U.S. Food and Drug Administration Bacteriological Analytical Manual. Chapter 7 *Campylobacter.* January 2001. Content current as of: 03/26/2018.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
11. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:250:1.

PACKAGING

Product	Type	REF	Pack
Campylobacter Blood Agar Base	Dehydrated medium	4012852	500 g (12.5 L)
Preston Antimicrobic Supplement	Freeze-dried supplement	4240017	10 vials, each for 500 mL of medium

IFU, rev 1, 2020/09

m-CCDA Agar
CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON (m-CCDA)
BOLTON CCDA ANTIMICROBIC SUPPLEMENT CAMPYLOBACTER BLOOD FREE AGAR (CCDA BOLTON)

Dehydrated and ready-to-use culture medium and selective supplement

INTENDED USE

Selective medium for the isolation of *Campylobacter* spp. in foodstuffs and other samples.

COMPOSITION*

CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON - DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	10.00 g
Peptone	10.00 g
Tryptone	3.00 g
Sodium chloride	5.00 g
Charcoal	4.00 g
Sodium deoxycholate	1.00 g
Ferrous sulphate	0.25 g
Sodium pyruvate	0.25 g
Agar	15.50 g

BOLTON CCDA ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefoperazone	16 mg
Amphotericin B	5 mg

CAMPYLOBACTER BLOOD FREE AGAR (CCDA BOLTON)
READY-TO-USE PLATES, TYPICAL FORMULA

Campylobacter Blood Free Medium Base Bolton	49 g
Cefoperazone	32 mg
Amphotericin B	10 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Since the early 1970s, when *C. jejuni* and *C. coli* have been recognised as agents of gastrointestinal infections associated with food poisoning, several liquid and plated culture media have been developed, originally designed for the examination of faeces and then extended to the detection of *Campylobacter* in food and water.[1]
The selective media for isolation of *Campylobacter* consist of a non-selective base to be used with or without animal blood and of a mixture of antimicrobial compounds; among the isolation media proposed in the literature, the review by Corry and Atabay[1] mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.
Blood free formulations (e.g., mCCDA, Karmali) appear to have better performances than blood containing media.[2]
Campylobacter Blood Free Medium CCDA Bolton is prepared according the formulation proposed by Bolton, Hutchinson and Coates[3] and later modified by the replacement of cefazolin with cefoperazone to improve the selectivity properties.[4] The medium is recommended by ISO 10272[5,6] for detection and enumeration of *Campylobacter* spp. in samples of the food chain and by ISO 17995[7] for detection and enumeration of thermotolerant *Campylobacter* spp, in water. It is included by APHA[8] and FDA-BAM[9] in the range of selective isolating agars for the detection of *Campylobacter* in food.
The medium is known also as “modified charcoal cefoperazone deoxycholate agar (mCCD agar)”
Beef extract, tryptone and peptone provide nitrogen, carbon, minerals and amino acids for the microbial growth. Charcoal, sodium pyruvate and ferrous sulphate, enhance the isolation and the oxygen tolerance of *Campylobacter* spp. by quenching superoxide anions and hydrogen peroxide which occur spontaneously in the culture medium[10]; sodium chloride maintains the osmotic balance. The selective agents of the medium are: sodium deoxycholate active against Gram-positive bacteria, cefoperazone which mainly suppresses the growth of Gram-negative bacteria and amphotericin B, included as an antifungal compound.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 24.5 g of Campylobacter Blood Free Medium Base Bolton in 500 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes and cool to 47-50°C. Add the contents of one vial of Bolton CCDA Antimicrobic Supplement (REF 4240020) reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Campylobacter Blood Free Medium Base Bolton

Dehydrated medium appearance	black, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	black opaque
Final pH at 20-25 °C	7.4 ± 0.2

Bolton CCDA Antimicrobic Supplement

Freeze-dried supplement appearance	short, yellowish pastille
Reconstituted supplement appearance	pale yellow, opalescent

SPECIMENS

Water, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable International Standards[5-7] for the collection, transport, storage and preparation of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

Detection of *Campylobacter* in food samples

According to ISO 10272-1[5], depending on the type of sample and the purpose of the test, three different detection procedures of *Campylobacter* can be used:

A. detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters: enrichment in Bolton broth* with incubation in a microaerobic atmosphere at 37°C for 4 h to 6 h and then at 41.5 ± 1 °C for 44 ± 4 h.
B. detection of *Campylobacter* by enrichment, in samples with low numbers of campylobacters and high level of background microflora: enrichment in Preston broth^ with incubation in a microaerobic atmosphere at 41.5 ± 1 °C for 44 ± 4 h.
C. detection of *Campylobacter* by direct plating, in samples with high numbers of campylobacters.

Detection procedure A: from the enrichment culture in Bolton Broth, inoculate two selective solid media:
1) mCCD Agar, 2) any other solid selective *Campylobacter* medium using a different selective principle.
Detection procedure B: from the enrichment culture in Preston Broth inoculate the plates of mCCDA agar.
Detection procedure C: the test portion is plated directly or after suspending in an appropriate amount of liquid onto the plates of mCCD agar.
The selective isolation agars are incubated at 41.5 ± 1 °C in a microaerobic atmosphere and examined after 44 h to detect the presence of suspect *Campylobacter* colonies.

Enumeration of *Campylobacter* in food samples

According to ISO 10272-2[6], well dried mCCDA plates are inoculated with 0.1 mL of the test portion if the product is liquid or of the initial suspension in the case of other products.
Other plates are prepared under the same conditions, using decimal dilutions of the test portion or of the initial suspension.
If it is necessary to estimate low numbers of Campylobacter, the limit of enumeration may be lowered by examining 1 mL of the initial suspension distributed either on the surface of mCCDA agar plates in a large Petri dish (140 mm or three regular plates (90 mm), in duplicate.
The inoculum is spread over the surface of the agar and the plates are incubated at 41.5 ± 1 °C in a microaerobic atmosphere and examined after 44 h to record the number of suspect *Campylobacter* colonies.

Detection of *Campylobacter* in water samples

In general, the detection of *Campylobacter* in water, according to ISO 17995, requires enrichment followed by isolation of colonies and their confirmation.[7] Samples with expected high contamination levels are inoculated directly into Preston broth; where the expected level of background microorganisms is low and samples cannot be processed by membrane filtration, Bolton broth may be used. If no information about the contamination level is available, both broths should be used.
The incubated enrichment broths are inoculated onto mCCDA plates and incubated at 41.5 ± 1 °C for 44 ± 4 h.

*Bolton broth: Campylobacter Bolton Broth Base REF 401286B2 + Bolton Broth Selective Supplement REF 4240025.
^Preston broth: Nutrient Broth n° 2 REF 401812S2 + Preston Antimicrobic Supplement REF 4240022 + Lysed Horse Blood REF 90HLX100.

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Campylobacter colonies usually are greyish on mCCD Agar, often with a metallic sheen, and are flat and moist, with a tendency to spread. Colonies tend to spread less on drier agar surfaces. Other forms of colonies may occur.
The suspect *Campylobacter* colonies are examined for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirmed by detection of oxidase activity and an aerobic growth test at 25°C.
Characteristics of *Campylobacter*:
morphology: small curved bacilli, motility: characteristic corkscrew darting, aerobic growth at 25°C: negative, oxidase test: positive.
C. coli, *C. jejuni, C. lari, C. upsaliensis* can be differentiated by catalase test, hydrolysis of hippurate and indoxyl acetate test
As an alternative, or in addition, to the confirmation and identification tests, other tests such as PCR test, serological methods, MALDI-TOF-MS analysis, can be used.
For a complete explanation of the identification criteria and methods, refer to the quoted reference.[5-9]

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. jejuni	ATCC 33291	40.5-42.5°C / 40-48h / M	good growth
C. coli	ATCC 43478	40.5-42.5°C / 40-48h / M	good growth
E. coli	ATCC 25922	40.5-42.5°C / 40-48h / M	inhibited
S. aureus	ATCC 25923	40.5-42.5°C / 40-48h / M	inhibited

M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The most numerous contaminants found in the mCCD agar medium are *Enterobacteriaceae*, which are resistant to cefoperazone when present in high numbers, especially *Klebsiella oxytoca*.[1]
- The procedures described above target the thermotolerant *Campylobacter* spp. relevant for human health. The most frequently encountered strains are *C. jejuni* and *C. coli*. However, other species have been described (*C. lari, C. upsalinesis* and others).[5]
- The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat.[5]

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at 2-8°C away from direct light.
According to ISO 10272 the self-prepared mCCDA plates can be stored undried at 5 ± 3 °C in the dark for up to 1 month.[5,6]
Ready-to-use plates
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Corry JEL, Atabay HI. Culture Media for the Isolation of Campylobacters, Helicobacters and Arcobacters. *in* Handbook of Culture Media for Food and Water Microbiology, edited by Corry JEL, Curtis GDW, Baird RM. Published by the Royal Society of Chemistry, 3rd Edition 2012.
2. Fitzgerard C, Nachamamkin I. Campylobacter and Arcobacter. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.998.
3. Bolton FJ, Hutchinson DN, Coates D. A blood-free selective medium for the isolation of C.jeuni from faeces. J Clin Microbiol 1984; 19:169.
4. Hutchinson DN, Bolton FJ. Improved blood-free medium for the isolation of Campylobacter jejuni from faecal specimens. J. Clin. Pathol. 1984, 37 pp. 956 – 957
5. ISO 10272-1:2017+A1:2023. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method
6. ISO 10272-2:2017+A1:2023. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 2: Colony-count technique
7. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant Campylobacter spp
8. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
9. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. Chapter 7: Campylobacter. Content current as of: 08/03/2021
10. Karmali, M.A., Simor, A.E., Roscoe, M., Fleming, P.C., Smith, S.S., Lane, J. (1986) J. Clin. Microbiol. 21, 456-59

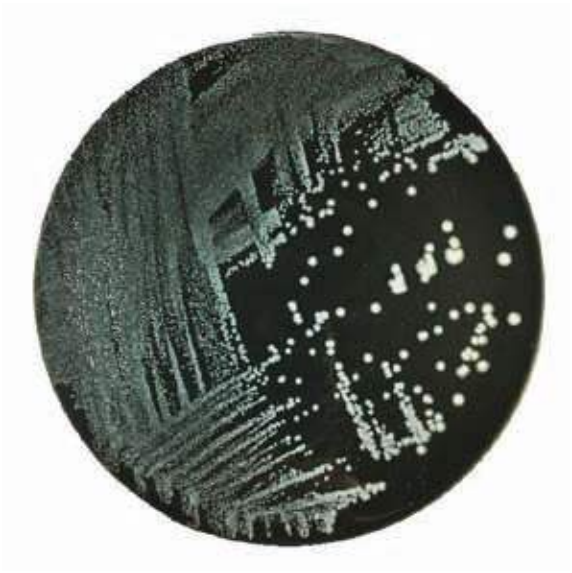
PACKAGING

Product	Type	REF	Pack
Campylobacter Blood Free Medium Base Bolton (m-CCDA)	Dehydrated medium	4012822	500 g (10.2 L)
Bolton CCDA Antimicrobic Supplement	Freeze-dried supplement	4240020	10 vials, each for 500 mL of medium
Campylobacter Blood Free Agar (CCDA Bolton)	Ready-to-use plates	541113	2 x 10 plates ø 90 mm

IFU rev 2, 2023/02

CAMPYLOBACTER BLOOD FREE MEDIUM BASE (KARMALI)
KARMALI ANTIMICROBIC SUPPLEMENT
CAMPYLOBACTER BLOOD FREE AGAR (KARMALI)

Dehydrated culture medium and selective supplement



Campylobacter coli on
Campylobacter Blood Free Medium (Karmali)

INTENDED USE

In vitro diagnostics. Basal medium, selective supplement and ready-to-use plates for the isolation of thermotolerant *Campylobacter* spp. from clinical and other specimens.

COMPOSITION *

CAMPYLOBACTER BLOOD FREE MEDIUM BASE (KARMALI), DEHYDRATED MEDIUM TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10.000 g
Tryptose	10.000 g
Peptone	3.000 g
Maize starch	1.000 g
Sodium chloride	5.000 g
Charcoal	4.000 g
Haematin	0.032 g
Sodium pyruvate	0.100 g
Cycloheximide	0.100 g
Agar	14.00 g

KARMALI ANTIMICROBIC SUPPLEMENT (VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefoperazone	16 mg
Vancomycin	10 mg

CAMPYLOBACTER BLOOD FREE AGAR (KARMALI) READY-TO-USE PLATES, TYPICAL FORMULA

Campylobacter Blood Free Medium Base (Karmali)	48.2 g
Cefoperazone	32.0 mg
Vancomycin	20.0 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Campylobacter spp. are Gram-negative, oxidase-positive, non-spore forming, S-shaped, or spiral rods, 0.2–0.9 µm wide and 0.5–5 µm long. Organisms are usually motile by means of a single polar unsheathed flagellum at one or both ends, that gives them a very characteristic "corkscrew" motility.[1] An atmosphere containing reduced oxygen (5 to 6%) is required for microaerobic growth. The species most commonly associated with disease in humans are thermotolerant: they will grow at 42°C - 43°C and 37°C, but not at 25°C. *Campylobacter jejuni* subspecies *doylei*, *Campylobacter fetus* and *C. fetus* subspecies *venerealis* do not grow at 42°C.[2]

In *Campylobacter* infection (campylobacteriosis), the symptoms usually range from none to severe, including fever, abdominal cramping, and diarrhoea (with or without blood/faecal white cells); nausea and vomiting may accompany the diarrhoea. Extraintestinal infections have been reported following *Campylobacter* enteritis in less than 0.15% of patients, usually in very old or very young subjects, and include bacteraemia, hepatitis, pancreatitis, meningitis, endocarditis, septic arthritis, abortion, neonatal sepsis; *C.jejuni* is the most often recognized infection preceding the development of Guillain-Barré syndrome.[1]
Campylobacter infections are acquired by ingestion of undercooked poultry, seafood, meat and produce, by the contact with animals and by drinking untreated water or milk. Most infections are caused by *C. jejuni* subsp. *jejuni* and *C. coli*: other species which sometimes cause diarrhoea are *C. lari*, *C. fetus subsp. fetus*, *C. jejuni subsp. doylei* and *C. upsaliensis*.
Since the early 1970s, when *C. jejuni* and *C. coli* have been recognised as agents of gastrointestinal infections associated with food poisoning, several liquid and plated culture media have been developed, originally designed for the examination of faeces and then extended to the detection of *Campylobacter* in food and water.[3] The selective media for isolation of *Campylobacter* consist of a non-selective base to be used with or without animal blood and of a mixture of antimicrobial compounds; among the isolation media proposed in the literature, the review by Corry and Atabay[3] mentions the following media: Skirrow, Blaser Wang, Preston, mCCD Bolton, mCCD Hutchinson and Bolton, Karmali, Line TTC.
Campylobacter Blood Free Medium Base Karmali and the selective supplement Karmali Antimicrobic Supplement are prepared according to the formulation devised by Karmali in 1986.[4] Campylobacter Blood Free Medium Karmali and Karmali Supplement are intended for the isolation of thermotolerant *Campylobacter* spp. from faeces and other non-clinical samples.
The medium of Karmali *et al*. is a variation of mCCDA of Bolton, Hutchinson and Coats[5], using haematin rather than ferrous sulphate, vancomycin instead of sodium deoxycholate and cycloheximide instead of amphotericin B.
The selective agents of the medium are vancomycin, with a strong inhibitory activity against Gram positive bacteria, cefoperazone, which mainly suppresses the growth of Gram-negative bacteria and cycloheximide, included as an antifungal compound. Charcoal (in substitution of animal blood), haematin and sodium pyruvate stimulate the growth of *Campylobacter*, increase its aero tolerance and inhibit the toxic compounds that are formed during the growth.
Karmali Medium (KM) was compared to Skirrow medium (SKM) for the recovery of *C. jejuni* and *C. coli* from stool of patients with diarrhea.[3] These campylobacters were isolated from 35 (2.9%) of 1,227 stools tested (29 on both media, 5 on KM alone, and one on SKM alone). Whenever *C. jejuni* and *C. coli* were recovered, growth was pure on 29 KM cultures (85%), but on only 11 SKM cultures (37%). Complete suppression of "contaminating" flora occurred in 704 KM cultures (57%) compared with 426 SKM cultures (35%).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 24.1 g in 500 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 118°C for 15 minutes and cool to 47-50°C. Add the contents of one vial of Karmali Antimicrobic Supplement (REF 4240035) reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Campylobacter Blood Free Medium Base Karmali

Dehydrated medium appearance	black, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	black opaque
Final pH at 20-25 °C	7.4 ± 0.2

Karmali Antimicrobic Supplement

Freeze-dried supplement appearance	short, dense, white pastille
Reconstituted supplement appearance	limpid, colourless

SPECIMENS

Faecal specimens are preferred for isolating *Campylobacter* spp. from patients with gastrointestinal infections; however, rectal swabs are acceptable for culture.[3] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied. For non-clinical samples, refer to the applicable international standards.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Solid faeces: faeces may be diluted 1:4 in sterile saline solution or 0.1% peptone water. It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens.[2] Inoculate 3-5 drops on the medium surface.
Liquid stool: inoculate 3 drops on the medium surface.
Rectal swabs: roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
For all type of specimens, streak with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Incubate in a microaerobic atmosphere consisting approximately of 5% O_2, 10% CO_2, and 85% N_2, at 39-42°C for 40-48 hours.[2]

READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Campylobacter colonies usually are grey/white or creamy grey in colour, swarming and moist in appearance. They may appear as a layer of growth over the surface of the agar. Colonies are usually non-pigmented.
Campylobacter species are oxidase positive. If a colony phenotypically resembling *Campylobacte*r species is oxidase negative, subculture to blood agar and retest after 24hr incubation.[6]
The presumptive identification of thermophilic and enteropathogenic Campylobacter can be done on the basis oxidase test (+) and the characteristic motility.
For a complete explanation of the identification criteria and methods, refer to the quoted reference.[6]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. jejuni	ATCC 33291	39-42°C / 40-48h / M	good growth
C. coli	ATCC 43478	39-42°C / 40-48h / M	good growth
E. coli	ATCC 25922	39-42°C / 40-48h / M	partially or totally inhibited
S. aureus	ATCC 25923	39-42°C / 40-48h / M	inhibited

A: aerobic incubation; M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The most numerous contaminants found in the Karmali medium are *Enterobacteriaceae*, which are resistant to cefoperazone when present in high numbers, especially *Klebsiella oxytoca*.[1]
- To achieve the highest yield of *Campylobacter* from stool samples, a combination of media that includes Karmali medium and a second selective medium, based on a different selective system, appears to be the optimal method (e.g., Skirrow medium).[8]
- Extending the incubation time from 48 to72 h leads to an increase in the isolation rate.[8]
- Blood free formulations (e.g., Karmali, CCDA) appear to have better performances than blood containing media.[3]
- The clinical advantage of enrichment broths formulated to enhance the recovery of *Campylobacter* has not been studied adequately.[3] Enrichment seems not to be necessary for samples collected in the acute campylobacteriosis phase, while *Campylobacter* recovery increases in asymptomatic patients, in studies involving low numbers of the target organism, in samples not readily sent to the laboratory and in samples taken in the convalescence phase after an episode of diarrhea.[9,10]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at 2-8°C away from direct light in a dry place.
Selective supplement and ready-to-use plates
Store the products in the original package at 2-8°C away from direct light.

REFERENCES

1. Corry JEL, Atabay HI. Culture Media for the Isolation of Campylobacters, Helicobacters and Arcobacters. *in* Handbook of Culture Media for Food and Water Microbiology, edited by Corry JEL, Curtis GDW, Baird RM. Published by the Royal Society of Chemistry, 3rd Edition 2012.
2. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. ID30. Issue 8.1. 2014
3. Fitzgerard C, Nachamamkin I. Campylobacter and Arcobacter. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.998.
4. Karmali, M.A., Simor, A.E., Roscoe, M., Fleming, P.C., Smith, S.S., Lane, J. (1986) J. Clin. Microbiol. 21, 456-59
5. Bolton FJ, Hutchinson DN, Coates D. A blood-free selective medium for the isolation of C.jeuni from faeces. J Clin Microbiol 1984; 19:169.
6. Public Health England. Identification of Campylobacter species. ID23. Issue 3.1. 2018
7. Varoli, O., Gatti M. (1989) Personal communication.
8. Endtz HP, Ruijs GJ, et al. Comparison of six media including a semisolid agar for the isolation of various Campylobacter species from stool specimens. J Clin Microbiol 1991; 29:1007
9. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982; 35:462
10. Hutchinson DN, Bolton FJ. Is enrichment culture necessary for the isolation of Campylobacter jejuni from faeces? J Clin Pathol 1983; 36:1350-1352

PACKAGING

Product	Type	REF	Pack
Campylobacter Blood Free Medium Base Karmali	Dehydrated medium	4012832	500 g (10.4 L)
Karmali Antimicrobic Supplement	Freeze-dried supplement	4240035	10 vials, each for 500 mL of medium
Campylobacter Blood Free Agar Karmali	Ready-to-use plates	541136	2 x 10 plates ø 90 mm

IFU rev 4, 2022/04

Bolton broth

CAMPYLOBACTER BOLTON ENRICHMENT BROTH BASE

BOLTON BROTH SELECTIVE SUPPLEMENT

Dehydrated culture medium and selective supplement

INTENDED USE

Selective liquid medium for the enrichment of *Campylobacter* spp. in food and water samples.

COMPOSITION*

CAMPYLOBACTER BOLTON ENRICHMENT BROTH BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of animal tissue	10.00 g
Lactalbumin hydrolysate	5.00 g
Yeast extract	5.00 g
Sodium chloride	5.00 g
Sodium pyruvate	0.50 g
Sodium metabisulfite	0.50 g
Sodium carbonate	0.60 g
α- ketoglutaric acid	1.00 g
Haemin	0.01 g

BOLTON BROTH SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefoperazone	10 mg
Vancomycin	10 mg
Trimethoprim lactate	10 mg
Amphotericin B	5 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Bolton broth, prepared with Campylobacter Bolton Enrichment Broth Base and the addition of Bolton Broth Selective Supplement and lysed horse blood, corresponds to the medium proposed by Bolton[1] to aid resuscitation of sub-lethally damaged campylobacters.
The current ISO method[2] for foods in general recommends the use of Bolton broth as a selective enrichment medium for samples with low numbers of campylobacters and low level of background microflora and/or with stressed campylobacters (ISO detection procedure A) and the use of Preston broth for samples with low numbers of campylobacters and high level of background microflora (ISO detection procedure B). There is a similar ISO method for water which uses Bolton broth as well as Preston broth.[3] The selective enrichment in Bolton Broth is also recommended by FDA-BAM.[4]
The enzymatic digest of animal tissue and lactalbumin hydrolysate provide nitrogen, carbon, minerals and amino acids for the microbial growth. The yeast extract is a source of vitamins particularly the B-group. Sodium pyruvate aids in resuscitation of stressed cells and with sodium metabisulfite and sodium carbonate enhances the isolation and the oxygen tolerance of *Campylobacter* spp. Sodium chloride maintains the osmotic balance. Alpha-ketoglutarate, hemin and lysed horse blood provide specific and essential nutritional factors for the growth of campylobacters. The selective agents of the medium are vancomycin active against Gram-positive bacteria, trimethoprim and cefoperazone which mainly suppresses the growth of Gram-negative bacteria and amphotericin B, included as an antifungal compound.

Directions for medium preparation

Suspend 13.8 g in 470 mL of purified water; heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool below 47 °C and add the contents of one vial of Bolton Broth Selective Supplement (REF 4240025) reconstituted with 5 mL of 50% ethanol/sterile purified water, together with 25 mL of lysed horse blood (REF 90HLX100). Mix well and distribute into sterile tubes or flasks.

Physical characteristics

Campylobacter Bolton Enrichment Broth Base

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow limpid
Prepared tubes appearance	dark red, limpid
Final pH at 20-25 °C	7.4 ± 0.2
Bolton Broth Selective Supplement	
Freeze-dried supplement appearance	short, pale-yellow pastille
Reconstituted supplement appearance	yellow, opalescent with precipitate

Specimens

Water, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable International Standards[2-4] for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

Test procedure

Food samples[2]

1. In general, for preparing the initial suspension, combine a quantity of 10 g or 10 mL of the test portion with 90 mL of Bolton broth, so as to obtain a 1 in 10 dilution, and homogenize.
2. Incubate in a microaerobic atmosphere at 37°C ± 1°C for 4 h to 6 h, then at 41.5°C ± 1°C for 44 h ± 4 h.
3. Using the culture obtained in the enrichment medium inoculate with a sterile 10 µl loop the surface of two selective isolation media: mCCD agar* and a second Campylobacter selective isolation medium using a different selective principle.
4. Incubate the selective solid media at 41.5°C ± 1°C in a microaerobic atmosphere and examine after 44 ± 4 h to detect the presence of suspect *Campylobacter* colonies.

Water samples[3]

1. In general, the detection of *Campylobacter* in water according to ISO 17995 requires enrichment followed by isolation of colonies and their confirmation.
2. Samples are inoculated either directly or after concentration using membrane filtration into one of two selective enrichment broths depending on the expected level of background microorganisms: Bolton broth for clean water and Preston broth^ for more heavily contaminated water. A single sample volume is processed for *Campylobacter* detection and, where necessary, at least three 10-fold volumes (for example 10 mL, 100 mL and 1000 mL) are used for a semi-quantitative determination. For a quantitative (MPN) determination, volumes of 500 mL, 5 x 100 mL, 5 x 10 mL and, where counts may be high, smaller volumes are used or the initial sample is diluted. The broths are then incubated microaerobically at 37 ± 1 °C for 44 ± 4 h.
3. From the enrichment broth cultures, liquid selective media are inoculated onto modified charcoal cefoperazone deoxycholate agar (mCCDA)*. The mCCDA plates are then incubated at 41.5 ± 1 °C for 44 ± 4 h in a microaerobic atmosphere.

Notes
* mCCDA agar: Campylobacter Blood Free Medium Base Bolton REF 401282 + Bolton CCDA Antimicrobic Supplement REF 42400120
^Preston broth: Nutrient Broth n° 2 REF 401812S2 + Preston Antimicrobic Supplement REF 4240022 + Lysed Horse Blood REF 90HLX100.

Reading and interpretation

Microbial growth in Bolton broth is evidenced by the development of turbidity.
After incubation of isolation plated media, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Campylobacter colonies usually are greyish on mCCD Agar, often with a metallic sheen, and are flat and moist, with a tendency to spread. Colonies tend to spread less on drier agar surfaces. Other forms of colonies may occur.
The suspect *Campylobacter* colonies are examined for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirmed by detection of oxidase activity and an aerobic growth test at 25°C.
Characteristics of *Campylobacter*:
morphology: small curved bacilli, motility: characteristic corkscrew darting, aerobic growth at 25°C: negative, oxidase test: positive
C. coli, *C. jejuni, C. lari, C. upsaliensis* can be differentiated by catalase test, hydrolysis of hippurate and indoxyl acetate test
As an alternative, or in addition, to the confirmation and identification tests, other tests such as PCR test, serological methods, MALDI-TOF-MS analysis, can be used.
For a complete explanation of the identification criteria and methods, refer to the quoted references.[2-4]

User quality control

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control according to ISO 10272-1[2]:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS (AFTER SUB-CULTURE)
C. jejuni 33291+*E. coli* 25922+*S. aureus* 6538	37±1°C-41.5 / 5 h+44±4 h / M	> 10 characteristic colonies on mCCD agar
C. coli 43478+*E. coli* 25922+ *S. aureus* 6538	37±1°C-41.5 / 5 h+44±4 h / M	> 10 characteristic colonies on mCCD agar
E. coli ATCC 25922	37±1°C-41.5 / 5 h+44±4 h / M	no growth after subculture on Tryptic Soy Agar
P. mirabilis ATCC 29906	37±1°C-41.5 / 5 h+44±4 h / M	no growth after subculture on Tryptic Soy Agar

M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The procedures described above target the thermotolerant *Campylobacter* spp. relevant for human health. The most frequently encountered strains are *C. jejuni* and *C. coli*. However, other species have been described (*C. lari, C. upsalinesis* and others).[2]
- The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat.[2]

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Freeze-dried supplement

Store the product in the original package at 2°C /+8°C away from direct light.

According to ISO 10272-1 the complete Bolton broth should be stored at 5 ± 3°C for not more than 7 days.[2] According to ISO 17995, the basal broth should be stored at 5 ± 3°C for not more than six months in the dark in airtight bottles.[3]

REFERENCES

1. Bolton, F. J. Methods for the isolation of campylobacters from humans, food and water. In: The Increasing Incidence of Human Campylobacteriosis. Report and Proceedings of a WHO Consultation of Experts, Copenhagen, Denmark, 21-25 November 2000, pp. 87 – 93
2. ISO 10272-1:2017+A1:2023. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method
3. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant Campylobacter spp
4. FDA-BAM Chapter 7: Campylobacter. Content current as of: 08/03/2021

PACKAGING

Product	Type	REF	Pack
Campylobacter Bolton Enrichment Broth Base	Dehydrated medium	401286B2	500 g (18.1 L)
Bolton Broth Selective Supplement	Freeze-dried supplement	4240025	10 vials, each for 500 mL of medium

IFU rev 3, 2023/02

CAMPYLOBACTER ENRICHMENT BROTH PRESTON (according to ISO Standards): Preston broth

see Nutrient Broth n° 2

CAMPYLOBACTER ENRICHMENT BROTH BASE
PRESTON ANTIMICROBIC SUPPLEMENT
(According to ISTISAN 86/95)
Dehydrated culture medium, selective supplement and ready-to-use tubes

INTENDED USE

Medium base and selective supplement for the enrichment of *Campylobacter* spp. in food samples.

COMPOSITION*

CAMPYLOBACTER ENRICHMENT BROTH BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10 g
Beef extract	10 g
Sodium chloride	5 g
Agar	1 g

PRESTON ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cycloheximide	50 mg
Rifampicin	5 mg
Trimethoprim lactate	5 mg
Polymyxin B	2500 IU

CAMPYLOBACTER ENRICHMENT BROTH, READY-TO-USE TUBES
TYPICAL FORMULA

Peptone	10 g
Beef extract	10 g
Sodium chloride	5 g
Agar	1 g
Cycloheximide	50 mg
Rifampicin	5 mg
Trimethoprim lactate	5 mg
Polymyxin B	2500 IU
Lysed horse blood	50 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Campylobacter Enrichment Broth Base with the addition of Preston Antimicrobic Supplement and lysed horse blood, corresponds to the medium proposed by ISTISAN Report 86-95[1] to aid resuscitation of sub-lethally damaged campylobacters. The medium represents a modification of the original formula described by Bolton and Robertson [2] and recommended by ISO 17995[3] as it contains 1 g/L of agar.

Peptone and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Agar, included at a concentration of 0.1 %, aids in initialization of the growth of *Campylobacter* and allows their growth from low inocula; it also retards the dispersion of CO_2, diffusion of oxygen and reducing substances. The selective agents of the medium are polymyxin B, active against Gram-negative bacteria, trimethoprim which mainly suppresses the growth of *Proteus* spp. and other Gram-negative bacteria, rifampicin active against Gram-negative and Gram-positive bacteria and cycloheximide, included as an antifungal compound. Lysed blood horse provides specific and essential nutritional factors for the growth of campylobacters.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 13 g in 500 mL of purified water; heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes.
Cool below 47°C and add the contents of one vial of Preston Antimicrobic Supplement (REF 42540017) reconstituted with 2 mL of 50% acetone/sterile purified water, and 25 mL of lysed horse blood (REF 90HLX100). Mix well and distribute into sterile tubes or flasks under aseptic conditions.

PHYSICAL CHARACTERISTICS

Campylobacter Enrichment Broth Base

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Prepared tubes appearance	dark red, limpid
Final pH at 20-25 °C	7.5 ± 0.2
Preston Antimicrobic Supplement	
Freeze-dried supplement appearance	short, red-brown pastille
Reconstituted supplement appearance	red limpid solution

SPECIMENS

Food samples. Refer to applicable International Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

1. In general, for preparing the initial suspension, combine a quantity of 25 g or 25 mL of the test portion with 225 ml of the enrichment medium, so as to obtain a 1 in 10 dilution, and homogenize.
2. Incubate the initial suspension in a microaerobic atmosphere at 42 °C for 18 hours.
3. Place a pipette about 2 cm below the meniscus of the broth and take an aliquot.
4. Sow 3-5 drops on the surface of a plate of Skirrow medium and a second selective medium such as Karmali or CCDA Bolton.
5. Streak the entire agar surface to obtain well-isolated colonies and incubate at 42°C for 24-48 hours in a microaerophilic atmosphere.
6. Select 5 typical colonies from the selective media and proceed to confirmation tests.

READING AND INTERPRETATION

Microbial growth in the selective enrichment broth is evidenced by the development of turbidity.
After incubation of isolation plated media, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
The suspect *Campylobacter* colonies are examined for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirmed by detection of oxidase and catalase, an aerobic growth test at 25°C, acid and gas in Triple Sugar Iron Agar (TSI), test with cephalothin discs. Optionally, the *Campylobacter* species are identified by specific biochemical tests and/or molecular methods.
The criteria for the identification of thermophilic *Campylobacter* and for the differentiation between species are indicated in the following tables.

Main characteristics of thermophilic *Campylobacter*

Cell morphology	small, slender, curved to spiral, Gram-negative rods
Motility	typical darting motility
Growth at 25°C	negative
Oxidase	positive
Catalase	positive
Acid/gas in TSI	negative
Cephalothin	resistant

Differential tests for *C. jejuni, C. coli, C. lari*

Test	***C. jejuni***	***C. coli***	***C. lari***
H_2S in TSI	-	-	-
Nalidixic Acid	*S*	*S*	*R*
Hippurate Hydrolysis	+	-	-

S = susceptible R = resistant

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. jejuni 33291+*E. coli* 25922+*P. mirabilis* 29906	42ºC 18 h / M	> 10 characteristic colonies on mCCD agar
C. coli 43478+*E. coli* 25922+*P. mirabilis* 29906	42ºC 18 h / M	> 10 characteristic colonies on mCCD agar
E. coli ATCC 25922	42ºC 18 h / M	no growth after subculture on Tryptic Soy Agar
P. mirabilis ATCC 29906	42ºC 18 h / M	no growth after subculture on Tryptic Soy Agar

M: microaerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at 2-8°C away from direct light.
According to ISO 17995[3] the complete Preston broth should be stored at 5 ± 3°C for not more than 7 days.

REFERENCES

1. Istituto Superiore di Sanità -Metodi di analisi per il controllo microbiologico degli alimenti – Raccolta a cura di D.De Medici, L.Fenicia, L.Orefice, A.Stacchini 1996, iv, 166 p. Rapporti ISTISAN 96/35
2. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982 Apr; 35(4):462-7.
3. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant Campylobacter spp
4. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

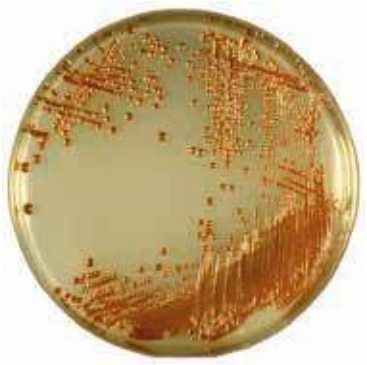
PACKAGING

Product	Type	REF	Pack
Campylobacter Enrichment Broth Base	Dehydrated medium	4012862	500 g (19.2 L)
Preston Antimicrobic Supplement	Freeze-dried supplement	4240017	10 vials, each for 500 mL of medium
Campylobacter Enrichment Broth	Ready-to-use tubes	551977	20 x 9 mL

IFU rev 1, 2022/11

CANDIDA AGAR (NICKERSON)

Dehydrated culture medium



Candida Agar Nickerson : *Candida albicans*

INTENDED USE

In vitro diagnostic. Selective medium for the isolation and differentiation of *Candida* spp. from clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Glycine	10.00 g
Bismuth ammonium citrate	5.00 g
Sodium sulphite	3.00 g
Glucose	10.00 g
Yeast extract	1.00 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Candida Agar (Nickerson), also known as BiGGY (Bismuth Glucose Glycine Yeast) Agar is prepared on the basis of the formulation described in 1953 by Nickerson.[1]
Candida Agar (Nickerson) is used for the isolation and presumptive differentiation of species of the genus *Candida* on the basis of colony morphology and colour from clinical specimens.[2-4]
Bismuthyl hydroxy sulphite complex produced into the medium by heat, is extracellularly reduced by *Candida* spp. to sulphide in a neutral or acidic environment and this reduction, depending on the intensity, results in brown to black pigmentation of the yeast colonies.[1]
Bismuth sulphite, bismuth ammonium citrate, glycine at high concentrations, act as selective compounds and the medium is not favourable for the development of schizomycetes; yeast extract and glucose are the nutritive bases.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 44 g in 1000 ml of cold purified water and mix thoroughly. Heat with frequent agitation until boiling and continue to boil for 30 to 60 seconds to dissolve the agar and obtain a uniform suspension. Cool to approximately 50°C, mix gently to disperse the precipitate evenly and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	white with flocculent precipitate
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Candida Agar (Nickerson) is intended for the bacteriological processing of non-sterile clinical specimens such as mouth, throat, pharyngeal, vaginal swabs. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[5]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.
Incubate in aerobic condition at 28-30°C for up to 5 days and examine daily for evidence of sulphite reduction.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record each specific morphological and chromatic characteristic of the colonies. The development of brown to black colonies allows a presumptive diagnosis of the genus. This diagnosis must be confirmed at least by microscopic examination (fresh preparation, clarified with methyl blue lactophenol: branched pseudomycelium, budding yeast cells, presence of chlamydospores, absence of ascospores). Presumptive species identification can be made by taking into account the colony characteristics of the main *Candida* species, summarised below.[2]
Candida albicans: smooth, circular or hemispherical brown-black colonies, slight mycelial fringe; no colour diffusion into surrounding medium; no sheen.
Candida tropicalis: smooth, discrete dark brown colonies with black coloured centres; slight mycelial fringe; diffuse blackening of medium after 72 hours; sheen.
Candida krusei: large, flat, wrinkled silvery brown-black colonies with brown peripheries; yellow halo diffused into medium.
Candida pseudotropicalis: medium size, flat, dark reddish-brown glistening colonies; slight mycelial fringe; no diffusion.
Candida parakrusei: medium size, flat, wrinkled, glistening dark reddish-brown colonies with light peripheries; extensive yellow mycelial fringe.
Candida stellatoidea: medium size, flat, dark brown colonies; very light mycelial fringe.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 60193	30°C / 72 H / A	growth with brown-black colonies
C. tropicalis NCPF 8841	30°C / 72 H / A	growth with dark brown colonies and metallic sheen
E. coli ATCC 25922	30°C / 72 H / A	growth inhibited
S. aureus ATCC 25923	30°C / 72 H / A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Pigmented bacteria and yeast-like fungi are usually inhibited on the medium. If they grow brown colonies, they are easily distinguished by microscopic observation; dermatophytes and moulds rarely grow and are distinguishable by the formation of aerial mycelia.[2]
- The medium should be prepared fresh, just prior to use.[2]
- Results in test tubes are not satisfactory.[1]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the plates should be freshly prepared just prior to use.[2]

REFERENCES

1. Nickerson, W. J. 1953. Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of Candida. J. Infect. Dis. 93:43.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
4. Lindsley M. Reagents Stains and Media: Mycology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. Berkow EL, McGowan KL. Specimen Collection, Transport and Processing: Mycology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
Candida Agar (Nickerson)	Dehydrated medium	4012802	500 g (11.4 L)

IFU rev 1, 2022/04

CARY-BLAIR TRANSPORT MEDIUM

Dehydrated culture medium

INTENDED USE

Medium for collection and transport of samples for microbiological analysis of enteric pathogens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Disodium hydrogen phosphate	1.1 g
Sodium thioglycolate	1.5 g
Sodium chloride	5.0 g
Calcium chloride	0.09 g
Agar	5.6 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Cary-Blair Transport Medium, prepared according to the formulation described by Cary and Blair,[1] is a medium with a defined composition intended for the storage and shipment of samples for microbiological analysis of enteric pathogens.
The sodium glycerophosphate present in Stuart's medium is replaced in the Cary-Blair formulation with a phosphate buffer to prevent overgrowth in the medium of contaminating bacteria possessing the enzyme glycerophosphate dehydrogenase (*Escherichia coli*, *Citrobacter freundii* or *Klebsiella aerogenes*). The medium is particularly suitable for shipment faecal samples for the detection of *Salmonella, Shigella, Vibrio* and *Campylobacter*.[2]
Cary and Blair report recovery of *Vibrio cholerae* up to 22 days of storage, of *Salmonella* and *Shigella* after 48 days and of *Yersinia pestis* up to 75 days with storage at 28°C.[1]
Neumann reports a survival in Cary Blair Medium of *Vibrio parahaemolyticus* of up to 35 days, with storage at 15-21°C.[3] Studies by Wells and Morris showed that recovery of *Shigella* is higher at 4°C or -20°C, especially when held longer than 3 days.[4]
The medium can also be used to transport anaerobic bacteria: in this case Cary Blair Medium must be prepared as sterilised, pre-reduced (PRAS) medium. PRAS medium production methods are described by Holdeman and Moore.[6]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 13.3 g in 1000 ml of cold purified water. Bring slowly to the boil and dispense into test tubes or vials with screw caps. Sterilise by flowing steam for 15 minutes. Allow to solidify and close the caps tightly.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	light yellow, opaque
Final pH at 20-25 °C	8.0 ± 0.5

SPECIMENS

Cary-Blair Transport Medium is suitable for collection and transport of faecal samples containing enteric pathogens.

TEST PROCEDURE

- Insert the swab into the medium to one-third of the medium depth.
- Cut or break the swab stick if longer than the tube.
- Screw the cap firmly.
- Transport to the laboratory as soon as possible or preferably within 24 hours.
- Transfer to appropriate isolation media depending on specimen source.
- Incubate plated media using proper microbiological procedures for cultivation of the suspected pathogens.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of varying morphology and size on the isolation media. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. flexneri ATCC 12022	20-25°C / 24h	good recovery after subculture to Blood Agar

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The survival of bacteria in a transport medium depends on many factors, including the type and concentration of bacteria in the sample and the temperature during the transport. Optimal growth and typical morphology can only be predicted following direct inoculation of the specimen and the use of an adequate isolation medium. Amies Transport medium, however, provides an adequate level of microbial survival in specimens that cannot be immediately forwarded to the laboratory.
- Cell viability may decrease during the storage period and some degree of multiplication of contaminating microorganisms may occur, especially for faecal specimens that contain a considerable number of coliforms.
- The condition of the sample received by the laboratory for culture is a significant variable in the recovery and final identification of the suspected pathogen. An unsatisfactory sample (invaded by contaminants, containing non-viable organisms or with a significantly reduced number of pathogens) may lead to incorrect or inconclusive results.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin[2] and Morris *et al*[7], prepared tubes may be stored at +2°C/+8°C or at room temperature for up to 19 months.

REFERENCES

1. Cary, S.G., Blair, E.B. New transport medium for shipment of clinical specimens. I Feacal specimen. J Bact 1964; 88: 96-98
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Neumann DA, Benenson MW, Hubster E,Tuan NTN. Cary-Blair, a transport medium for Vibrio parahemolyticus Am J Clin Pathol 1972 Jan;57(1):33-4
4. Wells JG and Morris GK. Evaluation of transport methods for isolating Shigella spp. J Clin Microbiol. 1981 Apr;13(4):789-90.
5. Barry A.L., Fay G.D., Sauer R.L. Efficiency of a transport medium for the recovery of aerobic and anaerobic bacteria from applicator swabs Appl Microbiol. 1972 Jul;24(1):31-3.
6. Holdeman LV and Moore WEC. Anaerobe Laboratory Manual, Virginia Polytechnic Institute, Anaerobe Laboratory, 3rd Ed.1975
7. Morris GK, Heck J. Quality of Cary Blair transport medium after aging nineteen months. J Clin Microniol 1978; 8:616.

PACKAGING

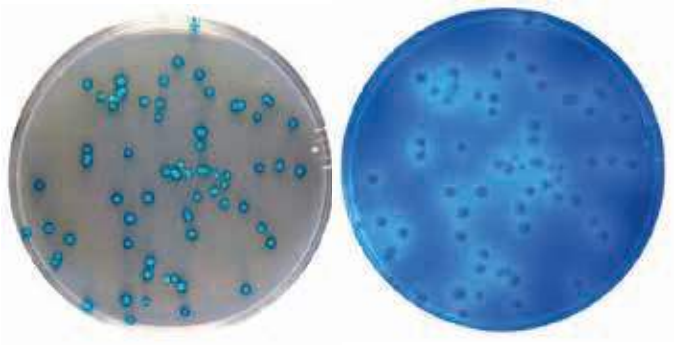
Product	Type	REF	Pack
Cary-Blair Transport Medium	Dehydrated medium	4012872	500 g (37.6 L)

IFU rev 4, 2023/05

ChromArt

C-EC AGAR

Dehydrated and ready-to-use culture medium



On the left: *E. coli* colonies in sunlight; on the right: the same plate under Wood's lamp.

INTENDED USE

Chromogenic and fluorogenic medium for the simultaneous detection of coliforms and *Escherichia* coli in water.

COMPOSITION – TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES AND FLASKS

Tryptose	10.00 g
Tryptophan	1.00 g
Peptocomplex	5.00 g
Yeast extract	3.00 g
Sodium chloride	5.00 g
Bile Salts n. 3	1.50 g
Isopropyl ß-D-1-thiogalactopyranoside (IPTG)	0.10 g
5-Bromo-4-chloro-3-indolyl beta-galactoside (X-GAL)	0.08 g
4-Metilumbelliferil-β-D-glucuronide (MUG)	0.05 g
Agar	13.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Faecal pollution is the major cause of waterborne diseases, since most of the pathogens associated with transmission reside in human and warm-blooded animal faeces. Examination of samples of sanitary significance for the presence of *E. coli* and coliform bacteria provides an indication of such pollution.
C-EC Agar medium allows the simultaneous quantitative determination of total coliforms and *E. coli* with incubation at 37°C for 18-24 hours, or the detection of *E. coli* and faecal coliforms with incubation at 44°C. C-EC Agar is included in the APAT guidelines for water analysis.[1]
Tryptose and Peptocomplex provide nitrogen, carbon, amino acids and minerals for the microbial growth, yeast extract is a source of vitamins, particularly of group B. Sodium chloride maintains the osmotic balance. Sodium pyruvate stimulates a quick bacterial growth and aids in resuscitation of stressed cells. Bile salts no. 3 inhibit the growth of Gram-positive bacteria.
Detection of coliform bacteria is based on the ability of β-D-galactosidase to cleave the substrate X-GAL with the formation of blue-green colonies. Enumeration of *E. coli* is based on the detection of β-D-glucuronidase, in addition to β-D-galactosidase, which cleaves the fluorogenic substrate MUG, with the formation of dark blue colonies, fluorescent under Wood's lamp. The hydrolysis of X-GAL in enhanced by IPTG, a lactose operon inducer. The indole test can confirm the presence of *E. coli* by adding a drop of Kovacs' reagent to the colonies.
C-EC Agar was one of the first chromogenic culture media proposed in the early 1990s for microbial isolation and differentiation and has been the subject of published trials by Bonadonna *et al.*[2,3], Jermini *et al.*[4], Cesaroni *et al.*[5].

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 38.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation until completely dissolved. Sterilize in the autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and dispense in sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates and flasks appearance	pale yellow, limpid or slightly opalescent
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

SPECIMENS

Water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and norms.

TEST PROCEDURE

Filter 100 mL (or other volumes, e.g., 250 mL for bottled water) of the sample using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and, preferentially, with grid lines. The minimum volume for filtration is 10 mL of sample or dilutions thereof to ensure even distribution of the bacteria on the membrane filter.
After filtration place the membrane filter on the C-EC Agar, ensuring that no air is trapped underneath, invert petri dish, and incubate at 36 ± 1 °C for 18-24 h (*E. coli* and coliforms detection) or at 44 ± 1°C for 18-24 hours for *E. coli* and faecal coliforms detection.[1]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies in natural light and under a Wood's lamp in semi-dark surroundings and count:
All blue-green colonies (positive for β-D-galactosidase reaction) as presumptive coliform or faecal coliform bacteria (depending on the incubation temperature).
All blue-green colonies (β-D-galactosidase positive) and fluorescent under Wood's lamp (β-D-glucuronidase positive) as *E. coli*.
Confirmation of *E. coli* identification can be performed by the indole test (+), directly on the plate with Kovacs' Reagent (REF 19171000).

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C/ 18-24H/A	growth, blue-green colonies, fluorescent under Wood's lamp
E. aerogenes ATCC 13048	37°C/ 18-24H/A	growth, pink colonies, non-fluorescent under Wood's lamp
P. aeruginosa ATCC 27853	37°C/ 18-24H/A	growth, colourless colonies
E. faecalis ATCC 19433	37°C/ 18-24H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of Salmonella subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia, Enterobacter* and *Yersinia* strains (1-5%).[6-8]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[9,10] Consequently, these strains, being positive for β-galactosidase, will grow with blue-green colonies not fluorescent and be counted as coliforms.
- In addition to expressing β-D-glucuronidase, *E. coli* is able to produce indole from tryptophan. Therefore, in case of any doubt of *E. coli* colonies on the primary agar medium, indole test may be used as an additional confirmation.[9]
- To avoid false-positive results, caused by oxidase positive bacteria, for example, *Aeromonas* spp., the presumptive coliforms colonies shall be confirmed by a negative oxidase reaction (Oxidase Test Strips, REF 191040ST)
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium

Store at +2°C /+8°C away from direct light in a dry place.
According to APAT guidelines the self-prepared plates of C-EC Agar can be stored at 2-8°C for up to 2 weeks.[1]

Ready-to-use plates and flasks

Store plates or flasks in their original pack at 2-8°C away from direct light.

REFERENCES

1. APAT, IRSA-CNR. Metodi analitici per le acque. Volume Terzo, Sezione 7000 - Determinazione di microorganismi. APAT Manuali e Linee Guida 29/2003.
2. Bonadonna L, Chiaretti G, Coccia AM, Semproni M. Valutazione comparativa di procedure analitiche per il rilevamento di Enterobacteriaceae in acque marine costiere. ISSN 1123-3117, Rapporti ISTISAN 97/3.
3. Bonadonna L, Villa L. Un substrato cromogeno per l'isolamento dei coliformi totali nelle acque: il C-EC-MF Plate. Notiziario. Metodi Analitici per le Acque, Anno 13, N. 1, 1993.
4. Jermini M, Domeniconi F, Jaeggll M. 1994. C-EC-Agar, a Modified mFC-Agar for the Simoultaneous Enumeration of Fecal Coliforms and E. coli in water samples. Letters App Microbiol. 1994; 19: 332-335.
5. Cesaroni D, Felicori M, Marcon A, Piscolla F, Bertaccini E, Biserni R, Cirillo G, Gironi A. Valutazione preliminare di un nuovo substrato colturale per la determinazione dei coliformi e di *E coli* nelle acque. Convegno Microbiologia Alimentare: Aspetti Analitici e Legislativi - Bologna 25/2/1993.
6. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
7. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288
8. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Production of Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
9. ISO 9308-1:2014 Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora.
10. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288

PACKAGING

Product	Type	REF	Pack
C-EC Agar	Dehydrated medium	4012982	500 g (12.9 L)
C-EC MF Plate	Ready-to-use plates	497101	3 x 10 plates ø 55 mm
C-EC Agar	Ready-to-use flasks	5112982	6 x 100 mL

IFU rev 1, 2022/12

CHAPMAN STONE MEDIUM

Dehydrated culture medium

S. aureus on Chapman Stone Medium

INTENDED USE

Selective medium for the isolation and differentiation of staphylococci.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10 g
Yeast Extract	2 g
Gelatin	30 g
D-Mannitol	10 g
Sodium Chloride	55 g
Ammonium Sulphate	75 g
Dipotassium Hydrogen Phosphate	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Chapman Stone Medium is prepared on the basis of the formula described by Chapman[1] in 1948, which represents a modification of the Chapman's Staphylococcus Medium described in 1946[2], in which sodium chloride concentration is reduced to 5.5% and the ammonium sulphate is incorporated into the medium rather than applied to the plate after incubation. The medium was developed by Chapman, following Stone's observations of 1935[3] on the positivity to gelatinase test by staphylococci.

Chapman Stone Medium is a selective medium for the isolation and differentiation of staphylococci, based on the tolerance to high concentrations of sodium chloride, fermentation of mannitol and liquefaction of gelatin.
The medium is suitable for isolation and differentiation of staphylococci and for studies of food-poisoning outbreaks.[4] Chapman Stone Medium is included by Atlas in the reviews of culture media for isolation and differentiation of staphylococci from food[5] and environmental samples[6].
Tryptone and yeast extract provide nitrogen, carbon, minerals and vitamins for microbial growth. Potassium phosphate prevents pH changes. The selectivity of the medium is due to the presence of a relatively high NaCl content which allows a good growth of staphylococci and a partial to total inhibition of Gram-negative bacteria and enterococci. Mannitol is included as a fermentable carbohydrate: *Staphylococcus aureus* ferments mannitol producing the acidification around the colony; its fermentation can be detected by adding a few drops of bromo-cresol purple on the area of removed colonies from the agar plate, resulting in the production of a yellow colour. Gelatin serves as a substrate for gelatinase activity: gelatin hydrolysis is observed as clear zones around colonies.

Directions for medium preparation
Suspend 202 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

Physical characteristics
Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution appearance	yellowish, hazy
Prepared plates appearance	yellowish, opaque
Final pH at 20-25 °C	7.0 ± 0.2

Specimens
For food samples, refer to the applicable international standards.
Good laboratory practices for collection, transport and storage of specimens should be applied.

Test procedure
Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate inoculated plates in aerobic atmosphere at 30-35°C for 44-48 hours.

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
The presumptive differentiation between *S. aureus* and *S. epidermidis* is performed by reading the mannitol fermentation and gelatinase tests.
Mannitol fermentation: add 1 drop of 0,04% solution of bromocresol purple to the area where the colony has been removed: positive results are indicated by yellow colour (acid production).
Gelatinase activity: the inoculated medium is opaque and whitish in colour, owing to precipitates that form between the gelatin and ammonium sulphate; a positive reaction is indicated by a clear zone around the colony.
Yellow-orange, gelatinase positive and mannitol positive colonies are presumptively identified as *S. aureus*
White colonies, gelatinase positive and mannitol negative are probably *S. epidermidis*.
Confirm a possible *S. aureus* by testing for coagulase production that must be performed after the colony subculture in Nutrient Broth or BHI Broth or on a blood agar plate and incubation at 30-35°C for 18-24 hours; do not perform coagulase test directly with colonies grown on Chapman Stone Medium as salt content ~~m~~ may interfere with coagulase results.
Emulsify 0.5 mL of broth culture or a colony from blood agar with 0.5 mL of rabbit plasma (Coagulase Plasma EDTA cat. no. 429937).
Incubate at 35-37°C and examine hourly up to 3-6 hours. Do not shake or agitate the tube. Gently slant and examine for a clot which gels the whole contents of the tube or forms a loose web of fibrin.
If negative by the end of 3-6 hours, incubate overnight and re-examine at 24 hours. This is because a small proportion of strains require longer than 4 hours for clot formation.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
S. aureus ATCC 25923	30-35°C / 44-48 H / A	growth, colonies with a clear zone, mannitol positive
S. epidermidis ATCC 12228	30-35°C / 44-48 H / A	growth, colonies with a clear zone, mannitol negative
E. coli ATCC 25922	30-35°C / 44-48 H / A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- *E. faecalis* and other enterococci may exhibit growth and slight mannitol fermentation; however, the colonies are tiny and are easily differentiated from staphylococci by Gram staining and catalase test (*E. faecalis*: catalase negative, cocci in chains; staphylococci: catalase positive, cocci in clusters).
- For complete identification, biochemical, immunological, molecular or mass spectrometry tests must be performed on the isolates.

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates may be stored at +2°C/+8°C for up to 6-8 weeks.[4]

References

1. Chapman GH. An Improved Stone medium for the isolation and testing of food-poisoning Staphylococci. Food Res 1948; 13:100.
2. Chapman GH. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation, and the Stone reaction. J Bacteriol 1946; 51:409-410.
3. Stone RV. A cultural method for classifying Staphylococci as of the “food poisoning” type. Proc Soc Exper Biol Med 1935; 33:185-187.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Atlas R. The Handbook of Microbiological Media for the Examination of Food. CRC Press,1995
6. Atlas R. The Handbook of Microbiological Media for Environmental Microbiology. CRC Press,1995

PACKAGING

Product	Type	REF	Pack
Chapman Stone Medium	Dehydrated medium	4013002	500 g (2.5 L)

IFU rev2, 2022/05

CHLORAMPHENICOL GLUCOSE YEAST EXTRACT AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

Selective medium for the isolation and enumeration of yeasts and moulds in milk, dairy products and other foodstuffs.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED AND READY-TO-USE FLASKS

Yeast extract	5.0 g
Glucose	20.0 g
Chloramphenicol	0.1 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Antibiotic supplemented media has been found superior to acidified media for the enumeration of yeasts and moulds in foodstuffs: they are less inhibitory to injured cells, more effective in inhibiting bacterial growth and less likely to cause precipitation of food particles because of their higher pH.[1] Chloramphenicol Glucose Yeast Extract Agar is recommended by ISO 6611 and by FIL-IDF for the isolation and enumeration of yeasts and moulds in milk and dairy products.[2] Yeast extract is a source of vitamins, particularly of the B-group and provides the basic nutrients for microbial growth. Glucose is a carbon and energy source. Chloramphenicol is a thermostable broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 40.1 in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121° C for 15 minutes. Cool to 45-50°C, mix well and distribute in sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (MEDIUM IN FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared flasks appearance	pale yellow, limpid
Final pH at 20-25 °C	6.6 ± 0.2

SPECIMENS

Milk, dairy products and other foodstuffs. Refer to applicable International Standards and regulations and operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

Prepare the sample suspension and the further decimal dilutions with the suitable diluent.[2]
Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1mL of each decimal dilution, in duplicate, to the centres of empty Petri dishes.
Add to each plate 15 mL of medium pre-cooled to 45°C. Mix the inoculum with the melted agar and leave to solidify.
Incubate the plates in an inverted position at 25°C and enumerate the colonies after 3-5 days.
To prevent the spreading of the colonies, some precautions should be taken such as: 1) an addition of an overlayer of culture medium after solidifying or 2) an addition of a drop of glycerol on filter paper on the lid of the dish.[2]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies, distinguishing yeasts from moulds. Select plates containing 10-150 colonies and count the colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	25°C/3 days /A	good growth, typical colonies
A. brasiliensis ATCC 16404	25°C/3 days /A	good growth, typical colonies
P. cyclopium ATCC 16025	25°C/3 days /A	good growth, typical colonies
E. coli ATCC 25922	25°C/3 days /A	inhibited
B, subtilis ATCC 6633	25°C/3 days /A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The method described above is not suitable for a large number of thermolabile yeasts in fresh cheeses. In such case the agar surface plating method is preferred.[2]

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use medium in flasks
Store flasks in their original pack at 2-8°C away from direct light.

REFERENCES

1. Beuchat LR. Media for detecting and enumerating yeasts and mould. Int J Food Protect 1992; 17:145:58.
2. ISO 6611:2004 [IDF 94:2004] Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 degrees.

PACKAGING

Product	Type	REF	Pack
Chloramphenicol Glucose Yeast Extract Agar	Dehydrated medium	4012892 4012894	500 g (12,5 L) 5 kg (125 L)
Chloramphenicol Glucose Yeast Extract Agar	Ready-to-use flasks	5112892	6 x 100 mL

IFU rev 1, 2022/06

CHOCOLATE AGAR BACITRACIN

Ready-to-use plates

INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of *Haemophilus* spp. from clinical specimens with mixed flora.

COMPOSITION - TYPICAL FORMULA *

Peptocomplex	15 g
Corn starch	1 g
Dipotassium hydrogen phosphate	4 g
Potassium dihydrogen phosphate	1 g
Sodium chloride	5 g
Agar	12 g
80°C heated defibrinated horse blood	70 mL
Vancomycin	5 mg
Bacitracin	20000 UI
Purified water	1000 mL
Biovitex Enrichment Supplement	
Nicotinamide adenine dinucleotide (NAD)	2.50 mg
Cocarboxylase	1.00 mg
p-aminobenzoic acid	0.13 mg
Thiamine	0.03 mg
Vitamin B12	0.10 mg
L-glutamine	100.00 mg
L-cystine	11.00 mg
L-cysteine HCl	259.00 mg
Adenine	10.00 mg
Guanine HCl	0.30 mg
Ferric nitrate.6H20	0.20 mg
Glucose	1.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Haemophilus influenzae, a fastidious Gram-negative bacillus, is frequently found in the upper respiratory tract of healthy humans. In addition to various systemic life-threatening infections, *H. influenzae* is a common cause of serious diseases of the upper and lower respiratory tract.
In 1969 Hovig and Aandahl[1] formulated a selective medium for the isolation of *Haemophilus* spp. from respiratory tract, incorporating bacitracin 300 mg/L into chocolate agar. The use of the selective medium increased the isolation rate of *Haemophilus* sp. from all specimens: for nose swabs the isolation rate increased from 32% to 41.3%, for throat swabs from 30.7% to 98.7%, for sputum samples from 3.4% to 61.4%.[1]
Chocolate Agar Bacitracin is a selective medium prepared with GC Medium base, supplemented with heated defibrinated horse blood, Biovitex and bacitracin, for the isolation and cultivation of *Haemophilus* spp. from specimens contaminated by less fastidious commensal bacteria.[2-4]
Peptocomplex provides carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, dibasic and monobasic potassium phosphates buffer prevent pH changes due to amine production, corn starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth. Heated horse blood provides hemin (X factor) required for growth of *Haemophilus spp*. The medium is supplemented with Biovitex that provides V factor (NAD) vitamins, amino acids, coenzymes, dextrose, ferric ions and other factors which improve the growth of species requiring V factor for the growth: *H. influenzae H. ducrey, H. aegytpius, H. haemolyticus*.[2] Bacitracin suppresses the growth of most strains of streptococci, staphylococci, micrococci and *Neisseria*, vancomycin is active against Gram positive bacteria.

PHYSICAL CHARACTERISTICS

Medium appearance	brownish, opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Chocolate Agar Bacitracin plates can be directly inoculated with clinical specimens collected from non-sterile human sites such as ear and respiratory tract.[2-4]. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[3-5]

TEST PROCEDURE

Allow plates to come to room temperature. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate at 35-37°C in a moist atmosphere in the presence of 5-10% CO_2 and record the results after 24 and 44-48 hours, to obtain satisfactory growth of *H. influenzae* and most other *Haemophilus* species. When specimens for *H. aegyptius* and *H. ducreyi* are cultured, incubation may be necessary for up to 5 days.[2] Further, when *H. ducrey* is suspected in the specimen, plates should be incubated at 30-33°C in 5% CO_2 in a high-moisture environment.[2]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic characteristics of the colonies.
The colonies' morphology of *Haemophilus* spp. are summarized here below.[2]
Colonies of *H. influenzae* have a diameter of about 1-2 mm, are smooth, low, convex, greyish, and translucent, with a characteristic "mousy" odour (non-indole producing strains) or a strong amine-like odour (indole producing strains)
Colonies of *H. aegytpius* reach a colony size of 0.5 mm after 48 hours of incubation; colonies are low, convex, translucent with a smooth entire surface.
Colonies of *H. parainfluenzae* are typically off-white to yellow and, like *H. influenzae*, 1 to 2 mm in diameter. The colony appearance is extremely varied.
Colonies of *H. haemolyticus* are translucent, smooth, and convex.
Colonies of *H. ducrey* are small, flat, grey, and smooth.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
H. influenzae	ATCC	10211	35-37°C / 24-48H / CO_2	good growth
S. pyogenes	ATCC	19615	35-37°C / 44-48H / CO_2	growth inhibited

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The presence of small particles may sometimes be observed in the agar. However, this phenomenon does not affect the performance of the medium.
- *E. coli*, some *Neisseria* and *Candida* species, *Klebsiella, Proteus*, and *Pseudomonas* spp., as well as other Gram-negative bacteria may grow on this medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Hovig B, Aandahl EH. A selective method for the isolation of Haemophilus in material from the respiratory tract. Acta Pathol Microb Scand 1969; 77:676-84
2. Gonzales MD, Ledeboer NA. Haemophilus. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
3. Public Health England- UK Standards for microbiology investigations (UK SMI) Investigation of Ear Infections and Associated Specimens. B 1 Issue 9, 2014
4. Public Health England- UK Standards for microbiology investigations (UK SMI) Investigation of bronchoalveolar lavage, sputum and associated specimens B 57, Issue n° 3.5, 2019
5. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology. *In* In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
Chocolate Agar Bacitracin	Ready-to-use plates	541519	2 x 10 plates ø 90 mm

IFU rev 1, 2020/05

Chocolate Agar Bacitracin:
colonies of *Haemophilus influenzae*

CHOCOLATE AGAR ENRICHED

Ready-to-use plates

Neisseria gonorrhoeae
on Chocolate Agar Enriched

INTENDED USE

In vitro diagnostic device. Non selective, general-purpose medium for the isolation and cultivation of nutritionally fastidious microorganisms, from clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Peptocomplex	15 g
Corn starch	1 g
Dipotassium hydrogen phosphate	4 g
Potassium dihydrogen phosphate	1 g
Sodium chloride	5 g
Agar	12 g
80°C heated defibrinated sheep blood	50 mL
Purified water	1000 mL
Biovitex Enrichment Supplement	
Nicotinamide adenine dinucleotide (NAD)	2.5 mg
Cocarboxylase	1 mg
p-aminobenzoic acid	0.13 mg
Thiamine	0.03 mg
Vitamin B12	0.1 mg
L-glutamine	100 mg
L-cystine	11 mg
L-cysteine HCl	259 mg
Adenine	10 mg
Guanine HCl	0.3 mg
Ferric nitrate.$6H_20$	0.2 mg
Glucose	1 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In 1945, Johnston[1] described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 hours as opposed to previous 48 hours methods. This medium was later modified by Carpenter and Morton[2] using GC Medium Base with the addition of haemoglobin and a yeast concentrate. The medium was further improved by replacing yeast concentrate with a chemically defined supplement formulated specifically to facilitate the growth of gonococci.[3]

Chocolate Agar Enriched is a non-selective, general-purpose medium, prepared with GC Medium Base, supplemented with heated defibrinated horse blood and Biovitex, for the isolation and cultivation of nutritionally fastidious microorganisms from clinical specimens.[4,5]

Peptocomplex provides carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, dibasic and monobasic potassium phosphates buffer prevents pH changes due to amine production, corn starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth.[4] Heated horse blood provides hemin (X factor) required for growth of *Haemophilus* and enhances growth of *Neisseria*. The medium is supplemented with Biovitex that provides V factor (NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria.*

PHYSICAL CHARACTERISTICS

Medium appearance	brown, opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Chocolate Agar Enriched plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens types, related to specific infections.[6-8] Chocolate Agar Enriched is not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[6]

TEST PROCEDURE

Allow plates to come to room temperature. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate at 35-37°C in aerobic conditions with 5 -10% CO_2, and record the results after 18-24 and 48 hours.

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols. Consult the procedures outlined in the references for further information.[7,8]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic characteristics of the colonies.

Colonies of *Haemophilus influenzae* have a diameter of about 1-2 mm, are colourless, transparent, moist and tend to be translucent, with a characteristic “mousy” odour.

Colonies of *N. gonorrhoeae* are of variable diameter (0.5 - 2 mm), moderately convex, raised, finely granular, glistening, with entire or lobate margins. For other fastidious microorganisms, refer to appropriate references and procedures for results reading and interpretation.[7,8]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
H. influenzae ATCC 10221	35-37°C / 18-24H / CO_2	good growth
N. gonorrhoeae ATCC 43069	35-37°C / 18-24H / CO_2	good growth

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The presence of small particles may sometimes be observed in the agar. However, this phenomenon does not affect the performance of the medium.
- The growth on Chocolate Agar Enriched depends on the metabolic requirements of each microorganism; it is possible that some strains are unable to grow on the medium.
- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional selective media such as Thayer Martin for the isolation for gonococcus and chocolate agar with bacitracin for the isolation of *H. influenzae*.
- For the growth of *N. gonorrhoeae,* it is necessary that the surface of the plates is moist; if it appears dry, humidify with a few drops of sterile purified water. Place damp gauze or paper towels in the CO_2 container before incubation or use an incubator with humidifier.[4]
- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N. gonorrhoeae*.[4]
- The gonococci are one of the most fragile Gram-negative bacteria. It is recommended that any suspected *Neisseria* containing specimen should be inoculated onto primary isolation medium immediately on collection to avoid any loss in viability and/or overgrowth of contaminants; if this is not possible *N. gonorrhoeae* swabs are better held at 4-6° C for not more than 3 hours.[4]
- If *N. gonorrhoeae* is suspected, the incubator temperature should be set at 35-36,5°C with 5% CO_2, because many strains of *N. gonorrhoeae* will not grow well at 37°C and grow poorly with 10% CO_2.[4,9]
- The presence of colonies on Chocolate Agar Enriched is not an indication, by itself, of the presence of pathogenic microorganisms: user must differentiate potential pathogens requiring biochemical, immunological, molecular, or mass spectrometry testing for identification and antimicrobial testing from contaminants that represent members of normal microbiota.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Johnston J. Comparison of gonococcus cultures read at 24 and 48 hours. J Vener Dis Inform 1945; 26:239.
2. Carpenter CM, Morton HE. An improved medium for isolation of the gonococcus in 24 hours. Proc. N.Y. State Assoc. Public Health Labs 1947; 27:58-60.
3. Martin JE Jr, Billings TE, Hackney JF, Thayer JD. Primary isolation of N. gonorrhoeae with a new commercial medium. Public Health Rep. 1967; 82:361-363.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Atlas R, Snyder J. Media Reagents and Stains. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.345.
6. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
7. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
8. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019.
9. CDC: Morbidity and Mortality Weekly Report (MMWR). Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae. Infections Recommendations and Reports. October 18, 2002 / Vol. 51 / No. RR-15

PACKAGING

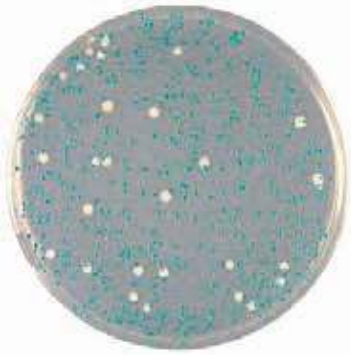
Product	Type	REF	Pack
Chocolate Agar Enriched	Ready-to-use plates	541521	2 x 10 plates ø 90 mm

IFU rev 1, 2020/05

ChromArt

CHROMALBICANS AGAR

Dehydrated and ready-to-use culture medium



Chromalbicans Agar: *C. albicans* (blue-green colonies) and *C. tropicalis* (colourless colonies)

INTENDED USE

In vitro diagnostic. Selective and chromogenic medium for the isolation of *Candida* spp. from clinical specimens and for the differentiation of *Candida albicans* and *Candida dubliniensis* from other species of *Candida* genus.

COMPOSITION TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Growths factors	18.5 g
Chloramphenicol	0.05 g
Gentamicin	0.1 g
Tryptone	20 g
Glucose	1 g
Agar	13 g
Chromogenic substrate	0.1 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Since the early 90s advances have been made in laboratory methods for diagnosis of *Candida* species, especially *Candida albicans*, resulting in more rapid and reliable identification.[1-3] One of these methods was the incorporation of chromogenic substrates directly into the growth agar media. A common principle among these media is the inclusion of a chromogenic substrate for β-hexosaminidase thus allowing the differentiation and presumptive identification of the most frequent and clinically important species, *C. albicans*.[4]

Chromalbicans Agar is a “first generation” chromogenic and selective medium for the isolation of *Candida* spp. from clinical specimens and the differentiation of *C. albicans - C. dubliniensis* group from other species of *Candida* genus. The selectivity of the medium is due to the presence of chloramphenicol and gentamicin which suppress the growth of bacteria. Differentiation is obtained by the presence of a single chromogenic compound for the detection of β-hexosaminidase enzymatic activity of *C. albicans* and *C. dubliniensis*. The hydrolysis of the compound results in the release of an insoluble blue-green chromophore that remains inside the colonies giving them a typical colour.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 52,75 g in 1000 mL of cold purified water; bring to boiling with frequent agitation. Sterilize by autoclaving at 115°C for 15 minutes. Cool to 47-50ºC mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	whitish, opalescent
Final pH at 20-25°C	6.2 ± 0.2

SPECIMENS

Chromalbicans Agar is intended for the bacteriological processing of non-sterile clinical specimens such as mouth, throat, pharyngeal, vaginal swabs. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate by rolling the swab over a small area of the surface at the edge; then streak from this inoculated area to obtain well isolated colonies.
Incubate inoculated plates in aerobic conditions at 35-37°C for 18-24 or 48 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
C. albicans and *C. dubliniensis* grow with blue or blue-green colonies.
Other species of the genus *Candida* grow with colourless colonies.
Gram-positive and Gram-negative bacteria are almost inhibited.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans	ATCC	10231	35-37°C /18- 24h / A	good growth, blue-green colonies
C. tropicalis	NCPF	8841	35-37°C /18- 24h / A	good growth, colourless colonies
P. mirabilis	ATCC	10005	35-37°C /18- 24h / A	inhibited
P. aeruginosa	ATCC	27853	35-37°C /18- 24h / A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCPF: Public Health England, National Collection of Pathogenic Fungi.

PERFORMANCES CHARACTERISTICS

The performances characteristics of Chromalbicans Agar was evaluated by Carillo-Munoz *et al.*[5] with 723 clinical isolates and type culture collection strains from different genera including *Candida, Cryptococcus, Pichia, Rhodotorula, Saccharomyces, Trichosporon* and *Zygosaccharomyces*. Presumptive identification was confirmed by germ tube test and carbohydrate assimilation on API-ATB ID 32C Growth on Chromalbicans Agar was very useful for the presumptive identification of *C. albicans/C. dubliniensis* isolates, and sensitivity and specificity values were significantly high (>97%), since a very low number of isolates were found to be false negative or false positive. Sensitivity of *C. albicans/C. dubliniensis* detection: 97.09%; specificity *of C. albicans/C. dubliniensis* detection: 97.63%.
Predictive value of the negative result: 97.38%; predictive value of the positive result: 97.37%.

LIMITATIONS OF THE METHOD

- *C. dubliniensis* is ß-hexosaminidase positive and grows with blue-green colonies and therefore it is not differentiable from *C. albicans*.
- The medium contains a single chromogenic substrate for the detection of ß-hexosaminidase positive strains (*C. albicans* and *C. dubliniensis*) and therefore doesn’t allow the differentiation between other species of the genus *Candida* which grow with colourless colonies.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
Ready-to-use plates
Store plates in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Polacheck I, Melamed M, Bercovier H, Salkin IF. Beta-Glucosidase in Candida albicans and its application in yeast identification. J Clin Microbiol 1987;25:907-10.
2. Perry JL, Miller GR. Umbelliferyl-labeled galactosaminide as an aid in identification of Candida albicans J Clin Microbiol 1987;25:2424-5.
3. Willinger BW, Manafi M, Rotter ML. Comparison of rapid methods using fluorogenic-chromogenic assays for detecting Candida albicans. Letters App Microbiol 1994; 18:47-49
4. Perry JD Freydie AM. The application of chromogenic media in clinical microbiology. J App Micrrobiol 2007; 103:2046
5. Carrillo-Muñoz AJ, Quindós G, Cárdenes CD, Alonso-Vargas R, Arévalo P, Brió S, Madariaga L. Evaluation of Chromalbicans Agar for presumptive identification of Candida albicans. Rev Iberoam Micol 2001; 18:105-8.

PACKAGING

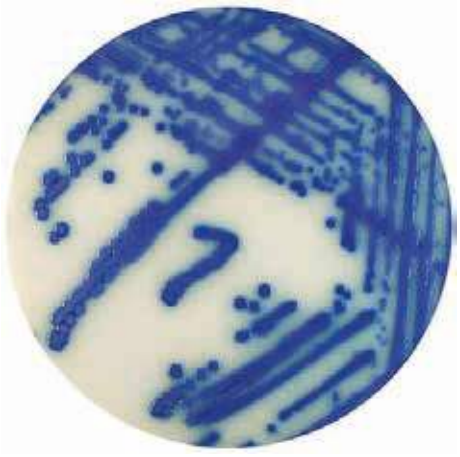
Product	Type	REF	Pack
Chromalbicans Agar	Dehydrated medium	4080002 4080004	500 g (9.5 L) 5 kg (95 L)
Chromalbicans Agar	Ready-to-use plates	548000	2 x 10 plates ø 90 mm

IFU rev 3, 2022/01

ChromArt

CRE-ESBL AGAR BASE
ESBL SUPPLEMENT - CRE SUPPLEMENT
CHROMART CRE – CHROMART ESBL
Dehydrated culture medium and supplements, ready-to use plates

ESBL Medium: *Escherichia coli*



CRE Medium: *Klebsiella pneumoniae*

INTENDED USE
In vitro diagnostics. Chromogenic basal medium, selective supplements and ready-to-use plates for the presumptive determination of *Enterobacteriaceae* resistant to carbapenems (CRE medium) and ESBL producing (ESBL medium), in clinical specimens.

COMPOSITION - TYPICAL FORMULAS *

DEHYDRATED CRE-ESBL AGAR BASE (REF 4080252)
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptones	16.0 g
Growth factors	5.0 g
Opacifier compounds	10.0 g
Tryptophan	2.0 g
Chromogenic mix	0.4 g
Agar	16.0 g

VIAL CONTENTS
ESBL Supplement (REF 4240080) (for 500 mL of medium)

Antimicrobials mix	0.21 g

VIAL CONTENTS
CRE Supplement (REF 4240082) (for 500 mL of medium)

Antimicrobials mix	0.21 g

ChromArt CRE (ready-to-use plates)

Peptones	16.00 g
Growth factors	5.00 g
Opacifying compound	10.00 g
Tryptophan	2.00 g
Chromogenic mix	0.40 g
Antimicrobials mix	0.42 g
Agar	16.00 g
Purified water	1000 mL

ChromArt ESBL (ready-to-use plates)

Peptones	16.0 g
Growth factors	5.0 g
Opacifying compound	10.0 g
Tryptophan	2.0 g
Chromogenic mix	0.4 g
Antimicrobials mix	0.42 g
Agar	16.0 g
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
CRE-ESBL Agar Base, with the addition of the suitable supplement, can be used for the preparation of CRE medium or ESBL medium. The use of chromogenic media is the preferred option for the detection of ESBL-producers or carbapenem resistant strains in faecal screening.[1,2] Bacterial differentiation is obtained with a mixture of chromogenic compounds designed to detect specific enzymatic activities (β-galactosidase, β-glucosidase, tryptophanase), of *E. coli,* of bacteria of the KESC group (*Klebsiella, Enterobacter, Serratia, Citrobacter*) and of the *Proteus-Morganella-Providencia* group. The grey and opaque background of the medium allows a better observation and colour reading of the colonies.

ESBL Medium

The Extended Spectrum Beta Lactamases (ESBLs) are acquired class A β-lactamases that hydrolyse and (usually) confer resistance to 2nd and 3rd generation cephalosporins, (e.g., cefuroxime, cefotaxime, ceftazidime and ceftriaxone), and 4th generation cephalosporins (e.g., cefepime, cefpirome), but not cephamycins (e.g., cefoxitin) or carbapenems.[1] ESBLs have become globally disseminated within species of *Enterobacteriaceae*.[2]

ESBL Medium is a chromogenic and selective screening medium for the isolation and differentiation of ESBL-producing *Enterobacteriaceae*. The selectivity of the medium is due to the presence of an inhibitory mixture of antibiotics against Gram-positive bacteria, fungi and Gram-negative bacteria susceptible to 3rd or 4th generation cephalosporins.

CRE Medium

Mechanisms of carbapenem-resistance among Gram-negative organisms are heterogeneous but are primarily broken down into two broad categories: carbapenemase-producing and non-carbapenemase-producing mechanisms. For the latter, carbapenem resistance is mediated by porin mutations or efflux pumps or the combination of these with ESBL and/or AmpC production depending of the Gram-negative organism.[3] Carbapenemase production is the primary mechanism mediating increased carbapenem resistance among Gram-negative bacteria.[3] Carbapenemases are β-lactamases that hydrolyze penicillins, in most cases cephalosporins, and to various degrees carbapenems and monobactams (the latter are not hydrolyzed by metallo-β-lactamases).[4]

The early identification of carbapenem-resistant organisms in clinical samples is a determining factor in preventing or limiting their spread and preserving the therapeutic efficacy of carbapenems.

Chromogenic media are recommended for the detection of gastro-intestinal colonisation of carbapenem-resistant organisms. [3,5,6]

CRE Medium is a chromogenic and selective screening medium for the isolation and differentiation of carbapenem-resistant *Enterobacteriaceae* (CRE). The selectivity of the medium is due to the presence of an inhibitory mixture of antibiotics against Gram-positive bacteria, fungi and Gram-negative bacteria susceptible to carbapenems

DIRECTIONS FOR MEDIA PREPARATION

Suspend 49.4 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C.

ESBL Medium: dissolve the contents of one vial of ESBL Supplement (4240080) with 5 mL of sterile purified water. Add to 500 mL of medium base cooled to 47-50°C under aseptic conditions. Mix well and distribute into sterile Petri dishes.

CRE Medium: dissolve the contents of one vial of CRE Supplement (4240082) with 5 mL of sterile purified water. Add to 500 mL of medium base cooled to 47-50°C under aseptic conditions. Mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

CRE-ESBL Agar Base	
Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	greyish, opaque
Final pH at 20-25 °C	7.2 ± 0.2
ESBL SUPPLEMENT	
Appearance of the lyophilized	high, homogeneous, reddish pastille
Appearance of the solution	opalescent reddish
CRE SUPPLEMENT	
Appearance of the lyophilized	high, homogeneous, reddish pastille
Appearance of the solution	limpid or slightly opalescent, reddish
ChromArt CRE and Chromart ESBL	
Ready-to-use plates	
Appearance	greyish, opaque
Final pH	7.2 ± 0.2

SPECIMENS

ESBL medium is intended for screening clinical specimens such as stools, rectal or peri-rectal swab and for processing other clinical specimens such as urine, wounds and respiratory secretions.[1]

CRE Medium: any sample type can be used; however, stool and rectal swab are the most sensitive for detecting CRE colonisation; if a rectal swab is not feasible or acceptable any clinical specimen such as blood, wound swab or urine is suitable. [5]

Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; collect specimens before antimicrobial therapy where possible.

TEST PROCEDURE

Allow plates to come to room temperature. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate in air at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

CRE isolates and ESBL producing *Enterobacteriaceae* show the following characteristic colonies:

Pink / red-magenta colonies: *E. coli*

Blue / green-blue / blue-violet / grey-violet colonies: *Klebsiella, Enterobacter, Serratia, Citrobacter*

Brown colonies with brown halo: *Proteus-Morganella-Providencia*

CRE isolates shall be subjected to confirmatory tests. Consult the listed references.[1-3]

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
ESBL Medium			
K. pneumoniae SHV-18	ATCC 700603	35-37°C / 18-24H / A	growth with blue colonies
E. coli	ATCC 25922	35-37°C / 18-24H / A	inhibited
C. albicans	ATCC 10231	35-37°C / 18-24H / A	inhibited
CRE Medium			
K. pneumoniae	ATCC BAA-1705	35-37°C / 18-24H / A	growth with blue colonies
E. coli	ATCC 25922	35-37°C / 18-24H / A	inhibited
C. albicans	ATCC 10231	35-37°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

PERFORMANCES CHARACTERISTICS

ESBL Medium

The performances of ESBL medium were evaluated in a clinical study by a Clinical Microbiology Laboratory in northern Italy[7] on 2500 urine cultures and 38 samples from other body sites. The results are summarized in the tables below.

The data demonstrate the capacity of ESBL medium to detect ESBL-producing *Enterobacteriaceae* with high sensitivity (98.82%) and specificity (98.29%).

CRE Medium

The performances of CRE Medium were evaluated in a clinical study by a Clinical Microbiology Laboratory in northern Italy[8] on 110 strains of carbapenem-resistant Gram-negative bacteria, 50 strains of 3rd generation cephalosporin-resistant Enterobacteria or ESBL-producing bacteria.

The published data demonstrate that CRE medium detects carbapenem-resistant Gram-negative bacteria with high sensitivity (98.2%) and specificity (100%) while it does not allow the growth of carbapenems susceptible organisms possessing other mechanisms that can cause resistance to beta-lactam antibiotics, such as ESBL or overproduction of AmpC.

If the research target is the determination of carbapenemase producing strains, the sensitivity is 100% and the specificity is reduced (85.1%) as the medium allows the growth of carbapenem resistant strains caused by membrane impermeability due to porin loss.

LIMITATIONS OF THE METHOD

- ESBL Chromogenic agar media are likely to be less specific, particularly in areas where ESBL producers are common.[1]
- Some *Enterobacteriaceae* strains hyperproducing cephalosporinases, some multi drug resistant *Pseudomonas* spp. and *Acinetobacter* spp. may grow on the ESBL medium.
- Growth on the medium depends on the metabolic requirements of each microorganism and on the resistance to the antimicrobials present; some target strains may not be able to grow on ESBL medium or may show a delayed growth (e.g., *Proteus* spp.).
- Some Gram-negative bacteria resistant to carbapenem due to membrane impermeability mechanism may grow on CRE medium.
- Multidrug resistant Gram-negative bacteria other than carbapemen-resistant *Enterobacteriaceae* (*Acinetobacte*r and *Pseudomonas*) may grow on CRE medium.
- There is very little evidence that extended incubation enhances the sensitivity of chromogenic media for CRE, but there is evidence to show that specificity is decreased.[3]
- Screening for intestinal carriage of CRE is of significant importance for the development of infection control strategies. However, the optimal screening modality remains to be established for each location and for each specific purpose.[9]
- Culture-based methods may not be optimal for the detection of low-level carbapenemase production, which is important for epidemiological purposes.[9]
- Agar-based procedures always require confirmatory testing to detect the type of *bla* gene present after a potentially resistant isolate is detected.
- Growth on CRE medium depends on the metabolic requirements of each microorganism and on the resistance to the antimicrobials present; some target strains may not be able to grow on the medium or may show a delayed growth.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. On the isolates, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

CRE-ESBL Agar Base

Store at +2°C /+8°C away from direct light in a dry place.

ESBL Supplement - CRE Supplement

Store the product in the original package at +2°C /+8°C away from direct light.

CromArt CRE and ChromArt ESBL, ready-to-use plates

Store the plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Public Health England. UK Standards for Microbiology Investigations (SMI) B 59: Detection of *Enterobacteriaceae* producing extended spectrum β lactamases.2016
2. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev. 2017; 30:449-479.
3. Simner PJ, Humphries R. Special phenotypic methods for detecting antibacterial resistance. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance Version 2.01, July 2017.
5. Public Health England. UK Standards for Microbiology Investigations (SMI) B 60: detection of bacteria with carbapenem hydrolysing β-lactamases (carbapenemases); September 2020.
6. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev. 2017; 30:449-479.
7. Comi C, Bracco S, Colombo L, Bartesaghi P, Barletta R, Silva M, Luzzaro F. Valutazione del terreno ESBL (Biolife) per la rilevazione degli Enterobatteri produttori di ESBL in campioni clinici. XLIII Congresso AMCLI, Sezione Poster, 2014.
8. Bracco S, Mauri C, Meroni E, Principe L, Pini B, Luzzaro F. Valutazione del terreno CRE (Biolife) per la rilevazione di batteri Gram-negativi resistenti ai carbapenemi. XLIII Congresso AMCLI, Sezione Poster, 2014.
9. Viau R, Frank KM, Jacobs MR, Wilson B, Kaye K, Donskey CJ, Perez F, Endimiani A, Bonomo RA. Intestinal Carriage of Carbapenemase-Producing Organisms: Current Status of Surveillance Methods. Clin Microbiol Rev. 2016; 29:1-27

PACKAGING

Product	Type	REF	Pack
CRE-ESBL Agar Base	Dehydrated medium	4080252	500 g (10.1 L)
ESBL Supplement	Freeze-dried supplement	4240080	10 vials, each for 500 mL of medium base
CRE Supplement	Freeze-dried supplement	4240082	10 vials, each for 500 mL of medium base
ChromArt CRE	Ready-to-use plates	548015	2 x 10 plates ø 90 mm
ChromArt ESBL	Ready-to-use plates	548020	2 x 10 plates ø 90 mm

IFU rev 2, 2021/12

ChromArt

CHROMOGENIC BACILLUS CEREUS AGAR BASE
CHROMOGENIC BACILLUS CEREUS SUPPLEMENTS
Dehydrated culture medium, selective supplement and enrichment

Typical *Bacillus cereus* colonies

INTENDED USE
For the enumeration of *Bacillus cereus* Group in foods and environmental samples.

COMPOSITION*
BACILLUS CEREUS AGAR BASE
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Peptones	20.0 g
Sodium chloride	5.0 g
Chromogenic mix	0.2 g
Agar	15.0 g

CHROMOGENIC B.CEREUS SELECTIVE SUPPLEMENT (4240090S)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Antimicrobial mix	75 mg

CHROMOGENIC B.CEREUS ENRICHMENT SUPPLEMENT (4240090E)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Phospholipids	10 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Bacillus cereus is a group of ubiquitous facultative anaerobic, spore forming, Gram-positive rods, commonly found in soil, on vegetables, and in many raw and processed foods. The group consists of at least eight closely related species: *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. cytotoxicus*, *B. anthracis*, and *B. toyonensis*.[1]
B. cereus food poisoning may occur when foods, such as cooked meat and vegetables, boiled or fried rice, vanilla sauce, custards, soups, and raw vegetable sprouts, are prepared and held without adequate refrigeration for several hours before serving, with *B. cereus* reaching >10^6 cells/g.[2] *Bacillus cereus* may cause an emetic or a diarrhoeal type of food-associated illness; the emetic disease is a food intoxication caused by cereulide, a small ring-formed dodecapeptide while the diarrhoeal syndrome is an infection caused by vegetative cells, ingested as viable cells or spores, thought to produce protein enterotoxins in the small intestine.[3]
The current method recommended by ISO Standards for the enumeration and identification of *B. cereus* includes growth on MYP and PEMBA media.[4,5] Problematic issues with traditional media include a lack of characteristic colony morphology, masked by the presence of background flora, such as *Bacillus* species other than *B. cereus* group and *S. aureus*.[2]
Chromogenic B. Cereus Agar inhibits the growth of background flora and includes a specific chromogenic compound for the detection of β-glucosidase enzyme and a substrate for the detection of phospholipase. Colonies of *B. cereus* and *B. cereus* Group are blue-green with a typical zone of precipitation. The antimicrobial mix strongly reduces the background Gram negative and Gram-positive flora and allows to isolate *B. cereus* Group often in pure culture. Biochemical testing is necessary to delineate to the species level.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 20.1 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 42-45 °C and, under aseptic conditions, add the following supplements: the contents of one vial of Chromogenic B. Cereus Enrichment Supplement (4240090E) and the contents of one vial of Chromogenic B. Cereus Selective Supplement (4240090S), reconstituted with 5 ml of sterile purified water. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Prepared plates appearance	yellow, opaque
Freeze-dried selective supplement	dense, white, pellet
Reconstituted selective supplement	homogeneously cloudy solution
Enrichment supplement appearance	yellow suspension, homogenously opaque
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

TEST PROCEDURE
Prepare the test sample in accordance with the specific International Standard appropriate to the product concerned.
Distribute 0.1mL of test sample if the product is liquid, or of the initial suspension if solid onto the surface of two agar plates (90mm). Repeat the procedure using further decimal dilutions.
If low number of *B. cereus* is expected, distribute 1mL of test sample if the product is liquid or 1mL of the initial suspension if solid to each of two agar plates (140 mm) or over the surface of three 90 mm plates.
Incubate at 30°C ± 1°C in aerobic conditions for 24 ± 2 hours. If colonies are not visible incubate the plates for further 24 hours before counting.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Count the presumptive *B. cereus* Group colonies in the plates with less than 150 colonies, that have the following characteristics: large, blue-green and generally surrounded by a zone of precipitation (indicating the production of phospholipase).
According to ISO Standards[4,5] typical and atypical colonies must be confirmed by means of the haemolysis test on sheep blood agar (+): select five presumptive colonies from each plate and streak the selected colonies onto the surface of sheep blood agar, incubate at 30 °C for 24 h ± 2 h and interpret the haemolysis reaction.

According to FDA-BAM typical and atypical colonies must be confirmed with Gram staining (*B. cereus* will appear as large Gram-positive bacilli in short-to-long chains; spores are ellipsoidal, central to subterminal, and do not swell the sporangium), Phenol red glucose broth (+), Nitrate broth (+), Modified VP medium (+), Tyrosine agar (+), Lysozyme broth (+)
Thanks to the high selectivity of Chromogenic B. Cereus Agar, the confirmation tests on typical colonies could be omitted.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains		Incubation T°/ t - ATM	Expected results
B. cereus	ATCC 11778	30 °C / 24 h - A	Blue-green colonies with opaque halo
B. subtilis	ATCC 6633	30 °C / 48 h - A	Inhibited
E. coli	ATC 25922	30 °C / 48 h - A	Inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Some strains of *B. cereus* produce only little or no phospholipase. Colonies of these strains will not be surrounded by a precipitation zone.[4]
- *Bacillus megaterium* may grow with blue-green colonies but without opaque halo.[2]
- Some bacteria can also grow as blue-green coloured colonies on the medium but without expression of the phospholipase activity. The absence of the opaque halo will make them easily distinguishable from the *Bacillus cereus*.

Storage conditions

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
Freeze-dried and liquid supplements
Store the products in the original package at +2°C /+8°C away from direct light.

References

1. Ehling-Schulz M, Koehler TM, Lereclus D. The Bacillus cereus Group: Bacillus species with Pathogenic Potential. Microbiol Spectr. 2019 May; 7(3): 10.
2. FDA-BAM, Chapter 14: Bacillus cereus. Content current as of: 06/29/2021
3. Stenfors Arnesen LP, Fagerlund A, Granum PE. From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiol Rev. 2008 Jul;32(4):579-606
4. ISO 7932:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive Bacillus cereus - Colony-count technique at 30 degrees C.
5. ISO 21871:2006 Microbiology of food and animal feeding stuffs - Horizontal method for the determination of low numbers of presumptive Bacillus cereus- Most probable number technique and detection method.

Packaging

Product	Type	REF	Pack
Chromogenic B.Cereus Agar Base	Dehydrated medium	4080202	500 g (12.4 L)
Chromogenic B.Cereus Supplements	Freeze-dried and liquid supplements	4240090	4 + 4 vials (each vial is for 500 mL of medium base)

IFU rev 1, 2022/06

ChromArt

CHROMOGENIC CANDIDA AGAR

Dehydrated and ready-to-use culture medium

Chromogenic Candida Agar:
C. albicans (green-blue colonies), *C. tropicalis* (blue-grey colonies) and *C. krusei* (large pink-violet colonies)

Intended use

In vitro diagnostics. Selective and chromogenic medium for the isolation of *Candida* spp. from clinical specimens and for the differentiation of *Candida albicans / Candida dubliniensis* group from *Candida tropicalis* and other species of the genus *Candida*.

Composition - typical formula* (after reconstitution with 1 L of water)

Peptones	10.30 g
Growth factors	11.70 g
Inorganic salts	4.60 g
Chloramphenicol	0.50 g
Chromogenic mix	0.36 g
Agar	12.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Yeast infections require prompt diagnosis to allow the early initiation of appropriate antifungal therapies. Since the 90s, advances have been made in laboratory methods for diagnosis of *Candida* species, especially *Candida albicans*, resulting in more rapid and reliable identification.[1-3] One of these methods was the incorporation of chromogenic substrates directly into the isolation media. A common principle of these media is the use of a chromogenic substrate for β-hexosaminidase, to differentiate *C. albicans / C. dubliniensis* group from other yeasts and a second chromogenic substrate (usually to detect phosphatase or β-glucosidase) to provide further discrimination between species.[4] The main advantage of such chromogenic media is their ability to detect mixed yeast cultures, because different species often form colonies with different colours.
Chromogenic Candida Agar is a “second generation” chromogenic and selective medium for the isolation of *Candida* spp. from clinical specimens and for the differentiation of clinically important *Candida* spp.: *C. albicans - C. dubliniensis* group from *Candida tropicalis, Candida krusei* and other *Candida* species. The selectivity of the medium is due to the presence of chloramphenicol which suppresses the growth of bacteria. Differentiation is obtained by the presence of two chromogenic compounds. The hydrolysis of the substrate for the detection of β-hexosaminidase

enzyme of *C. albicans* and *C. dubliniensis* results in the release of an insoluble chromophore that remains inside the colonies giving them a typical green-blue colour. The hydrolysis of the second chromogenic substrate results in the release of an insoluble pink chromophore and orients in the identification of other species: *Candida tropicalis* splits both the compounds with the formation of blue-grey colonies while other species of the genus *Candida* hydrolyse only the second chromogenic compound and grow with colonies with different shades of pink.

Directions for medium preparation
Suspend 39.5 g in 1000 mL of cold purified water; heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to approximately 47-50ºC, mix well and pour into sterile Petri dishes.

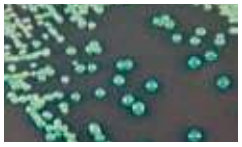
Physical characteristics
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25°C	6.0 ± 0.2

Specimens
Chromogenic Candida Agar is intended for the bacteriological processing of non-sterile clinical specimens such as mouth, throat, pharyngeal, vaginal swabs. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.

Test procedure
Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate by rolling the swab over a small area of the surface at the edge; then streak from this inoculated area to obtain well isolated colonies.
Incubate inoculated plates in aerobic conditions at 35-37°C for 18-24 and 48 hours.

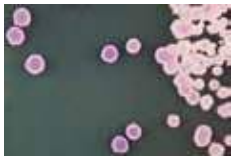
Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Here below a short interpretation guide is reported.

Brilliant green-blue colonies: characteristic of *C. albicans / C. dubliniensis.* (*C. albicans* here below)



Grey-blue colonies with purple tinges and/or a violet halo: characteristic of *C. tropicalis.*

Enlarged, flat pink-red or violaceous colonies, with a rough fine texture: characteristics of *C. krusei.*



White or pink or pink-purple colonies: characteristics of other *Candida* species (*C. glabrata* here below)

Candida kefir produces violet-red colonies. *Candida parapsilosis* complex produces pink, pink-violet colonies.
Gram-positive and Gram-negative bacteria are almost inhibited.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains		Incubation T°/ t / ATM	Expected results
C. albicans	ATCC 10231	35-37°C / 44-48 h / A	good growth, green-blue colonies
C. tropicalis	NCPF 8841	35-37°C / 44-48 h / A	good growth, blue-grey colonies
E. coli	ATCC 25922	35-37°C / 44-48 h / A	inhibited
S. aureus	ATCC 25923	35-37°C / 44-48 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCPF: Public Health England, National Collection of Pathogenic Fungi.

Performances characteristics
The performances characteristics of Chromogenic Candida Agar were evaluated by Andreoni *et al.*[5] with 82 yeast strains isolated from human specimens, identified with a phenotypic system and confirmed with a spectrometric method and stored at -80°C and with 80 clinical specimens isolated from respiratory material, vaginal exudates, urine and positive blood cultures. Chromogenic Candida Agar was compared with a chromogenic medium of the market. The conclusions have been the following: for yeast strains isolated from human specimens, the comparison between the two media, in general showed a better growth, in terms of colony dimension at 24 and 48 hours, on Chromogenic Candida Agar; the colony colour as well, in terms of tonality and intensity, resulted more evident on Chromogenic Candida, allowing a better differentiation between species with similar colours. The findings confirmed that the Chromogenic Candida Agar can substantially ensure the presumptive identification of frequent clinical isolation species, allowing an orientation for presumptively identifying yeasts species of lower isolation frequency. The rapid growth and the colour intensity moreover guarantee a morphological and colour evaluation in a shorter time.

Limitations of the method
- *C. dubliniensis* is ß-hexosaminidase positive and grows with green-blue colonies and therefore it is not differentiable from *C. albicans*.[5]
- Chromogenic Candida agar does not differentiate between *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis.*[5]
- The best colour differentiation of *Candida* spp. is obtained after 48 hours of incubation.[5]

- *Candida* spp. other than *C. albicans / C. dubliniensis* and *C. tropicalis* appear as a variety of pink/grey/violet colours, due to the mixture of natural pigmentation and the released chromophores. The experience of the microbiologist can help to differentiate these species by colour and morphology of the colonies.
- Growth depends on the requirements of each individual microorganisms. It is possible that yeasts with specific metabolic requirements may not grow or may not produce colour.
- Some rare bacterial strains which may be resistant to chloramphenicol may grow on the medium with coloured colonies.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions

Dehydrated medium
Store at +2°C/+8°away from direct light in a dry place.
Ready-to-use plates
Store plates in their original pack at +2°C/+8°Caway from direct light.

References

1. Polacheck I, Melamed M, Bercovier H, Salkin IF. Beta-Glucosidase in Candida albicans and its application in yeast identification. J Clin Microbiol 1987;25:907-10.
2. Perry JL, Miller GR. Umbelliferyl-labeled galactosaminide as an aid in identification of Candida albicans J Clin Microbiol 1987;25:2424-5.
3. Willinger BW, Manafi M, Rotter ML. Comparison of rapid methods using fluorogenic-chromogenic assays for detecting Candida albicans. Letters App Microbiol 1994; 18:47-49
4. Perry JD. A Decade of Development of Chromogenic Culture Media for Clinical Microbiology in an Era of Molecular Diagnostics. Clin Microbiol Rev. 2017 Apr;30(2):449-479.
5. Andreoni S., Molinari G.L., Ruzza P., Dellera A. Evaluation of Chromogenic Candida Agar for isolation and presumptive identification of yeasts. XLI AMCLI Italian Clinical Microbiologists Association Congress Rimini, November 13-16, 2012.

Packaging

Product	Type	REF	Pack
Chromogenic Candida Agar	Dehydrated medium	4080052 4080054	500 g (12.65 L 5 kg (126 L)
Chromogenic Candida Agar	Ready-to-use plates	548005	2 x 10 plates ø 90 mm

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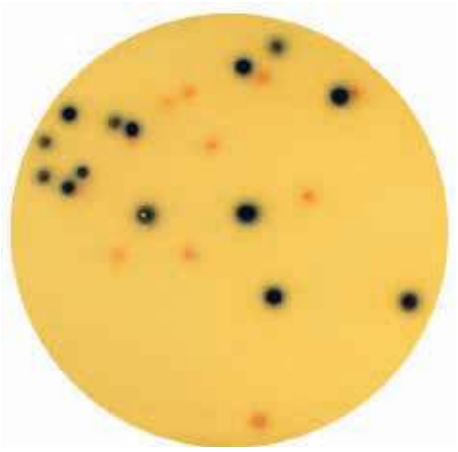
CHROMOGENIC COLIFORM AGAR

Dehydrated and ready-to-use culture medium



Chromogenic Coliform Agar: *E. coli* blue-grey colonies;
E. aerogens salmon colonies

Intended use

Chromogenic medium for the simultaneous enumeration of *Escherichia coli* and coliform bacteria.

Composition – typical formula* (after reconstitution with 1 L of water)

Tryptose	10.00 g
Tryptophane	0.10 g
Peptocomplex	5.00 g
Yeast Extract	3.00 g
Sodium Chloride	5.00 g
Bile Salts n.3	1.50 g
Isopropyl-β-D-thiogalactopyranoside (IPTG)	0.10 g
X-β-glucuronide CHX salt	0.06 g
Salmon-β-D-galactoside	0.15 g
Agar	13.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Faecal pollution is the major cause of waterborne disease, since most of the pathogens associated with transmission reside in human and warm-blooded animal faeces. Examination of water samples for the presence of *E. coli* and coliform bacteria provides an indication of such pollution.
Chromogenic Coliform Agar is reported in the review of water analysis media ISSN:1125-2464[1] and in the APAT-IRSA[2] review for the simultaneous detection and enumeration of β-glucuronidase-positive *E. coli* and β-D-galactosidase positive coliform bacteria from water samples.
Peptocomplex and tryptose provide nitrogen, carbon, amino acids and minerals for the microbial growth, yeast extract is a source of vitamins, particularly of group B. Sodium chloride maintains the osmotic balance. Bile salts n° 3 act as a selective agent, inhibiting the growth of Gram-positive bacteria.
Detection of coliform bacteria is based on the ability of β-D-galactosidase to cleave the substrate salmon-β-D-galactoside with the formation of salmon red colonies. Enumeration of *E. coli* is based on the detection of two enzymatic activities, β-D-glucuronidase and β-D-galactosidase, which cleave the chromogenic substrates salmon-β-D-galactoside and X-β-glucuronide, with the formation of dark blue-grey colonies. The hydrolysis of X-GAL in enhanced by IPTG, a lactose operon inducer. The tryptophane in the medium, allows the indole test to be performed directly on colonies with the addition of Kovacs' reagent, for confirmation of *E. coli*.

Directions for medium preparation (dehydrated medium)

Suspend 37.9 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation until completely dissolved and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and dispense in sterile Petri dishes.

Directions for medium preparation (ready-to-use flasks)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates and flasks appearance	pale yellow, limpid or slightly opalescent
Final pH of complete medium (at 20-25°C)	6.8 ± 0.2

Specimens

Water samples and other materials of sanitary importance. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards and norms.

Test procedure

Perform simultaneous enumeration of coliforms and *Escherichia coli*, applying normal laboratory methods with inoculation by pour plate method either by surface spread technique or by membrane filtration.
Incubate for 18-24 hours at 37°C.
The APAT-IRSA[2] review of microbiological methods for the determination of *E. coli* in water reports the following procedure:
Filter an aliquot of the sample or a volume of its dilutions using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0.45 µm and, preferentially, with grid lines. After filtration place the membrane filter on medium surface, ensuring that no air is trapped underneath, invert Petri dish, and incubate at 36°C ± 1°C for 18-24 hours.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and count:
- All pink to red colonies (positive for β-D-galactosidase reaction) as presumptive coliform bacteria other than *E. coli*.
- All blue-grey, indole-positive colonies (positive for β-D-galactosidase and β-D-glucuronidase reactions) as *E. coli*.

To avoid false-positive results, caused by oxidase positive bacteria, for example, *Aeromonas* spp, the presumptive colonies shall be confirmed by a negative oxidase reaction (Oxidase test Strips, cat. N° 191040ST)
The count of total coliform bacteria is the sum of all oxidase negative pink to red colonies plus all blue-grey colonies.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 25922	35-37°C/18-24H/A	growth, blue-grey colonies
E. aerogenes ATCC 13048	34-38°C/18-24H/A	growth, salmon colonies
E. faecalis ATCC 19433	34-38°C/18-24H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of Salmonella subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia, Enterobacter* and *Yersinia* strains (1-5%).[3-5]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[6] Consequently, these strains, being positive for β-galactosidase, will grow with red-pink colonies and be counted as coliforms.
- In addition to expressing β-D-glucuronidase, E. coli is able to produce indole from tryptophan. Therefore, in case of any doubt of *E. coli* colonies on the primary agar medium, indole test may be used as an additional confirmation.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

Storage conditions

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
Ready-to-use plates and flasks
Store in their original pack at +2°C /+8°C away from direct light.

References

1. APAT-IRSA Metodi analitici per le acque, vol 3 n° 7000 – Metodi per la determinazione di microrganismi indicatori di inquinamento e di patogeni. n° 7030, *2003, Escherichia coli.*
2. Bonadonna L. *Escherichia coli* nelle acque significato sanitario e metodologie di analisi. ISSN:1125-2464, 2001
3. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
4. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288
5. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Production of Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
6. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288

Packaging

Product	Type	REF	Pack
Chromogenic Coliform Agar	Dehydrated medium	4012992	500 g (13.2 L)
Chromogenic Coliform Agar	Ready-to-use plates	491299	3 x 10 plates, ø 55 mm
Chromogenic Coliform Agar	Ready-to-use plates	541299	2 x 10 plates, Ø 90 mm
Chromogenic Coliform Agar ISO Formulation	Ready-to-use flasks	5112972	6 x 100 mL

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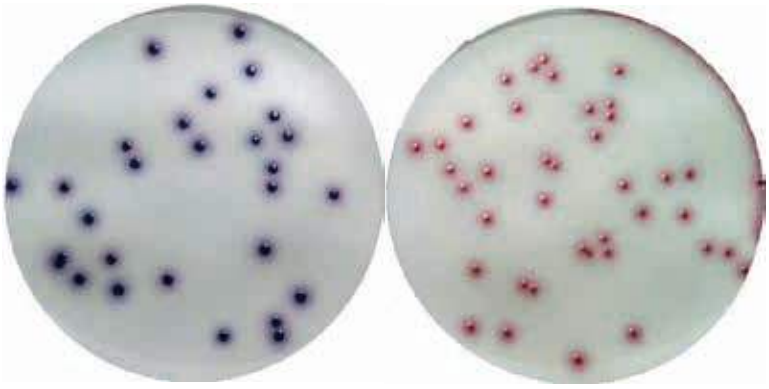
CHROMOGENIC COLIFORM AGAR ISO FORMULATION

Dehydrated and ready-to-use culture medium



Chromogenic Coliform Agar ISO Formulation.
Escherichia coli : blue colonies*; Citrobacter freundii:* red colonies

INTENDED USE

Chromogenic medium for the enumeration of *Escherichia coli* and coliform bacteria with membrane filtration technique on waters with low bacterial background, according to ISO 9308-1.

COMPOSITION – TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	1.00 g
Yeast extract	2.00 g
Sodium chloride	5.00 g
Sodium dihydrogen phosphate x $2H_2O$	2.20 g
Di-sodium hydrogen phosphate	2.70 g
Sodium pyruvate	1.00 g
Sorbitol	1.00 g
Tryptophane	1.00 g
Tergitol® 7	0.15 g
Salmon-β-D-galactoside	0.20 g
X-β-glucuronide CHX salt	0.10 g
Isopropyl-β-D-thiogalactopyranoside (IPTG)	0.10 g
Agar	10.60 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Faecal pollution is the major cause of waterborne disease, since most of the pathogens associated with transmission reside in human and warm-blooded animal faeces. Examination of water samples for the presence of *E. coli* and coliform bacteria provides an indication of such pollution.
Chromogenic Coliform Agar ISO Formulation is a selective and differential medium for the simultaneous detection and enumeration of β-glucuronidase-positive *E. coli* and β-D-galactosidase positive coliform bacteria from water samples with low bacterial numbers, such as drinking water, disinfected pool water, or finished water from drinking water treatment plant, according to ISO 9308-1.[1,2]
Enzymatic digest o casein provides nitrogen, carbon, amino acids and minerals for the microbial growth, yeast extract is a source of vitamins, particularly of group B. Sodium chloride maintains the osmotic balance while phosphates act as buffer of the medium. Sorbitol and sodium pyruvate stimulate a quick bacterial growth and aid in resuscitation of stressed cells. Tergitol 7 is a surfactant that acts as a selective agent, moderately inhibiting the growth of Gram-positive bacteria.
Detection of coliform bacteria is based on the ability of β-D-galactosidase to cleave the substrate salmon-β-D-galactoside with the formation of salmon red colonies. Enumeration of *E. coli* is based on the detection of two enzymatic activities, β-D-glucuronidase and β-D-galactosidase, which cleave the chromogenic substrates salmon-β-D-galactoside and X-β-glucuronide, with the formation of dark blue colonies. The hydrolysis of X-GAL in enhanced by IPTG, a lactose operon inducer.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 27.1 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation until completely dissolved. Do not autoclave, do not overheat. Cool to 47-50°C, mix well and dispense in sterile Petri dishes to a depth of at least 4 mm. Some turbidity may occur after boiling; it normally disappears when the temperature decreases to 47-50°C and doesn't affect the medium performances.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates and flasks appearance	pale yellow, limpid or slightly opalescent
Final pH of complete medium (at 20-25°C)	6.8 ± 0.2

SPECIMENS

Water samples with low bacterial numbers, such as drinking water, disinfected pool water, or finished water from drinking water treatment plant. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards and norms.

TEST PROCEDURE

Filter 100 mL (or other volumes, e.g., 250 mL for bottled water) of the sample using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and, preferentially, with grid lines. The minimum volume for filtration is 10 mL of sample or dilutions thereof to ensure even distribution of the bacteria on the membrane filter.
After filtration place the membrane filter on medium surface, ensuring that no air is trapped underneath, invert Petri dish, and incubate at 36 ± 2 °C for 21-24 h.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and count:
All pink to red colonies (positive for β-D-galactosidase reaction) as presumptive coliform bacteria other than *E. coli*.
All dark-blue to violet colonies (positive for β-D-galactosidase and β-D-glucuronidase reactions) as *E. coli*.
To avoid false-positive results, caused by oxidase positive bacteria, for example, *Aeromonas* spp, the presumptive colonies shall be confirmed by a negative oxidase reaction (Oxidase test Strips, cat. N° 191040ST)

Perform the oxidase test preferentially on all, or at least 10 selected pink to red colonies (coliform bacteria, different from *E. coli*): the test should be negative.
The count of total coliform bacteria is the sum of all oxidase negative pink to red colonies plus all dark-blue to violet colonies.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 25922	34-38°C/21-24H/A	growth, blue colonies
E. aerogenes ATCC 13048	34-38°C/21-24H/A	growth, pink colonies
P. aeruginosa ATCC 10145	34-38°C/21-24H/A	growth, colourless colonies
E. faecalis ATCC 19433	34-38°C/21-24H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of Salmonella subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia, Enterobacter* and *Yersinia* strains (1-5%).[3-5]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[1,4] Consequently, these strains, being positive for β-galactosidase, will grow with red-pink colonies and be counted as coliforms.[1]
- In addition to expressing β-D-glucuronidase, E. coli is able to produce indole from tryptophan. Therefore, in case of any doubt of *E. coli* colonies on the primary agar medium, indole test may be used as an additional confirmation.[1]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

Storage conditions

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
According to ISO 9301-1 the plates can be stored at 5 ± 3°C in the dark and protected against evaporation for at least one month.
Ready-to-use plates and flasks
Store in their original pack at +2°C /+8°C away from direct light.

References

1. ISO 9308-1:2014 Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora.
2. ISO 9308-1:2014/AMD 1:2016 Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora — Amendment 1
3. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
4. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288
5. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Production of Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.

Packaging

Product	Type	REF	Pack
Chromogenic Coliform Agar ISO Formulation	Dehydrated medium	4012972	500 g (18.4 L)
Chromogenic Coliform Agar ISO Formulation	Ready-to-use plates	491297	3 x 10 plates ø 55 mm
Chromogenic Coliform Agar ISO Formulation	Ready-to-use flasks	5112972	6 x 100 mL

IFU rev 1, 2022/06

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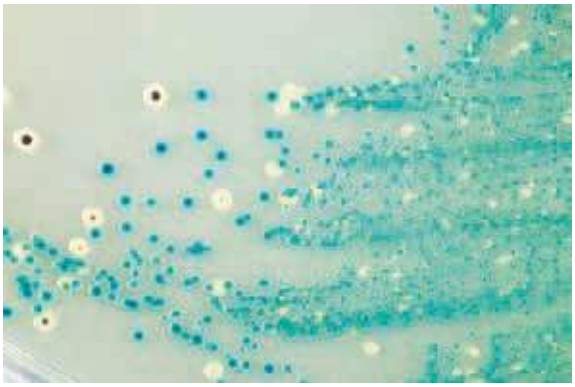
CHROMOGENIC CRONOBACTER ISOLATION (CCI) AGAR

Dehydrated culture medium and ready-to-use plates



CCI Agar: blue-green colonies: *Cronobacter sakazakii*; white colonies with grey or black centre: *Salmonella* Enteritidis

Intended use

For the detection (presence/absence) of *Cronobacter* spp. in samples from the food chain, according to ISO 22964.

Composition - typical formula * (after reconstitution with 1 L of water)

Tryptone	7.00 g
Yeast extract	3.00 g
Sodium chloride	5.00 g
5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside	0.15 g
Sodium deoxycholate	0.25 g
Ammonium iron (III) citrate	1.00 g
Sodium thiosulfate	1.00 g
Agar	14.50 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Cronobacter species (formerly known as *Enterobacter sakazakii*) are Gram-negative rod-shaped, motile pathogenic bacteria of the family *Enterobacteriaceae*. These organisms are regarded as opportunistic pathogens linked with life-threatening infections predominantly in neonates.[1]

Clinical syndromes of *Cronobacter* infection include necrotizing enterocolitis (NEC), bacteraemia and meningitis, with case fatality rates ranging from 40-80%.[1,2] The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, water, rice and others.[1,3,4]
Chromogenic Cronobacter Isolation (CCI) Agar is a culture medium for the determination of the presence or absence of *Cronobacter* spp. in samples from the food supply chain, according to ISO 22964.[5] It must be used combined with Cronobacter Screening Broth.
Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium chloride maintains the osmotic balance. The selective agent of the medium is sodium deoxycholate which inhibits the growth of Gram-positive bacteria. The presence of sodium thiosulfate/ammonium iron citrate indicator system allows the differentiation of thiosulfate reductase positive bacteria (e.g., *Proteus*, *Salmonella*) that produce colonies with a slight black centre. The medium contains the chromogenic compound 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside, a substrate for the detection of α-glucosidase: *Cronobacter* spp. possess α-glucosidase, cleave the chromogenic substrate so grow with blue to blue-green colonies

Directions for medium preparation
Suspend 31.9 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C mix well and pour into sterile Petri dishes

Physical characteristics
Dehydrated medium appearance	straw colour, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	dark amber, clear
Final pH at 20-25°C	7.3 ± 0.2

Specimens
Food products and ingredients intended for human consumption and the feeding of animals; environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[5]

Test procedure
Prepare the test sample in accordance with the relevant part of ISO 6887 dealing with the product concerned.
Add 10 g or 10 mL of sample to 90 mL of Buffered Peptone Water (REF 401278). For inoculum above 10 g preheat the broth to 34-38°C. Incubate the pre-enrichment broth at 36 ± 2 °C for 18 ± 2 hours.
Transfer 0.1 mL of pre-enriched broth into 10 mL of Cronobacter Screening Broth (REF 401355)
Incubate the enrichment broth tubes at 41.5 ± 1 °C for 24 ± 2 hours
Streak a loopful of enrichment broth (about 10 µL) on a plate of CCI Agar and incubate upside down at 41.5 ± 1 °C for 24 ± 2 hours.

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical *Cronobacter* colonies are small to medium-sized (1 mm to 3 mm) and blue to blue-green in colour.
Colonies of non-typical Gram-negative bacteria may develop on CCI Agar with the following characteristics: white, with or without a grey or black or green centre; some naturally pigmented colonies of non-*Cronobacter* may appear yellow or red.
Perform the confirmation tests on the typical colonies as reported by ISO 22964.[5]

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[5]

Control strains	Incubation T°/ t / ATM	Expected results
C. sakazakii ATCC 29544	41.5 °C ± 1°C /24 h ± 2 h / A	Good growth, blue-green colonies
C. muytjensis ATCC 51329	41.5 °C ± 1°C /24 h ± 2 h / A	Good growth, blue-green colonies
E. cloacae ATCC 13047	41.5 °C ± 1°C /24 h ± 2 h / A	White colonies
E. faecalis ATCC 19433	41.5 °C ± 1°C /24 h ± 2 h / A	Inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- *Cronobacter* may be present in low numbers in the samples, along with other *Enterobacteriaceae*, such as *E. cloacae*, which may interfere in the determination of the target microorganism.[5]
- The use of large sample sizes can compromise the recovery of stressed *Cronobacter* spp. when interfering microflora are present, such as probiotics [5]

Storage conditions
Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
According to ISO 22964[5] the plated medium may be kept at 5°C ± 3 ºC for up to 14 days.
Ready-to-use plates
Store plates in their original pack at +2°C /+8°C away from direct light.

References

1. Yan QQ, Condell O, Power K, Butler F, Tall BD, Fanning S. Cronobacter species (formerly known as Enterobacter sakazakii) in powdered infant formula: a review of our current understanding of the biology of this bacterium, J App Microbiol 2012; 113:1-15
2. Friedemann, M. Epidemiology of invasive neonatal Cronobacter (Enterobacter sakazakii) infections. Eur J Clin Microbiol Infect Dis 2009; 28:1297–1304.
3. Bowen AB, Braden CR. Invasive Enterobacter sakazakii disease in infants. Emerg Infect Dis 2006; 12:1185–1189.
4. Healy B, Cooney S, O'Brien S, Iversen C, Whyte P, Nally J, Callanan JJ, Fanning, S (2010) Cronobacter (Enterobacter sakazakii): an opportunistic foodborne pathogen. Foodborne Pathog Dis 2010; 7:339–350.
5. ISO 22964:2017 Microbiology of the food chain — Horizontal method for the detection of Cronobacter spp.

Packaging
Product	Type	REF	Pack
Chromogenic Cronobacter Isolation (CCI) Agar	Dehydrated medium	4080302 4080301	500 g (15.6 L) 100 g (3.1 L)
Chromogenic Cronobacter Isolation (CCI) Agar	Ready-to-use plates	548030	2 x 10 plates ø 90 mm

IFU rev 1, 2022/06

ChromArt

CHROMOGENIC E. COLI O157 AGAR

Dehydrated culture medium

INTENDED USE

Selective and chromogenic medium for the isolation and differentiation of *Escherichia coli* O157:H7, from food.

COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptones	17.0 g
Bile salts n°3	1.5 g
Chromogenic compounds	0.5 g
Agar	12.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

E. coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak investigation of haemorrhagic colitis.[1] Although more than 300 verotoxins or Shiga toxins producing serotypes are known, the infection is mainly caused by the motile serotype *E. coli* O157: H7 and its non-motile variant O157:NM (O157:H-).[2] The severity of illness presents different degrees, from uncomplicated diarrhoea to haemorrhagic colitis, up to haemolytic-uremic syndrome and thrombotic thrombocytopenic purpura; the infectious dose for O157:H7 is estimated to be 10-100 cells; the infection is particularly serious for the most vulnerable subjects, such as children and the elderly.[3] The strain virulence is substantially due to the production of one or both of the Shiga toxins Stx1 and Stx2 and, more rarely, of their variants. Infections are mostly food or water borne and have implicated undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice and sprouts and vegetables.[4] Direct contact with animals belonging to the reservoir species and person to person transmission may play a role in the spread of infection.[5]
E.coli O157:H7 strains are phenotypically distinct from *E.coli* as they exhibit slow or no fermentation of sorbitol and do not have glucuronidase activity; these characteristics led to the design of various culture media for primary isolation such as CT-SMAC (Cefixime Tellurite Sorbitol Mac Conkey Agar) and CT-SMAC MUG.[6] Following the findings about the isolation of sorbitol positive and β-glucuronidase positive *E.coli* O157 and strains that do not grow on CT-SMAC, [7,8,9] several chromogenic media, based on more specific differential mechanisms, have been proposed.
Chromogenic E. coli O157 Agar includes a mixture of chromogenic compounds to detect the enzymatic activities of *Enterobacteriaceae* (β-glucuronidase and β-glucosidase) and a specific enzymatic activity of *E. coli* O157.
The selective action of Chromogenic E. coli O157 Agar is due to the presence of bile salts n°3, which inhibit the growth of Gram-positive bacteria. To increase the selective properties and the specificity of the results, potassium tellurite and cefixime can be added to the medium: according to the data of Zadik[10] this addition completely or partially inhibits the growth of 67% of *E. coli* non-O157 and almost completely the growth of sorbitol non-fermenting Gram-negative bacteria other than *E. coli* O157.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 31 g in 1000 mL of cold purified water. Heat to boiling, stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes. If cefixime-tellurite addition is required, reconstitute one vial of Cefixime Tellurite O157 Supplement (REF 42ISEC) with 5 mL of sterile purified water and, under aseptic conditions, add to 500 mL of pre-cooled medium base.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greyish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Chromogenic E. coli O157 Agar, with or without Cefixime Tellurite O157 Supplement, is intended for the bacteriological processing of non-clinical samples; refer to the applicable international standards.[2,11]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

1. A test amount is enriched in nine times the weight of pre-warmed Modified Tryptic Soy Broth (REF 402155M2) plus novobiocin 20 mg/L (Novobiocin Antimicrobic Supplement -REF 4240045) at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h.
2. *E. coli* O157 cells are separated and concentrated using immunomagnetic beads coated with antibodies to *E. coli* O157 after 6 h and again, if necessary, after a further 12 to 18 h incubation.
3. 50 µl of immunomagnetic concentrated broth are sub-cultured onto CT-SMAC and onto Chromogenic E. coli O157 Agar.
4. Chromogenic E. coli O157 Agar is incubated in aerobic atmosphere at 35-37°C for 18-24 hours. CT-SMAC should be incubated following the IFU recommended procedure.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Pink to violet colonies can be presumptively identified as *E. coli* O157.
E. coli strains not belonging to the serogroup O157, *Citrobacter, Enterobacter, Klebsiella, Serratia* grow with blue or green-blue colonies.
Purify the typical colonies from Chromogenic E. coli O157 Agar by streaking onto Nutrient Agar and incubate at 37°C for 18 to 24 h.
For confirmation, ISO16654[11] requires indole test (+) and agglutination with *E. coli* O157 antiserum.
FDA BAM[2] requires β-galactosidase (+), β-glucuronidase (-) and indole (+) tests and the presence of the O157 and H7 antigens.
The pink to violet colony with the biochemical profile of *E. coli* and positive for the antisera O157 and H7 is confirmed as *E. coli* O157:H7.
If the isolate is O157 positive but H7 negative it may be a non-motile variant (O157: NM) and therefore requires a confirmation test of its toxigenic potential (for example with PCR technique). The colony can also be sub-cultured to blood agar plate to induce mobility and re-tested with H7 antiserum.
O157:H7 and O157: NM isolates that produce verocytotoxin are considered pathogenic. However, an O157: NM strain that does not produce shiga toxins or other EHEC (Enterohaemorrhagic *E. coli*) virulence factors is probably non-pathogenic. There are many *E. coli* O157 serotypes that carry other than H7 antigens (e.g.: H3, H12, H16, H38, H45, etc), and these often do not carry EHEC virulence factors.[2]
For a complete explanation of the identification criteria and methods, refer to the literature cited for clinical samples and for food samples.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli O157 ATCC 43894	35-37°C / 18-24 H / A	growth, violet colonies
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, blue colonies
S. aureus ATCC 25923	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Chromogenic E. coli O157 Agar does not detect EHEC strains of *E. coli* other than O157:H7.
- Follow the recommended times and temperatures as *E. coli* O157 does not grow at 44-45°C and because delayed observation of the colonies can lead to errors of interpretation.
- *Salmonella* spp., especially in the absence of the Cefixime Tellurite Supplement, may grow with colonies with a pale violet centre, that may not be distinguishable from *E. coli* O157 colonies.
- Some enterococci can develop small colonies with prolonged incubation beyond 24 hours.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Centers for Disease Control. 1984. Update: sporadic hemorrhagic colitis. Morbid. Mortal. Weekly Rep. 33:28
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual. Chapter 4a Diarrheagenic Escherichia coli. Rev October 2018
3. Griffin, P. M., S. M. Ostroff, R. V. Tauxe, K. D. Greene, J. G. Wells, J. H. Lewis, and P. A. Blake. 1988. Illnesses associated with Escherichia coli O157:H7 infections. A broad clinical spectrum. Ann. Intern. Med. 109:705–712.
4. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis. 2005; 11:603–609.
5. Johnson RP, Wilson JB, Michel P, Rahn K, Renwick SA, Gyles CL, Spika JS. Human infection with verotoxigenic *Escherichia coli* associated with exposure to farms and rural environments. In: Stewart CS, Flints HJ, editors. Escherichia coli O157 in Farm Animals. CABI Publishing; Wallingford, U.K: 1999. pp. 147–168.
6. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of Escherichia coli serotype O157. J Clin Microbiol. 1990; 28:2165–2168.
7. Díaz S, Vidal D, Herrera-León S, Sánchez S. Sorbitol-fermenting, β-Glucuronidase-positive, shiga toxin-negative Escherichia coli O157:H7 in free-ranging red deer in south-central Spain. Foodborne Pathog Dis 2018; 8:1313-1315
8. CDR Weekly. CDR. Sorbitol-fermenting Vero cytotoxin-producing E. coli (VTEC 0157). CDR. 2006
9. Gunzer F, Bohm H, Russmann H et al. Molecular detection of sorbitol-fermenting Escherichia coli 0157 in patients with hemolytic-uremic Ssyndrome. J Clin Microbiol 1992; 30:1807.
10. Zadik PM, Chapman PA, and Siddons CA. Use of tellurite for the selection of verocytotoxigenic Escherichia coli O157. J. Med. Microbiol 1993; 39:155-158
11. ISO 16654:2001. Microbiology of food and animal feeding stuffs- Horizontal method for detection of E.coli O157

PACKAGING

Product	Type	REF	Pack
Chromogenic E.coli O157 Agar	Dehydrated medium	4055812	500 g (16.1 L)

IFU rev 2, 2022/08

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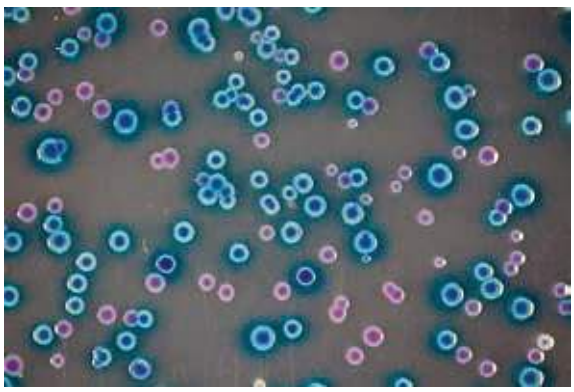
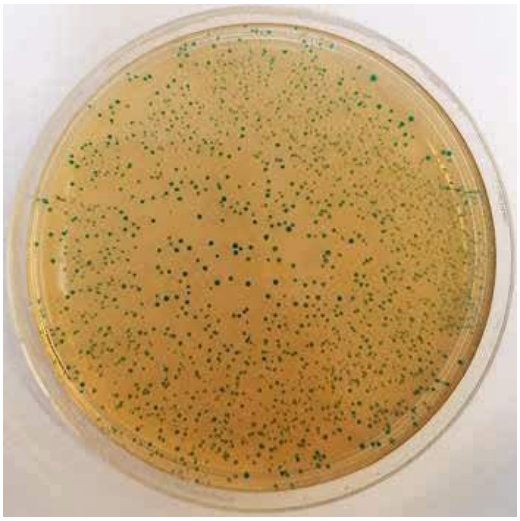


Chromogenic E.Coli O157 Agar*: E. coli* O157 (purple colonies), and *E. coli* non-O157 (blue colonies)

ChromArt

CHROMOGENIC LACTOBACILLUS ACIDOPHILUS AGAR (CLAA)

Dehydrated culture medium



L. acidophilus on CLAA

INTENDED USE

Chromogenic medium for the enumeration and differentiation of *Lactobacillus acidophilus* in yogurt and acid milk.

COMPOSITION TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Tryptone	10.00 g
Yeast Extract	5.00 g
Potassium dihydrogen phosphate	6.000 g
Di-ammonium citrate	2.000 g
Sodium acetate	15.00 g
Magnesium sulphate anhydrous	0.281 g
Ferrous sulphate	0.034 g
Manganous sulphate anhydrous	0.011 g
Glucose	20.00 g
Polysorbate 80	1.00 g
Agar	12.00 g
X-Glu^	0.02 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria
^ 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Yogurt-related milk products are increasingly being used as carriers of probiotic bacteria for their potential health benefits. To meet with a recommended level of ≥10^6 viable cells/g of a product, assessment of viability of probiotic bacteria in market preparations is crucial.[1]
Chromogenic Lactobacillus Acidophilus Agar (CLAA) is a selective and chromogenic medium for the enumeration and differentiation of *L. acidophilus* in yogurt-related milk products such as yogurt and acid milk.[2]
CLAA medium contains two peptones and yeast extract as sources of nitrogen, carbon and vitamins, necessary for microbial growth. Dextrose provides carbon and is source of energy. Polysorbate 80 acts as surfactant and provides fatty acids required for the metabolism of lactobacilli. Ammonium citrate and sodium acetate inhibit the growth of streptococci, moulds, and other oral microbial flora and restrict *Proteus* swarming. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate, ferrous sulphate and manganous sulphate are sources of inorganic ions for the optimal growth of lactobacilli. The detection principle is based on the specific visualization of the β-D-glucosidase activity of *L. acidophilus* via a chromogenic reaction of 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (X-Glu) which is cleaved with the formation of blue-green colonies.[2]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 71.3 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes, cool to 45-50°C and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5.8 ± 0.2

SPECIMENS

The specimens consist of yogurt-related milk products such as yogurt and acid milk. Refer to the applicable international Standards for samples collection and preparation.[3] The medium is not intended for microbiological examination of clinical specimens.

TEST PROCEDURE

Prepare the sample suspension and further decimal dilutions with Maximum Recovery Diluent or another suitable diluent. Transfer by means of a sterile pipette 0.1 mL of the test sample if liquid or 0.1 mL of the initial suspension in the case of other products, to each of two agar plates containing the CLAA medium. Repeat the procedure for further decimal dilutions if necessary. Carefully spread the inoculum as quickly as possible over the surface of the agar plate. Allow the medium to absorb the sample. Invert the plates and incubate anaerobically at 35-37°C for 72 h ± 3 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Characteristic *L. acidophilus* colonies are blue-green (β-glucosidase positive).
β-glucosidase negative lactobacilli, such as *L. casei* and *L. delbrueckii* subs *bulgaricus,* grow with white colonies.
Enumerate the colonies showing the features of characteristic microorganisms on plates having between 10 and 300 colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. acidophilus ATCC 314	35-37° / 70-74 H /AN	growth with blue-green colonies
L. delbrueckii subs *bulgaricus* DSM 20081	35-37° / 70-74 H /AN	growth with white colonies
L. casei ATCC 393	35-37° / 70-74 H /AN	growth with white colonies

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection: DSM: German Collection of Microorganisms and Cell Cultures

LIMITATIONS OF THE METHOD

- Other organisms such as enterococci, pediococci and *Leuconostoc* species may grow on CLAA medium.

STORAGE CONDITIONS

Store at +2°C /+8°C away from direct light in a dry place.

REFERENCES

1. Ashraf F., Shah NP. Selective and differential enumerations of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium* spp. in yoghurt--a review. Int J Food Microbiol. 2011 Oct 3;149(3):194-208.
2. Kneifel W, Pacher B. An X-Glu based agar medium for the selective enumeration of *Lactobacillus acidophilus* in yogurt-related milk products. International Dairy Journal 1993; 3:277
3. ISO 20128:2006 [IDF 192:2006]. Milk products -Enumeration of presumptive *Lactobacillus acidophilus* on a selective medium - Colony-count technique at 37 °C.

PACKAGING

Product	Type	REF	Pack
Chromogenic Lactobacillus Acidophilus Agar (CLAA)	Dehydrated medium	4015682 4015684	500 g (7 L) 5 kg (70 L)

IFU rev 0, 2021/05

ChromArt

CHROMOGENIC SALMONELLA AGAR BASE
SALMONELLA SELECTIVE SUPPLEMENT
CHROMOGENIC SALMONELLA AGAR

Dehydrated culture medium, supplements and ready-to-use plates

INTENDED USE

In vitro diagnostic. Selective and chromogenic medium base, used with selective supplements, and ready-to-use plates for the isolation and differentiation of *Salmonella* spp., from clinical and non-clinical specimens.

COMPOSITION*

CHROMOGENIC SALMONELLA AGAR BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Peptones	10.0 g
Selective compounds	12.0 g
Chromogenic mixture	0.9 g
Agar	15.0 g

SALMONELLA SELECTIVE SUPPLEMENTS

SALMONELLA SELECTIVE SUPPLEMENT VIAL A, VIAL CONTENTS FOR 500 ML OF MEDIUM

Emulsifying agents	5.7 mL

SALMONELLA SELECTIVE SUPPLEMENT VIAL B, VIAL CONTENTS FOR 500 ML OF MEDIUM

Cefsulodin	2.5 mg

CHROMOGENIC SALMONELLA AGAR, READY-TO-USE PLATES
TYPICAL FORMULA

Chromogenic Salmonella Agar Base	38.0 g
Opacifier	10.0 g
Emulsifying agents	11.4 mL
Cefsulodin	5.0 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Salmonella spp. remain one of the most important causes of foodborne gastroenteritis. Fluorogenic and chromogenic tests and media designed for the specific detection of *Salmonella* spp. have been available for at least 30 years. In 1987 a rapid fluorogenic screening reagent (MUCAP Test) for the identification of *Salmonella* colonies has been developed and proposed by Biolife Italiana, based on the detection of a specific *Salmonella* enzyme, C_8 esterase, by using a fluorogenic 4-methylumbelliferone-conjugated substrate.[1] Some years later, the same principle of detection of C_8 esterase enzyme has been exploited for the development of a chromogenic plating medium, Chromogenic Salmonella Agar, that demonstrated very high specificity and sensitivity for the detection of *Salmonella* spp.[2,3]
Chromogenic Salmonella Agar Base with Salmonella Selective Supplements is a selective and diagnostic medium, with a clear background, useful for the isolation of *Salmonella* spp. from clinical and non-clinical specimens and for the presumptive identification of the colonies. Chromogenic Salmonella Agar is included by ISTISAN Report[4] in the plating media range for the detection of *Salmonella* spp. and chromogenic media are included as the second plating medium in ISO Standards for detection of *Salmonella* in food and water.[5,6]
Peptones provide carbon, nitrogen, vitamins and trace elements for bacterial growth. The selective compounds incorporated in the medium are the following: cefsulodin, a third generation cephalosporin antibiotic that has very specific activity against *P. aeruginosa* and *S.aureus*, sodium

deoxycholate that suppresses the growth of Gram-positive and some Gram-negative bacteria and Tergitol 4, active mainly against the growth of *Proteus* spp.
Differentiation of *Salmonella* from other organisms that grow is achieved by:
- a chromogenic substrate for C_8 esterase enzyme, that is cleaved by *Salmonella* spp. with the release of an insoluble magenta-red chromophore.
- a chromogenic glucopyranoside derivative which is cleaved by β-glucosidase with the release of an insoluble blue-green chromophore.

Some *Enterobacteriaceae*, including *Klebsiella* and *Enterobacter,* but not *Salmonella*, are ß-glucosidase positive and if growing will form blue-green or dark blue colonies, even if they are esterase positive, which make them easy to differentiate from magenta-red *Salmonella* colonies.
The chromogenic and selective compounds of the medium also allow the detection of the rare lactose-positive *Salmonella* strains, missed on traditional media based on lactose fermentation. Chromogenic Salmonella Agar is useful also for the detection of *S*. Typhi and *S*. Paratyphi.
Because in the formulation is omitted the opaque compound, the prepared plates have a transparent background.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 19 g in 500 mL of cold purified water; add the content of one vial of Salmonella Selective Supplement Vial A, heat to boiling stirring constantly and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of Salmonella Selective Supplement Vial B, reconstituted with 2 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

CHROMOGENIC SALMONELLA AGAR BASE	
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	light yellowish, limpid
Final pH at 20-25 °C	7.2 ± 0.2
SALMONELLA SELECTIVE SUPPLEMENT VIAL A	
Appearance of solution	pale yellow slightly opalescent
SALMONELLA SELECTIVE SUPPLEMENT VIAL B	
Appearance of lyophilised pellet	short dense pastille
Appearance of solution	colourless limpid
CHROMOGENIC SALMONELLA AGAR	
Appearance of plates	whitish opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Chromogenic Salmonella Agar Base added with Salmonella Selective Supplements is intended for the bacteriological processing of clinical specimens such as faeces and rectal swabs. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[7] Consult appropriate standard methods for details of collection and preparation of non-clinical specimens.[5,6]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment step in Selenite Broth followed by sub-culture to Chromogenic Salmonella Agar and to a second plating medium.
Incubate inoculated plates with the specimen or with specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.
Consult appropriate references for the detection of *Salmonella* in food and water.[5,6]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Interpretation of colonies' colours:

Microorganism	Growth characteristics
Salmonella spp.	good growth, magenta-red colonies
Salmonella spp. lac+	good growth, magenta-red colonies
Salmonella Typhi	good growth, magenta-red colonies
E. coli	poor growth with colourless colonies
Enterobacter spp.	growth with blue-green colonies
Klebsiella spp.	poor growth with blue-green colonies
Pseudomonas spp.	inhibited
Proteus spp.	poor growth with pale brown or green colonies
Gram-positive bacteria	inhibited

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028	35-37°C / 18-24h / A	growth, magenta-red colonies
S. Enteritidis ATCC 13076	35-37°C / 18-24h / A	growth, magenta-red colonies
E. aerogenes ATCC 13048	35-37°C / 18-24h / A	growth, blue-green colonies
P. aeruginosa ATCC 27853	35-37°C / 18-24h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

PERFORMANCES CHARACTERISTICS

Chromogenic Salmonella Agar was evaluated by Babic-Erceg et al.[2] on 3,000 stool specimens, 45 of which positive for *Salmonella*, including SS Agar as the reference medium. The authors reported a sensitivity of 100% and a specificity of 99% in the isolation and preliminary identification of *Salmonella* colonies.
In another independent study[3], 50 pure cultures of *Salmonella* of clinical origin, gave all the specific chromatic reactions; among the other 80 strains of Gram-negative bacteria tested, not belonging to the *Salmonella* genus, 3 out of 3 strains of *P. aeruginosa* and 1 out of 3 strain of *A.*

baumannii provided chromatic results similar to *Salmonella* spp. (red-pink colonies), the remaining 76 strains of *Enterobacteriaceae* gave non-typical chromatic reactions; 20 out of 20 strains of Gram-positive bacteria were totally inhibited.
Chromogenic Salmonella Agar performance was evaluated with an in-house study, compared to Hektoen Enteric Agar (HEA). Productivity, selectivity and specificity have been evaluated by semi-quantitative ecometric technique, incubating at 35-37°C for 18-24 hours, using 43 bacterial strains: 8 target strains and 35 non target strains. 8 Salmonella strains, including 2 S. Typhi, showed a good growth with magenta-red colonies; 3 *Shigella* strains showed a poorer growth than on HEA with colourless colonies; 22 *Enterobacteriaceae* strains belonging to 9 genera showed a poorer growth than on HEA with colourless or blue-green colonies; 4 *P. aeruginosa* strains were totally inhibited; 2 non-fermenters strains were totally inhibited and *A. hydrophila* grew with magenta red colonies; 1 Gram-positive strain was totally inhibited and 1 yeast strain was partially inhibited showing colourless colonies.

Limitations of the method

- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella*, with lower selectivity such as Mac Conkey Agar should be used; other media for the isolation of other enteric pathogens should be inoculated with the specimen.
- Some strains of *Pseudomonas, Acinetobacter* and *Aeromonas,* resistant to antimicrobial agents of the medium, may grow with red-pink colonies, differentiable from *Salmonella* with oxidase test.
- The growth rate on the plates also depends on the nutritional requirements of *Salmonella*. It is possible that some strains with particular metabolic characteristics may not grow on the medium or grow colourless (e.g., *Salmonella enterica* serovar Dublin grows with white colonies).
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
Salmonella Selective Supplements
Store the product in the original package at +2°C /+8°C away from direct light.
Ready-to-use plates
Store plates in their original pack at +2°C/+8°C away from direct light

References

1. Pontello M, Russolo S, Carozzi F, Bottiroli U. Evaluation of a new rapid method (MUCAP Test) for the presumptive identification of Salmonella on primary isolation media. 5th Int. Simp. on Rapid Method and Aut. in Microb. and Immunol. Florence 4-6 nov. 1987
2. Babic-Erceg A et al. 12th European Congress of Clinical Microbiology and Infectious Diseases. Milan, April 24-27, 2002
3. Andreoni S. et al. Microbiologia Medica, 2002.
4. Istituto Superiore di Sanità Le infezioni da Salmonella: diagnostica, epidemiologia e sorveglianza. Caterina Graziani, Pasquale Galetta, Luca Busani, Anna Maria Dionisi, Emma Filetici, Antonia Ricci, Alfredo Caprioli, Ida Luzzi 2005, 49 p. Rapporti ISTISAN 05/27
5. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella
6. ISO19250:2010 Water quality — Determination of Salmonella species
7. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.

Packaging

Product	Type	REF	Pack
Chromogenic Salmonella Agar Base	Dehydrated medium	4053502 4053504	500 g (13,2 L) 5 kg (132 L)
Salmonella Selective Supplements	Selective supplements	4240013	5 vials A + 5 vials B, each for 500 mL of medium
Chromogenic Salmonella Agar	Ready-to-use plates	545350	2 x 10 plates ø 90 mm

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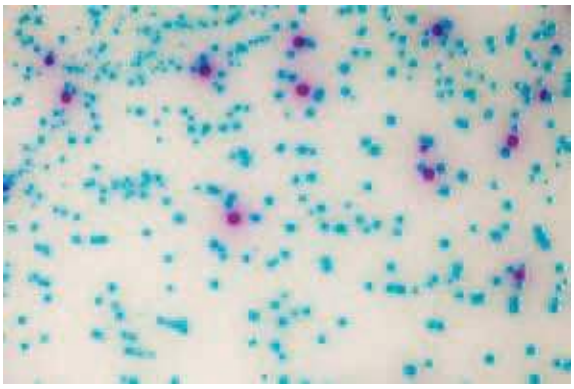

CSA: *Salmonella* sp. colonies (magenta-red),
E. aerogenes colonies (blue-green)

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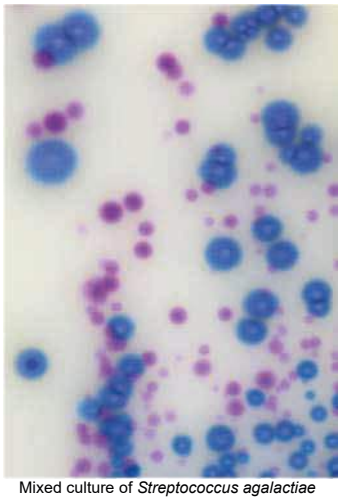
CHROMOGENIC STREPTO B AGAR BASE
CHROMOGENIC STREPTO B SUPPLEMENT
CHROMOGENIC STREPTO B AGAR

Dehydrated culture medium, selective supplement and ready-to-use plates



Mixed culture of *Streptococcus agalactiae* (pink-magenta colonies) and *Enterococcus* sp. (blue colonies)

INTENDED USE

In vitro diagnostics. Chromogenic basal medium, selective supplement and ready-to-use plates for the presumptive detection of Lancefield group B streptococci (*Streptococcus agalactiae*; GBS) carriage in clinical specimens.

COMPOSITION *

CHROMOGENIC STREPTO B AGAR BASE - DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptones	28.000 g
Buffer salts	5.250 g
Growth factors	6.700 g
Inorganic salts	8.500 g
Antimicrobial mix	0.027 g
Chromogenic mix	0.300 g
Opacifying compounds	6.500 g
Agar	15.000 g

CHROMOGENIC STREPTO B SUPPLEMENT
VIAL CONTENTS (FOR 500 mL OF MEDIUM)

Antimicrobial mix	0.04 g
Chromogenic mix	0.05 g

CHROMOGENIC STREPTO B AGAR - READY-TO-USE PLATES
TYPICAL FORMULA

Chromogenic Strepto B Agar Base	70.28 g
Antimicrobial mix	0.08 g
Chromogenic mix	0.10 g
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lancefield group B streptococci (GBS), or *Streptococcus agalactiae*, are facultatively anaerobic, oxidase-negative, catalase-negative, Gram-positive cocci occurring in chains, that cause invasive disease primarily in infants, pregnant or postpartum women, and older adults, with the highest incidence among young infants.[1,2]
Despite substantial progress in prevention of perinatal group B streptococcal disease since the 1990s, GBS remains the leading cause of early-onset neonatal sepsis. Universal screening at 35–37 weeks gestation for maternal GBS colonization and use of intrapartum antibiotic prophylaxis has resulted in substantial reductions in the burden of early-onset GBS disease among newborns.[2]
Optimum yield will be achieved by selective enrichment procedures and subculture to selective and non-selective media, applied to swabs obtained from the vagina and the anorectum which increase the likelihood of GBS isolation compared with vaginal or cervical culture alone.[1-3]
Chromogenic Strepto B Agar Base completed with Chromogenic Strepto B Supplement, is a selective and chromogenic medium for the isolation of Group B Streptococci (*S. agalactiae*) from clinical specimens and for the differentiation of the colonies based on a typical colour.
The medium consists in a buffered nutritive base containing antibiotics and chromogenic compounds. Gram-negative bacteria are strongly inhibited while the growth of Gram-positive organisms other than GBS is inhibited with different extent depending of genus and species of the organisms. The differential characteristics are based on specific enzymatic reactions, which allow the differentiation of *S. agalactiae* colonies (pink-magenta) from other bacteria not inhibited by selective agents (e.g. enterococci) which grow with green-blue, blue, without or with a pink halo or colourless colonies. The opaque white background helps in recognizing the colours of the colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.14 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and, under aseptic conditions, add the contents of one vial of Chromogenic Strepto B Supplement (4240053), reconstituted with 5 mL of sterile purified water, under aseptic conditions. Mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Chromogenic Strepto B Agar Base	
Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	white, opaque
Final pH at 20-25 °C	7.2 ± 0.2
Chromogenic Strepto B Supplement	
Appearance of the lyophilized	high, homogeneous, yellow pastille
Appearance of the solution	opalescent yellowish
Chromogenic Strepto B Agar	
Plates appearance	white, opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Specimens consist of maternal low vaginal and anorectal swabs collected and placed in appropriate transport medium (Amies or Stuart with or without charcoal).[1,4] While the culture counts decline to some extent, viability of *S. agalactiae* is preserved in transport medium kept at room temperature or 4°C for up to 4 days.[4] Maternal high vaginal swabs should not be collected as these have a lower sensitivity.[1] Good laboratory

practices for collection, transport and storage of the clinical specimens should be applied; collect specimens before antimicrobial therapy where possible.

TEST PROCEDURE

Chromogenic Strepto B Agar can be used according to two protocols:
- Inoculation of the plate after pre-enrichment in Todd Hewitt Broth supplemented with colistin and nalidixic acid (recommended because it is validated in the clinical study reported below and because it increases the sensitivity and specificity of the method).
- Direct inoculation of the specimen onto the agar surface.

Remove the cap aseptically from the specimen container and place the swab(s) in Todd Hewitt CNA Broth, break off (or cut) the swab stick(s) and replace the cap. Caps should be kept loose during incubation. Incubate at 35-37°C, 5% CO_2, for 18-24 hours.
Allow plates to come to room temperature in the dark. Subculture from the selective broth with a sterile loop and spread inoculum onto the agar surface.
For the direct inoculation, roll the swab(s) over a small area of the surface at the edge; then streak from this inoculated area.
Incubate the inoculated plates at 35 to 37°C, in air, for 24-48 hours.
Reading at 24 hours is possible in cases of urgency but increases the rate of false positivity. In any case, the final reading of the results must be made after incubation for full 48 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
- Typical *S. agalactiae* colonies: round colonies of varying size, pink or pink-magenta or magenta. Most strains develop good size (3-4 mm) round magenta colonies after 48 hours of incubation. At 24 hours some *Enterococcus* strains develop small pink or pink colonies with grey shades or have two types of small colonies: pink and grey. Colonies of these strains usually show a strong blue, grey-blue or purple colour at 48 hours.
- The presence of colourless, blue, green-blue, grey-blue, purple colonies with or without magenta halo should be interpreted as belonging to species other than *S. agalactiae* and the sample should be considered as negative.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. agalactiae	ATCC 13813	35-37°C / 44-48H / A	growth, pink-magenta colonies
E. faecalis	ATCC 19433	35-37°C / 44-48H / A	growth, blue colonies
P. aeruginosa	ATCC 27853	35-37°C / 44-48H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

PERFORMANCES CHARACTERISTICS

Chromogenic Strepto B Agar was evaluated by an independent Clinical Microbiological Laboratory in Italy on 225 anovaginal specimens. The medium was inoculated after enrichment of the specimen in Todd Hewitt CNA Broth. Reading was performed after 24 and 48 hours of incubation at 37°C. Chromogenic Strepto B Agar has been compared to a chromogenic medium of the market.
168 samples have been found “negative” with both chromogenic media; 44 samples have been found “positive” with both chromogenic media. 3 strains have been found “positive” with Chromogenic Strepto B Agar, “negative” with the reference medium and confirmed as Group B Streptococci by latex agglutination.
4 samples on Chromogenic Strep B Agar and 5 samples on the reference medium originated small pink colonies identified as Enterococci (false positive in the above table).
1 sample originated doubtful colonies on both media confirmed as non-Group B *Streptococcus* and considered in the above table as a “false positive”.
Chromogenic Strepto B Agar didn’t give any false negative result: sensitivity 100%
Chromogenic Strepto B Agar gave 5 false positive results: specificity: 97,2%
After 24 hours of incubation, 5 samples have been found “negative” on the Chromogenic Medium used as reference and originated typical colonies on Chromogenic Strepto B Agar; after 48 hours of incubation typical colonies were observed on the reference medium too.
The performance characteristics have been evaluated with 20 clinical collection *S. agalactiae* strains: all strains developed typical colonies on both media after 24 hours of incubation.

LIMITATIONS OF THE METHOD

- It is possible that few strains of *S. agalactiae* with specific growth requirements, may not grow on this medium. Optimum detection of GBS may require the use of more than one culture medium (e.g., selective medium and blood agar).[1]
- Some species (e.g., *Enterococcus* spp.) which are resistant to antibiotics may develop and produce colonies with an atypical colour. However, during the validation tests, 5 strains of enterococci produced small pink colonies.
- Group A streptococci and pneumococci may produce small pink colonies.
- The final reading and colonies interpretation shall be done after a full 48 hours incubation time.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. On the isolates, if relevant, perform antimicrobial susceptibility testing.
- The basal medium and the supplement are intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at +2°C /+8°C away from direct light.
Ready-to-use plates
Store plates in their original pack at 2-8°C in the dark

REFERENCES

1. Public Health England. UK Standards for Microbiology Investigations (SMI) Bacteriology, B58, Issue no:3, Issue date: 26.06.18 Detection of Carriage of Group B Streptococci (*Streptococcus agalactiae*).
2. Verani JR, McGee L, Schrag SJ. Prevention of Perinatal Group B Streptococcal Disease. MMWR Recomm. Rep. 2010 Nov 19; 59 (RR-10):1-36

3. Aila NA, Tency I, Claeys G, Saerens B, Cools P, Verstraelen H et al. Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infect Dis 2010;10:285.
4. Spellerberg B, Brandt C, Sendi P. *Streptococcus*. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
Chromogenic Strepto B Agar Base	Dehydrated medium	4080102	500 g (7.1 L)
Chromogenic Strepto B Supplement	Freeze-dried supplement	4240053	10 vials, each for 500 mL of medium base
Chromogenic Strepto B Agar	Ready-to-use plates	548010	2 x 10 plates ø 90 mm

IFU rev 3, 2022/01

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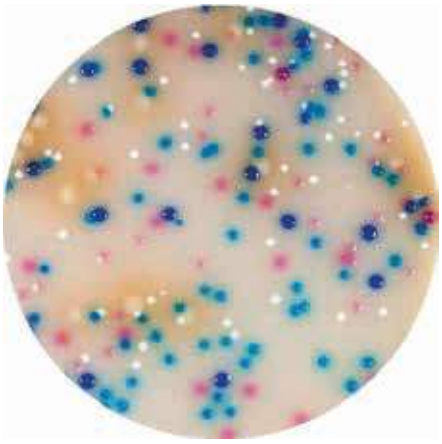
CHROMOGENIC URINE AGAR IV

Dehydrated culture medium



Mixed culture of UTI pathogens: *E.coli* (pink-magenta red colonies), *K.pneumoniae* (dark blue colonies), *Enterococcus* sp. (turquoise blue colonies), *S.aureus* (white colonies), *Proteus* sp. (light brown with brown halo).

INTENDED USE

In vitro diagnostic. Chromogenic culture medium for isolation, enumeration and presumptive rapid identification of microorganisms from urine.

COMPOSITION*

CHROMOGENIC URINE AGAR IV - DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Peptones and growth factors	24.0 g
Chromogenic mix	0.4 g
Opacifier compounds	6.2 g
Agar	15.0 g

CHROMOGENIC URINE AGAR IV – READY-TO-USE PLATES
TYPICAL FORMULA

Peptones and growth factors	24.0 g
Chromogenic mix	0.4 g
Opacifier compounds	10.0 g
Agar	15.0 g
Horse Serum	20.0 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In the last 25 years, chromogenic culture media have found widespread application in diagnostic clinical microbiology. Biolife proposed a chromogenic and fluorogenic medium for the diagnosis of urinary tract infections in 1997 named Chromogenic Urine Agar, in which the bacterial differentiation was obtained by the detection of β-glucuronidase, β-glucosidase and β-xylosidase. This first medium was subjected to incremental improvements over the years and new generations of media have been developed. The last one proposed by Biolife for urinary bacteriology is Chromogenic Urine Agar IV, a diagnostic medium intended for the isolation, enumeration and presumptive rapid identification of urinary tract pathogens such as *E. coli*, *Enterobacter-Klebsiella-Serratia*, *Proteus-Morganella-Providencia*, Enterococci, Staphylococci, yeasts.
The differentiation between the microbial species or genus is achieved by:

- A chromogenic substrate for β-galactosidase (GAL), which is cleaved by *E. coli* with the release of an insoluble pink-red chromophore.
- A chromogenic glucopyranoside derivative for β-glucosidase (GLU), which is cleaved by Enterococci with the formation of an insoluble blue-green dye.
- Tryptophan for the detection of tryptophan deaminase (TDA) and for performing rapid indole test.

Bacteria that produce both the enzymes (GAL and GLU), such as *Klebsiella/Enterobacter/Serratia* (KES) group, give dark blue or purple colonies. Tryptophan is present in the medium to detect members of the *Proteus* group, which generate a diffuse brown coloration as the result of tryptophan deaminase activity.
E. coli may be confirmed by indole spot test by adding a drop of Kovacs' Reagent to isolated colonies.
Main characteristics and advantages of CUA IV are: very good productivity obtained with selected and standardized peptones, optimized agar concentration to inhibit the swarming of *Proteus* spp., enhanced visual differentiation of the colonies due to strong chromatic reactions, grey opaque contrasting background and specific enzymatic reactions for presumptive identification of both Gram-positive and Gram-negative pathogens.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 45.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. The prepared plates shall be used within ten days from the date of preparation. For prolonged storage of the pre-poured plates add horse serum: cool the autoclaved medium to 47-50°C and add under aseptic conditions, 20 mL/L of Horse Serum. Mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	greyish, opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Chromogenic Urine Agar IV (CUA IV) is intended for the microbiological processing of clinical specimens such as urine. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[1,2]

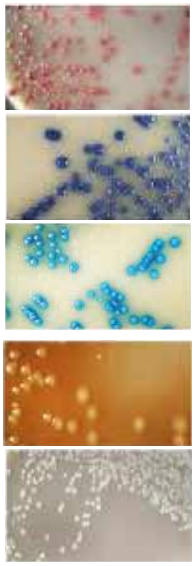
TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Mix the urine gently to avoid foaming. Dip the end of a sterile calibrated loop (e.g., 1µL or 10µL) in the urine to just below the surface and remove vertically, taking care not to carry over any on the shank. Use this to inoculate CUA IV plate from top to bottom in a vertical line and again from top to bottom perpendicular to this line in a back-and-forth fashion. The inoculum of urine is spread over the entire agar surface to simplify counting of colonies after growth.
Incubate at 35-37°C in air for 24 to 48 hours.
Although most urinary tract pathogens grow readily, slowly growing pathogens and those inhibited by the presence of antimicrobials in the patient specimen may not appear after overnight incubation (16 h). Perform leukocyte esterase and nitrite tests to determine which cultures get incubated for a full 48 hours. Urine cultures that are negative after overnight incubation but had one or both positive enzyme tests should be incubated for an additional day or re-inoculated on a blood agar plate.[1]

READING AND INTERPRETATION

After incubation, observe the bacterial growth, count the number of colonies (CFU) on the plate and record the specific morphological, chromatic, characteristics of the colonies. If a 1µL loop is used, one colony equals 1000 CFU/mL, if a 10µL loop is used, one colony equals 100 CFU/mL. Studies conducted in the 1950s remain the basis for interpreting urine culture results showing that bacterial counts of $\geq 10^5$ CFU/mL are indicative of an infection and counts below this usually indicate contamination.[2]
In specific patient groups, counts between 10^5 CFU/mL and 10^2 CFU/mL may be significant; a pure isolate with counts between 10^4 and 10^5 CFU/mL should be evaluated based on clinical information or confirmed by repeat culture.[2] For urine collected by suprapubic bladder puncture any CFU detected indicates an infection.[2]
Consult appropriate references for complete interpretation criteria of the microbial count.[1,2]

Typical colonial morphology and colours interpretation on Chromogenic Urine Agar IV are as follows:



Escherichia coli: pink colonies (β-galactosidase positive, β-glucosidase negative)
Indole test positive: *E. coli*
Indole test negative: proceed to the identification with conventional methods.

Klebsiella – Enterobacter - Serratia (KES): blue/blue-violet colonies:
(β-galactosidase positive, β-glucosidase positive)
Microscopic examination: Gram negative bacilli
For genus/species identification, proceed with conventional identification methods.

Enterococcus spp.: green to turquoise blue colonies (β-galactosidase neg., β-glucosidase pos.)
Microscopic examination: gram positive cocci.

Proteus-Morganella-Providencia: (brown colonies: tryptophan deaminase positive, β-galactosidase negative, β-glucosidase negative)
Indole test negative: *Proteus mirabilis*, to be confirmed with oxidase test which is negative.
Indole test positive: *Providencia* or *Morganella* or *Proteus* spp. indole + (proceed to the identification)

Staphylococci and yeasts: white colonies (β-galactosidase negative, β-glucosidase negative)
Microscopic examination: gram positive cocci or yeasts
Proceed to the identification with conventional methods.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth, pink colonies
E. faecalis	ATCC	29212	35-37°C / 18-24H / A	good growth, green colonies
S. aureus	ATCC	25923	35-37°C / 18-24H / A	good growth, white colonies
K. pneumoniae	ATCC	27736	35-37°C / 18-24H / A	good growth, dark blue colonies
P. mirabilis	ATCC	12453	35-37°C / 18-24H / A	good growth, bluish colonies not swarmed

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Gram staining is recommended to confirm any doubtful colour reactions.
- *Citrobacter* spp. may be presumptively identified as *E coli* because some strains are β-galactosidase positive and β-glucosidase negative The use of a spot indole test successfully eliminates some of these false positives[4]. The use of susceptibility data or the detection of pyrrolidonyl aminopeptidase (PYR test) may facilitate the differentiation of pink colonies of *Citrobacter* spp. from *E. coli*.[5]
- Between the *Proteus-Morganella-Providencia* group, *P. mirabilis* is indole negative and can be easy recognised.
- Biochemical identification is needed for genus/species identification within *Klebsiella, Enterobacter, Serratia* group.
- A pyrrolidonyl aminopeptidase (PYR test) might be necessary to differentiate enterococci from *S. agalactiae*.
- *S. saprophyticus* and *S. xylosus* produces small pink colonies.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, of indole test or Gram morphology, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use plates
Store plates in their original pack at +2°C/+8°C away from direct light.

References

1. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
2. Public Health England UK Standards for Microbiology Investigations. Investigation of urine. Bacteriology, B 41, 2019
3. Guidelines for assuring quality of medical microbiological culture media. The Australian Society for Microbiology, 2nd edition, 2012.
4. Perry JD, Butterworth LA, Nicholson A, Appleby MR, Orr KE. J Clin Pathol 2003; 56(7): 528–531
5. Fallon D, Andrews N, Frodsham D, Gee B, Howe S, Iliffe A, Nye KJ, Warren RE. J Clin Pathol 2002; 55(7): 524–529

Packaging

Product	Type	REF	Pack
Chromogenic Urine Agar IV	Dehydrated medium	409810G2	500 g (10.9 L)
Chromogenic Urine Agar IV	Ready-to-use plates	549810G	2 x 10 plates ø 90 mm

IFU rev 5, 2022/01

ChromArt

CHROMOGENIC URINE AGAR IV CLEAR

Dehydrated culture medium

Intended use

Chromogenic culture medium with a clear background for the isolation, enumeration and presumptive rapid identification of microorganisms from urine.

Composition - typical formula * (after reconstitution with 1 L of water)

Peptones and growth factors	24.0 g
Chromogenic mix	0.4 g
Agar	22.1 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Chromogenic Urine Agar IV Clear is a diagnostic medium, with a clear background, intended for the isolation, enumeration and presumptive rapid identification of urinary tract pathogens such as *E. coli*, *Enterobacter-Klebsiella-Serratia*, *Proteus-Morganella-Providencia*, enterococci, staphylococci, yeasts.

The differentiation between the microbial species or genus is achieved by:

- A chromogenic substrate for β-galactosidase (GAL), which is cleaved by *E. coli* with the release of an insoluble pink-red chromophore.
- A chromogenic glucopyranoside derivative for β-glucosidase (GLU), which is cleaved by enterococci with the formation of an insoluble blue-green dye.
- Tryptophan for the detection of tryptophan deaminase (TDA) and for performing rapid indole test.

Strains producing ß-galactosidase only, such as *E. coli* grow with pink-magenta colonies, strains producing β-glucosidase only, such as enterococci form green to turquoise blue colonies. Bacteria that produce both the enzymes (ß-galactosidase and β-glucosidase), such as *Klebsiella/Enterobacter/Serratia* (KES) group, give dark blue or purple colonies. Tryptophan is present in the medium to detect members of the *Proteus* group, which generate a diffuse brown coloration as the result of tryptophan deaminase activity.

E. coli may be confirmed by indole spot test by adding a drop of Kovacs' Reagent to isolated colonies.

Main characteristics and advantages of Chromogenic Urine Agar IV Clear are: very good productivity obtained with selected and standardized peptones, optimized agar concentration to inhibit the swarming of *Proteus* spp., enhanced visual differentiation of the colonies due to strong chromatic reactions.

Directions for medium preparation

Suspend 46.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add, under aseptic conditions, 20 mL of Horse Serum. Mix well and pour into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellowish, limpid
Final pH at 20-25 °C	7.2 ± 0.2

Specimens

Chromogenic Urine Agar IV Clear (CUA IV clear) is intended for the microbiological processing of urines. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[1,2]

Test procedure

Allow plates to come to room temperature and to dry the surface of the medium. Mix the urine gently to avoid foaming. Dip the end of a sterile calibrated loop (e.g., 1µL or 10µL) in the urine to just below the surface and remove vertically, taking care not to carry over any on the shank. Use this to inoculate CUA IV Clear plate from top to bottom in a vertical line and again from top to bottom perpendicular to this line in a back-and-forth fashion. The inoculum of urine is spread over the entire agar surface to simplify counting of colonies after growth.

Incubate at 35-37°C in air for 24 to 48 hours.

Although most urinary tract pathogens grow readily, slowly growing pathogens and those inhibited by the presence of antimicrobials in the patient specimen may not appear after overnight incubation (16 h). Perform leukocyte esterase and nitrite tests to determine which cultures get incubated for a full 48 hours. Urine cultures that are negative after overnight incubation but had one or both positive enzyme tests should be incubated for an additional day or re-inoculated on a blood agar plate.[1]

Reading and interpretation

After incubation, observe the bacterial growth, count the number of colonies (CFU) on the plate and record the specific morphological, chromatic, characteristics of the colonies. If a 1µL loop is used, one colony equals 1000 CFU/mL, if a 10µL loop is used, one colony equals 100 CFU/mL.

Studies conducted in the 1950s remain the basis for interpreting urine culture results showing that bacterial counts of ≥10^5 CFU/mL are indicative of an infection and counts below this usually indicate contamination.[2]
In specific patient groups, counts between 10^5 CFU/mL and 10^2 CFU/mL may be significant; a pure isolate with counts between 10^4 and 10^5 CFU/mL should be evaluated based on clinical information or confirmed by repeat culture.[2] For urine collected by suprapubic bladder puncture any CFU detected indicates an infection.[2] Consult appropriate references for complete interpretation criteria of the microbial count.[1,2]
Typical colonial morphology and colours interpretation on Chromogenic Urine Agar IV Clear:

Escherichia coli: pink colonies (β-galactosidase positive, β-glucosidase negative)
Indole test positive: *E. coli*
Indole test negative: proceed to the identification with conventional methods.

Klebsiella – Enterobacter - Serratia (KES): blue/blue-violet colonies:
(β-galactosidase positive, β-glucosidase positive)
Microscopic examination: Gram negative bacilli
For genus/species identification, proceed with conventional identification methods.

Enterococcus spp.: green to turquoise blue colonies (β-galactosidase neg., β-glucosidase pos.)
Microscopic examination: gram positive cocci.

Proteus-Morganella-Providencia: (brown colonies: tryptophan deaminase positive, β-galactosidase negative, β-glucosidase negative)
Indole test negative: *Proteus mirabilis*, to be confirmed with oxidase test which is negative.
Indole test positive: *Providencia* or *Morganella* or *Proteus* spp. indole + (proceed to the

Staphylococci and yeasts: white colonies (β-galactosidase negative, β-glucosidase negative)
Microscopic examination: g ram positive cocci or yeasts
Proceed to the identification with conventional methods.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli	ATCC 25922	35-37°C / 18-24H / A	good growth, pink colonies
E. faecalis	ATCC 29212	35-37°C / 18-24H / A	good growth, green colonies
S. aureus	ATCC 25923	35-37°C / 18-24H / A	good growth, white colonies
K. pneumoniae	ATCC 27736	35-37°C / 18-24H / A	good growth, dark blue colonies
P. mirabilis	ATCC 12453	35-37°C / 18-24H / A	good growth, bluish colonies not swarmed

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Gram staining is recommended to confirm any doubtful colour reactions.
- *Citrobacter* spp. may be presumptively identified as *E. coli* because some strains are β-galactosidase positive and β-glucosidase negative. The use of a spot indole test successfully eliminates some of these false positives[4]. The use of susceptibility data or the detection of pyrrolidonyl aminopeptidase (PYR test) may facilitate the differentiation of pink colonies of *Citrobacter* spp. from *E. coli*.[5]
- Between the *Proteus-Morganella-Providencia* group, *P. mirabilis* is indole negative and can be easy recognised.
- Biochemical identification is needed for genus/species identification within *Klebsiella, Enterobacter, Serratia* group.
- A pyrrolidonyl aminopeptidase (PYR test) might be necessary to differentiate enterococci from *S. agalactiae*.
- *S. saprophyticus* and *S. xylosus* produces small pink colonies.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, of indole test or Gram morphology, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +2°C /+8°C away from direct light in a dry place

REFERENCES

1. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
2. Public Health England UK Standards for Microbiology Investigations. Investigation of urine. Bacteriology, B 41, 2019
3. Guidelines for assuring quality of medical microbiological culture media. The Australian Society for Microbiology, 2nd edition, 2012.
4. Perry JD, Butterworth LA, Nicholson A, Appleby MR, Orr KE. J Clin Pathol 2003; 56(7): 528–531
5. Fallon D, Andrews N, Frodsham D, Gee B, Howe S, Iliffe A, Nye KJ, Warren RE. J Clin Pathol 2002; 55(7): 524–529

PACKAGING

Product	Type	REF	Pack
Chromogenic Urine Agar IV Clear	Dehydrated medium	409810C2	500 g (10.7 L)

IFU rev 2, 2022/07

ChromArt

CHROMOGENIC YERSINIA AGAR BASE
CHROMOGENIC YERSINIA SUPPLEMENT
Dehydrated culture medium and selective supplement

INTENDED USE
For the determination of the presence or absence of *Yersinia enterocolitica* in foods.

COMPOSITION*
CHROMOGENIC YERSINIA AGAR BASE
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Peptones	22.0 g
Cellobiose	20.0 g
Sodium pyruvate	2.0 g
Sodium chloride	5.0 g
Sodium deoxycholate	0.5 g
Neutral red	0.03 g
Crystal violet	0.001 g
Irgasan	0.004 g
5-Bromo-4-chloro-3-indolyl β-D-glucopyranoside (X-Glupy)	0.2 g
Growth factors	0.15 g
Agar	12.0 g

CHROMOGENIC YERSINIA SUPPLEMENT
VIAL CONTENTS FOR 500 mL OF MEDIUM

Antibiotic mix	0.0875 g

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Chromogenic Yersinia Agar is a modification of Cefsulodin-Irgasan-Novobiocin (CIN) Agar originally developed in 1979 by Schiemann,[1] devised to differentiate, with a chromogenic reaction, the colonies of some enterobacteria from those of *Yersinia enterocolitica*, thus increasing the specificity of the method.
Chromogenic Yersinia Agar, complete with the antibiotic mix of the selective supplement, may be used as the second plating medium in the procedure recommended by ISO 10273[2] for the determination of the presence or absence of pathogenic *Y. enterocolitica* in food chain samples.
Peptone and special growth factors provide nutrients for bacterial growth. Sodium chloride maintains the osmotic balance of the medium. Sodium pyruvate aids in the resuscitation of sub-lethally injured cells. Gram-positive and some Gram-negative bacteria (e.g. *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa*) are inhibited by the selective agents present in the medium base (sodium deoxycholate, crystal violet, irgasan) and in the lyophilized supplement. Cellobiose is present as a fermentable carbohydrate: cellobiose fermenting bacteria induce acidification of the medium with precipitation of deoxycholate and absorption of neutral red; *Y. enterocolitica* therefore cultivates with the characteristic aspect of the "bull's eye" colonies: the centre of the colony deep red with a transparent margin. Organisms that do not metabolize cellobiose to acid end-products will form colourless colonies. The medium also contains X-Glupy: the colonies of β-glucosidase positive strains hydrolyse the chromogenic compound with the formation of a green-blue chromophore. *C. freundii, P. rettgeri, S. marcescens, K. oxytoca* form blue or blue-green colonies.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 30.9 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and add the content of one vial of Chromogenic Yersinia Supplement (REF 4240095) dissolved with 5 mL of sterile purified water using aseptic precautions. Mix well and pour into sterile Petri dishes

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-purple, slightly opalescent
Freeze-dried selective supplement	short, dense, white, pellet
Reconstituted selective supplement	colourless, limpid
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

SPECIMENS
Food products and ingredients intended for human consumption and the feeding of animals; environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[2]

TEST PROCEDURE
Prepare the suspension of the sample with Yersinia PSB Broth (REF 402270) to obtain a 1:10 dilution (e.g.: 25 g of sample +225 mL of liquid medium).
The ISO 10273 method prescribes the direct inoculum of the sample suspension on CIN Agar plates and on a chromogenic medium or the inoculum of the sample after enrichment.
DIRECT PLATING
Using the initial PSB suspension, divide a volume of 1 mL onto two to four CIN agar plates and a volume of 1 mL onto two to four Chromogenic Yersinia Agar plates. Spread it over the plates with a spreader. Invert the plates and place them in the incubator set at 30°C for 24 h ± 2 h.
PLATING AFTER ENRICHMENT
Transfer 10 mL of the initial suspension in PSB (REF 402270) into 90 mL of Yersinia ITC Broth (REF 402265).
Incubate the initial suspension in PSB and the selective enrichment broth ITC at 25 °C for 44 h ± 4 h.
Using a sterile pipette, transfer 0.5 mL of the PSB enrichment into 4.5 mL of KOH solution (0.5 g in 100 mL of sterile saline solution) prepared the day before use, and mix. After 20 s ± 5 s of the addition of the PSB enrichment to the KOH solution, streak by means of a loop the surface of a CIN Agar plate and of a Chromogenic Yersinia Agar plate.
Repeat the procedure for ITC enrichment.
Invert the plates and place them in the incubator set at 30°C for 24 h ± 2 h.

Inoculate CIN Agar plates and Chromogenic Yersinia Agar plates also with untreated PSB and ITC cultures.

READING AND INTERPRETATION

Observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
After an incubation at 30°C for 24 h ± 2 h on Chromogenic Yersinia Agar, *Y. enterocolitica* grows with red colonies, with transparent surrounding rim. The colonies diameter changes from one strain to another but is the same within the same serotype.
K. oxytoca, C. freundii, P. rettgeri, S. marcescens can grow and form blue or green/blue colonies. *Aeromonas hydrophila* grows with pink colonies. Gram positive bacteria are totally inhibited. Perform the confirmation tests, according to ISO 10273[2], on the typical colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
Y. enterocolitica	ATCC	9610	30°C / 18-24H / A	good growth, colonies with red centre
S. marcescens	ATCC	8100	30°C / 18-24H / A	good growth, blue colonies
E. coli	ATCC	25922	30°C / 18-24H / A	inhibited
S. aureus	ATCC	25923	30°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- In case of dense growth of background flora on the plates, the colony size of *Y. enterocolitica* can be smaller and the typical red centre can be unclear or absent.
- *Y. intermedia, Y. frederiksenii,* and *Y. kristensenii* grow equally as well as *Y. enterocolitica* and exhibit the same colony morphology.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at 2-8°C away from direct light.

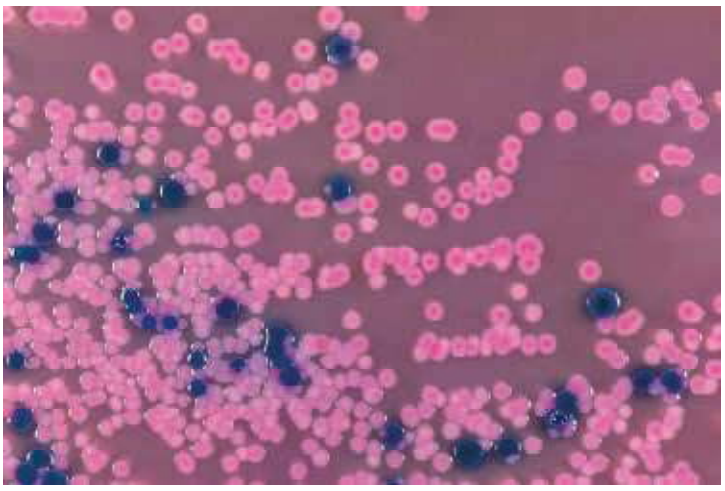
REFERENCES

1. Schiemann D A. Synthesis of a selective agar medium for Yersinia enterocolitica. Can J Microbiol 1979; 25(11):1298-1304,
2. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica

PACKAGING

Product	Type	REF	Pack
Chromogenic Yersinia Agar Base	Dehydrated medium	4080502	500 g (8.09 L)
Chromogenic Yersinia Supplement	Freeze-dried supplement	4240095	10 vials, each for 500 mL of medium

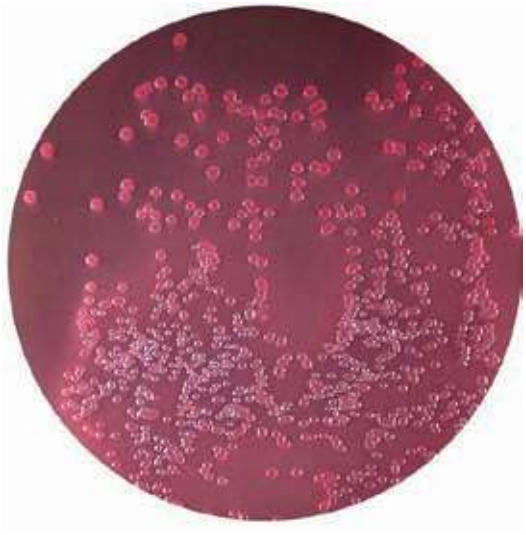
IFU rev 1, 2022/06



Chromogenic Yersinia Agar:
Yersinia enterocolitica with “bull’s eye” colonies;
Serratia marcescens: blue colonies.

CIN AGAR BASE
YERSINIA SELECTIVE SUPPLEMENT
YERSINIA SELECTIVE AGAR

Dehydrated culture medium, selective supplement and ready-to-use plates



Yersinia enterocolitica on CIN Agar

INTENDED USE

In vitro diagnostics. Selective and differential basal medium, selective supplement and ready-to-use plates for the isolation and characterisation of *Y. enterocolitica* from clinical and other specimens.

COMPOSITION *

CIN AGAR BASE, DEHYDRATED MEDIUM

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20.000 g
Yeast extract	2.000 g
Mannitol	20.000 g
Sodium pyruvate	2.000 g
Sodium chloride	1.000 g
Magnesium sulphate	0.010 g
Sodium deoxycholate	0.500 g
Irgasan	0.004 g
Neutral red	0.030 g
Crystal violet	0.001 g
Agar	12.000 g

YERSINIA SELECTIVE SUPPLEMENT

VIAL CONTENTS FOR 500 mL OF MEDIUM

Cefsulodin	7.50 mg
Novobiocin	1.25 mg

YERSINIA SELECTIVE AGAR, READY-TO-USE PLATES

TYPICAL FORMULA

CIN Agar Base	57.5 g
Cefsulodin	15.0 mg
Novobiocin	2.5 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The genus *Yersinia* includes 20 species, among them, only *Y. pestis*, *Y. pseudotuberculosis*, and certain strains of *Y. enterocolitica* are of pathogenic importance for humans, whereas the other species are of environmental origin. Of these, *Y. enterocolitica* is the most important as a cause of foodborne illness. *Y. enterocolitica* is a Gram-negative bacillus, motile at temperatures of 22–29°C but non-motile at 37°C. The most common form of disease due to *Y. enterocolitica* is gastroenteritis associated with consumption of contaminated food or water.[1] *Y. enterocolitica* is a heterogeneous group of strains, which are traditionally classified by biotyping into six biogroups on the basis of phenotypic characteristics, and by serotyping into more than 57 O serogroups, on the basis of their O (lipopolysaccharide or LPS) surface antigen. Of the six biotypes, five are recognised to be pathogenic (1B, 2-5). The most important *Y. enterocolitica* serogroup in many European countries is serogroup O:3 followed by O:9, whereas the serogroup O:8 is mainly detected in the United States.[2]

Cefsulodin-Irgasan-Novobiocin (CIN) Agar, originally developed in 1979 by Schiemann,[3] is a selective and differential medium for the isolation and characterisation of *Y. enterocolitica* from clinical[1,2] and non-clinical specimens[4,5]. The medium is recommended by ISO 10273[4] and by FDA-BAM[5] for the detection of *Y. enterocolitica* in food.

Compared with MacConkey agar, CAL agar and Y medium, CIN agar has been found to be the most effective medium for the recovery of *Y. enterocolitica*, inhibiting almost completely the faecal flora, while at the same time supporting luxuriant growth of *Y. enterocolitica*.[6]

The medium is highly selective: Schiemann[6] and Devenish[7] reported that only some strains of *C. freundii, S. liquefaciens* and *E. agglomerans* grow on CIN Agar; the colonies of these contaminants have an appearance similar to *Y. enterocolitica*.

Peptone and yeast extract provide nutrients for bacterial growth. Gram-positive and some Gram-negative bacteria (e.g., *E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa*) are inhibited by the selective agents present in the medium base (sodium deoxycholate, crystal violet, irgasan) and in the lyophilized supplement (cefsulodin and novobiocin). Mannitol is present as a fermentable carbohydrate: mannitol fermenting bacteria induce acidification of the medium with precipitation of deoxycholate and absorption of neutral red; *Y. enterocolitica* therefore cultivates with the characteristic aspect of the "bull's eye" colonies: the centre of the colony deep red with a transparent margin. Organisms that do not metabolize mannitol to acid end-products will form colourless colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 28.75 g in 500 mL of cold purified water. Heat to boiling stirring constantly, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of Yersinia Selective Supplement reconstituted with 5 mL of sterile purified water under aseptic conditions. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

CIN Agar Base

Dehydrated medium appearance	pinkish-beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.2

Yersinia Selective Supplement

Freeze-dried supplement appearance	short, dense, white pastille
Reconstituted supplement appearance	limpid, colourless

Yersinia Selective Agar, ready-to-use plates

Appearance	red-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

CIN Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swab[1,2] and non-clinical specimens such as food and animal feeding stuffs[4,5]. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[8] Collect specimens before antimicrobial therapy where possible. Consult appropriate standard methods for details on food sample collection and preparation.[4,5]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Clinical specimens

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Faeces may be diluted 1:4 in sterile saline solution or 0.1% peptone water. It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens.[2]

Incubate aerobically at 28-30°C for 24-48 hours.[2]

Food chain samples[4]

The general procedure involves:

- Direct plating of sample suspension prepared in PSB broth* on CIN Agar plate and incubation at 30°C ± 1°C for 24 h ± 2 h, or
- Enrichment in PSB Broth and in ITC broth** with incubation at 25°C ± 1°C for 44 h ± 4, followed by alkaline treatment of the cultures (0.5 mL of culture + 4.5 mL KOH 0.5% in saline solution for 20 s ± 5 s) and plating onto CIN Agar (incubation at 30°C ± 1°C for 24 h ± 2 h).
- A second plating medium may be chosen by the user (e.g. Chromogenic Yersinia Agar) ***

Notes

* Yersinia PSB Broth (REF 402270). ** Yersinia ITC Broth Base REF 402265 added with Potassium Chlorate Supplement (REF 4240065) and Ticarcillin Irgasan Antimicrobic Supplement (REF 4240060). *** Chromogenic Yersinia Agar Base (REF 408050) added with Chromogenic Yersinia Supplement (REF 4240095).

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Y. enterocolitica will ferment the mannitol and will develop colonies with deep red centres with sharp borders (irregular or entire), surrounded by an outer zone which is usually translucent ("bull's eye" colonies). The colony size, smoothness and the ratio of the border to centre diameter will vary considerably among serotypes.

Mannitol non-fermenters will grow with colourless or pale-yellow colonies.

Growth of non-*Yersinia* organisms is markedly to completely inhibited.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
Y. enterocolitica	ATCC	9610	28-30°C / 18-24H / A	good growth, colonies with red centre
P. aeruginosa	ATCC	27853	28-30°C / 44-48H / A	inhibited or partially inhibited, colourless colonies
E. coli	ATCC	25922	28-30°C / 44-48H / A	inhibited
E. faecalis	ATCC	19433	28-30°C / 44-48H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- In case of dense growth of background flora on the CIN plates, the colony size of pathogenic *Y. enterocolitica* can be smaller and the typical red centre can be unclear or absent.[4]
- *Y. intermedia, Y. frederiksenii, and Y. kristensenii* grow equally as well as *Y. enterocolitica* on CIN Agar and exhibit the same colony morphology.[10]
- *Serratia, Enterobacter* and *Citrobacter* are poorly inhibited. *Serratia* and *Enterobacter* develop raised and mucoid colonies with a diffuse pink pigmentation, although, occasionally, they can be confused with *Y. enterocolitica* colonies; *Citrobacter* colonies are the closest in appearance to *Yersinia* and cannot be distinguished only by their morphological characteristics.[10]
- The majority of *Y. pseudotuberculosis* strains are inhibited by the concentration of 15 mg/L of cefsulodin.[11]
- Some strains of *Y. enterocolitica* serovar O3 fail to grow on CIN Agar.[11]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to Baird et al. the self-prepared plates may be stored at +2°C/ +8° for 7 days.[12]

Selective supplement and ready-to-use plates

Store the products in the original package at +2°C/ +8°C away from direct light.

REFERENCES

1. Petersen MJ, Gladney LM, Schriefer ME. Yersinia. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.
2. Public Health England. Investigations of Faecal Specimens for Enteric Pathogens. UK Standards for Microbiology Investigations. 2014. B 30 Issue 8.1.
3. Schiemann D A. Synthesis of a selective agar medium for Yersinia enterocolitica. Can J Microbiol 1979; 25(11):1298-1304,
4. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 8: Yersinia enterocolitica. Rev 10/2017
6. Head CB, Whitty DH, Ratnam S. Comparative Study of Selective Media for Recovery of Yersinia enterocolitica. J Clin Microbiol 1982;16:615.
7. Schiemann DA. Development of a Two-Step Enrichment Procedure for Recovery of Yersinia enterocolitica from Food. Appl Environ Microbiol 1982; 43 :14-27

8. Devenish JA, Schiemann DA. An abbreviated scheme for identification of Yersinia enterocolitica isolated from food enrichments on CIN (cefsulodin–irgasan–novobiocin) agar. Can J Microbiol1981; 27: 937-941.
9. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
11. Fukushima H. Gomyoda M. Growth of Yersinia pseudotuberculosis and Yersinia enterocolitica Biotype 3B Serotype 03 Inhibited on Cefsulodin-Irgasan-Novobiocin Agar. J Clin Microbiol 1986, 24:116-120
12. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:208.

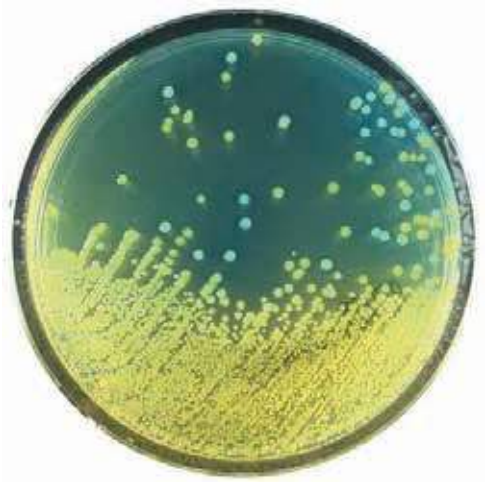
PACKAGING

Product	Type	REF	Pack
CIN Agar Base	Dehydrated medium	4013022	500 g (8.7 L
Yersinia Selective Supplement	Freeze-dried supplement	4240011	10 vials, each for 500 mL of medium
Yersinia selective aga	Ready-to-use plates	549997	2 x 10 plates ø 90 mm

IFU rev 1, 2020/09

CLED AGAR

Dehydrated and ready-to-use culture medium



CLED Agar: Lactose fermenting *E. coli* (yellow colonies) and non-lactose fermenting *Salmonella* (blue colonies)

INTENDED USE

In vitro diagnostics. Differential culture medium for isolation, enumeration and presumptive identification of microorganisms from urine.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Tryptone	4.000 g
Pancreatic digest of gelatin	4.000 g
Peptone	3.000 g
Lactose	10.000 g
L-cystine	0.128 g
Bromothymol blue	0.020 g
Agar	15.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

CLED Agar, is based on the “electrolyte deficient medium” first described by Sandys[1] for urinary bacteriology to prevent the swarming of *Proteus* spp. and later modified by Mackey and Sandys[2] by replacing the mannitol with 1% lactose and 0.2% sucrose and increasing the pH indicator and the agar concentrations. Mackey and Sandys[3] further modified the medium by the incorporation of L-cystine in order to enhance the growth of cystine-dependent “dwarf colony” coliforms and by deletion of sucrose. This final Cystine - Lactose - Electrolyte – Deficient (C.L.E.D.) medium is found to be ideal for the dip-slide inoculation and for urinary bacteriology in general, good colonial differentiation and easy recognition being particular features.[3] It supports the growth of all urinary potential pathogens. It also supports the growth of a number of essential contaminants such as diphtheroids, lactobacilli, and micrococci, which gives an indication of the extent of the contamination, and whilst being non-inhibitory it prevents the swarming of *Proteus* sp.[3]

Animal peptones provide carbon, nitrogen, vitamins and trace elements for microbial growth; cystine enhance the growth of "dwarf colony" coliforms[3]; lactose is present in the medium as a fermentable carbohydrate: lactose-fermenting bacteria acidify the medium with a colour change of bromothymol blue from blue-green to yellow.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 36.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green-grey, fine, homogeneous, free-flowing powder
Medium appearance of solution and plates	blue-green, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

CLED Agar is intended for the microbiological processing of clinical specimens such as urine. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[4,5]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Mix the urine gently to avoid foaming. Dip the end of a sterile calibrated loop (e.g. 1µL or 10µL) in the urine to just below the surface and remove vertically, taking care not to carry over any on the shank. Use this to inoculate CLED Agar plate from top to bottom in a vertical line and again from top to bottom perpendicular to this line in a back-and-forth fashion. The inoculum of urine is spread over the entire agar surface to simplify counting of colonies after growth.

Incubate at 35-37°C in air for 24 to 48 hours.

Although most urinary tract pathogens grow readily, slowly growing pathogens and those inhibited by the presence of antimicrobials in the patient specimen may not appear after overnight incubation (16 h). Perform leukocyte esterase and nitrite tests to determine which cultures get incubated

for a full 48 hours. Urine cultures that are negative after overnight incubation but had one or both positive enzyme tests should be incubated for an additional day or re-inoculated on a blood agar plate.[4]

READING AND INTERPRETATION

After incubation, observe the bacterial growth, count the number of colonies (CFU) on the plate and record the specific morphological, chromatic, characteristics of the colonies.
If a 1µL loop is used, one colony equals 1000 CFU/mL, if a 10µL loop is used, one colony equals 100 CFU/mL.
Studies conducted in the 1950s remain the basis for interpreting urine culture results showing that bacterial counts of $\geq 10^5$ CFU/mL are indicative of an infection and counts below this usually indicate contamination.[5]
In specific patient groups, counts between 10^5 CFU/mL and 10^2 CFU/mL may be significant; a pure isolate with counts between 10^4 and 10^5 CFU/mL should be evaluated based on clinical information or confirmed by repeated culture.[5] For urine collected by suprapubic bladder puncture any CFU detected indicates an infection.[5]
Consult appropriate references for complete interpretation criteria of the microbial count.[4,5]

Typical colonial morphology on CLED Agar is as follows[6]:

Escherichia coli	Yellow, opaque colonies, centre slightly deeper yellow
Klebsiella/Enterobacter	Yellow to whitish-blue colonies, mucoid
Salmonella	Blue, flat colonies
Proteus	Blue, translucent colonies
Pseudomonas	Green colonies, with typical rough (matted) surface and periphery
Enterococci	Small yellow colonies
Staphylococcus aureus	Deep yellow colonies, uniform in colour
Staphylococcus epidermidis	Pale yellow colonies, opaquer than *S. aureus*

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[7]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus	ATCC	25923	35-37°C / 18-24H / A	good growth, yellow colonies
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth, yellow colonies with yellow halo
P. vulgaris	ATCC	8427	35-37°C / 18-24H / A	good growth, bluish colonies not swarmed

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Medium is basically non-selective but, due to electrolyte exclusion, *Shigella* spp. usually do not grow on the medium.[6]
- If in the specimen the presence of genitourinary pathogens such as *Neisseria gonorrhoeae, Gardnerella vaginalis, Ureaplasma* is suspected, specific culture media must be inoculated.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates may be stored at +2°C/+8°C for 6-8 weeks.[6]
Ready-to-use plates
Store in their original pack at +2°C/+8°Caway from direct light.

REFERENCES

1. Sandys GH. A new method of preventing swarming of *Proteus* sp with a description of a new medium suitable for use in routine laboratory practice. J Med Lab Technol 1960; 17:224-233
2. Mackey JP, Sandys GH. Laboratory diagnosis of infection of the urinary tract in general practice by means of a dip-inoculum transport medium Br Med J 1965; 2:1286-1288
3. Mackey JP, Sandys GH. Correspondence. Diagnosis of urinary infections. Br Med J 1966; 1:1173
4. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
5. Public Health England UK Standards for Microbiology Investigations. Investigation of urine. Bacteriology, B 41, 2019
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

PACKAGING

Product	Type	REF	Pack
CLED Agar	Dehydrated medium	40129012 40129014	500 g (13.8L) 5 kg (138L)
CLED Agar	Ready-to-use plates	541901	2 x 10 plates ø 90 mm

IFU rev 3, 2022/02

CLOSTRIDIUM AGAR

(REINFORCED CLOSTRIDIAL AGAR)
Dehydrated culture medium

INTENDED USE

For the cultivation and enumeration of clostridia and other anaerobes.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	3.0 g
Beef extract	10.0 g
Peptone	10.0 g
Glucose	5.0 g
Soluble starch	1.0 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
L-cysteine HCl	0.5 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Reinforced Clostridium Medium has been formulated by Hirsch and Grinstead in 1954[1], while the solid version of the medium (Clostridium Agar or Reinforced Clostridial Agar) corresponds to the formulation described by Barnes and Ingram[2]
Clostridium Agar is very rich and is used for the cultivation and enumeration of clostridia, lactobacilli and other anaerobes and facultative anaerobes, in foodstuffs and other materials.[3]
Peptone and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group and glucose is a source of carbon and energy. Sodium chloride maintains the osmotic balance while sodium acetate buffers the medium. L-cysteine, a reducing agent, favours the growth of anaerobes. Soluble starch helps to detoxify metabolic by-products. Agar is the solidifying agent. According to Hirsch and Grinsted,[1] polymyxin B 0.02 g/L can be added to inhibit Gram-negative bacteria. According to APHA[4], for enumeration of lactic streptococci the medium must be supplemented with an additional 5 g/L glucose to bring to 10 g/L glucose per litre and prussian blue may be incorporated into basal medium to 0.3 g/L as needed.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 52.5 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 115°C for 20 minutes.
If required, cool to 45-50 °C and add 0.02 g/L of Polymyxin B in form of a filter-sterilized aqueous solution.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, slightly opalescent
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Water, food and pharmaceutical samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE,

Prepare the decimal dilutions of the sample and inoculate tubes or dishes by poured plate technique.
From each dilution, take an aliquot and add it to plates or tubes. Pour the melted medium at 45-50°C onto the sample and allow to solidify.
Incubate 2-5 days at an optimal temperature (e.g., 35 °C). An anaerobic environment can be achieved in tubes by covering with oil immediately after the medium is solidified. If plates are used, they must be incubated in an anaerobic atmosphere.

READING AND INTERPRETATION

Colonies of the bacteria growing on Clostridium Agar exhibit an opaque, yellowish colouration. The presence of rods with or without endospores that are negative in the catalase test indicates the presence of clostridia. The culture result must be confirmed by biochemical identification.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	30 or 35°C / 48h / AN	growth
C. sporogenes ATCC 19404	30 or 35°C / 48h / AN	growth

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The medium is not selective: other sporeforming anaerobes as well *C. butyricum*, lactobacilli and streptococci exhibit good growth.[3]
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the prepared medium may be stored at +2°C /+8°C for up to 2 weeks.[3]

REFERENCES

1. Hirsch A, Grinsted, E. Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. J Dairy Res 1954; 21: 101-110.

2. Barnes EM, Ingram M. The effect of redox potential on the grown Clostridium welchii strain isolated from horse muscle. J Appl Bact 1956; 19; 177-
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

PACKAGING

Product	Type	REF	Pack
Clostridium Agar	Dehydrated medium	4013032	500 g (9.5 L)

IFU rev 1, 2022/07

CLOSTRIDIUM BOTULINUM AGAR BASE
CLOSTRIDIUM BOTULINUM ANTIMICROBIC SUPPLEMENT
Dehydrated culture medium and selective supplement

INTENDED USE
Selective and differential medium for the isolation and presumptive identification of *Clostridium botulinum* from foods and other materials.

COMPOSITION*
CLOSTRIDIUM BOTULINUM AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	40 g
Yeast extract	5 g
Glucose	2 g
Disodium hydrogen phosphate	5 g
Sodium chloride	2 g
Agar	20 g
Magnesium sulphate	10 mg

CLOSTRIDIUM BOTULINUM ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 ML OF MEDIUM)

D-cycloserine	125 mg
Trimethoprim	2 mg
Sulphamethoxazole	38 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Clostridium botulinum is an anaerobic, rod-shaped sporeforming bacterium that produces a protein with characteristic neurotoxicity. Under certain conditions, these organisms may grow in foods, producing toxin(s).[1]
Clostridium Botulinum Agar Base completed with the antimicrobials of Clostridium Botulinum Antimicrobic Supplement corresponds to the formulation devised by Dezfulian et al. for the detection of *C. botulinum* from faeces and called CBI medium.[2]
According to Dezfulian, qualitative tests indicated complete recovery of *C. botulinum* types A, B, F, and G and the isolation of *C. botulinum* types A, B, and F from seeded faecal specimens was easily achieved with CBI medium.[1]
The Biolife medium has been used for the evaluation of a family outbreak of alimentary botulisms by Pavic et. al.[3] The authors reported that this medium is more suitable for the isolation of lecithinolytic *C. botulinum* type A, B and F than Sulphite Agar and Columbia Agar.
Pancreatic digest of casein provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins particularly of the B-group. Glucose is a source of carbon and energy. Sodium chloride maintains the osmotic balance. Disodium phosphate buffers the medium while magnesium sulphate helps for the sporulation of the organisms. Egg yolk is the substrate to detect lecithinase and lipase activities. Lecithinase degrades lecithin producing an insoluble, opaque precipitate in the medium surrounding the colonies. Lipase break down free fats causing an iridescent sheen on the surface of the colonies. The mixture of D-cycloserine, trimethoprim and sulphamethoxazole helps in the selective isolation of *C. botulinum* by inhibiting accompanying flora.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 37 g in 450 mL of cold purified water, heat to boiling with frequent agitation, and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 47-50°C and add, under aseptic conditions, the contents of one vial of Clostridium Botulinum Antimicrobic Supplement reconstituted with 2.5 mL of sterile purified water followed by 2.5 mL of acetone, and 50 mL of Egg Yolk Emulsion (cat. N° 4244160). Mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Clostridium Botulinum Agar Base	
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, opalescent
Prepared plates appearance	yellow, opaque
Final pH at 20-25 °C	7.4 ± 0.2
Clostridium Botulinum Antimicrobic Supplement	
Freeze-dried supplement appearance	high, dense, white pellet
Reconstituted supplement appearance	colourless, slightly opalescent

SPECIMENS
Foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Inoculate the food homogenate and the serial dilutions of the sample onto the surface of the plates. Incubate with anaerobic atmosphere at 35-37° for 48 hours.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
C. botulinum grows with large colonies surrounded by an opaque halo of egg yolk precipitation with an iridescent sheen on the surface of the growth.

USER QUALITY CONTROL
All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory.

LIMITATION OF THE METHOD
- The selectivity of the medium can interfere with the isolation of less resistant type G and nonproteolytic type F strains of *C. botulinum*.[2]
- Although *C. botulinum* type G can also be recovered on the medium, lack of lipase activity makes the differentiation of the colonies of this organism from those of other bacteria more difficult.[2]
- Although this medium seems to be superior to nonselective media, the simultaneous use of both media for the isolation of *C. botulinum* from the primary specimen, and if necessary, from the enriched culture, is recommended, to avoid missing drug-susceptible strains of *C. botulinum* such as some type E strains.[2]

STORAGE CONDITIONS
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at +2°C/+8°C away from direct light.

REFERENCES

1. FDA-BAM Chapter 17: Clostridium botulinum. Content current as of:10/31/2017
2. Dezfulian M, McCroskey LM, Hatheway CL, Dowell Jr VR. Selective medium for isolation of Clostridium botulinum from human feces. J Clin Microbiol 1981; Mar; 13(3):526-31.
3. Pavic, S et al. About suitability of C.Botulinum Agar for the isolation of B type C.botulinum from dry cured dalmatian ham and stools of patients during family outbreack. Zavod za javno zdravstvo Zupanije splitsko-dalmatinske. 2000.

PACKAGING
Product	Type	REF	Pack
Clostridium Botulinum Agar Base	Dehydrated medium	4013062	500 g (6.7 L)
Clostridium Botulinum Antimicrobic Supplement	Freeze-dried supplement	4240066	10 vials, each for 500 mL of medium

IFU rev 1, 2022/07

CLOSTRIDIUM BROTH
(REINFORCED CLOSTRIDIAL MEDIUM)
Dehydrated culture medium

INTENDED USE
For the cultivation and enumeration of clostridia and other anaerobes.

COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)
Yeast extract	3.0 g
Beef extract	10.0 g
Peptone	10.0 g
Glucose	5.0 g
Soluble starch	1.0 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
L-cysteine HCl	0.5 g
Agar	0.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Clostridium Broth, formulated by Hirsch and Grinstead in 1954,[1] is also known as Reinforced Clostridial Medium. It is recommended by European Pharmacopoeia[2] for the selective enrichment of clostridia from non-sterile pharmaceutical products and conforms to harmonized USP/EP/JP standards performance specifications.
Clostridium Broth is the basal medium for the preparation of Differential Reinforced Clostridial Medium of Gibbs and Freame,[3] recommended by ISO 6461-1[4] for detection and enumeration of the spores of sulphite-reducing anaerobes (clostridia) in water samples.
This medium has been described for the detection of clostridia in food products with the Most Probable Number method.[5]
The medium is very rich and permits the growth of most clostridia, and many other anaerobes and facultative anaerobes. Tryptone and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group and glucose is a source of carbon and energy. Sodium chloride maintains the osmotic balance while sodium acetate buffers the medium. L-cysteine, a reducing agent, and agar at low concentration favour the growth of anaerobes. Soluble starch helps to detoxify metabolic by-products. Sodium sulphite and ferric citrate are added to the medium and act as indicators: sulphite reducing clostridia produce sulphide from sulphite, which results in the formation of black medium. According to Hirsch and Grinsted,[1] polymyxin B 0.02 g/L can be added to inhibit Gram-negative bacteria.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 38 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation, distribute into screw-capped bottles, and sterilise by autoclaving at 115°C for 20 minutes.
For the preparation of Differential Clostridium Medium, prepare the Clostridium Broth at double strength reducing the water volume by half and transfer 10 mL and 50 mL aliquots into screw-capped bottles with a capacity slightly more than double. Prepare a 4% solution of sodium sulphite

and a 7% solution of ferric citrate; if necessary, heat the ferric citrate solution for 5 minutes to dissolve completely. Sterilise the two solutions by filtration, and store at 2-5 °C in closed bottles; the two solutions are stable for two weeks. The day of analysis, mix equal volumes of the two solutions and, under sterile conditions, add 0.5 mL of reagent to each 25 mL of medium. For the preparation of double strength Clostridium Broth add 0.4 mL of the mixture to each 10 mL and 2 mL to each 50 mL.
If required, add 0.02 g Polymyxin B/litre in form of a filter-sterilized aqueous solution to the pre-cooled medium to 45-50°C.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, slightly opalescent
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Water, food and pharmaceutical samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE, READING AND INTERPRETATION

For the determination of clostridia in non-sterile pharmaceutical products proceed as follows.[2]

1. Prepare a 1:10 dilution of the sample in Pharmacopoeia Diluent (REF 401395), with a minimum volume of 20 mL, using not less than 2 g or 2 mL of test product.
2. Divide this dilution into 2 aliquots of at least 10 mL each. Heat one aliquot to 80°C for 10 minutes and cool rapidly. Do not heat the second aliquot.
3. Inoculate 10 mL or the amount corresponding to 1 g or 1 mL of product to be examined from each of the two aliquots into Clostridium Broth.
4. Incubate under anaerobic conditions at 30°C - 35°C for 48 hours.
5. After incubation perform subcultures from each tube/flask on Columbia Agar and incubate under anaerobic conditions at 30°C - 35°C for 48-72 hours.
6. The presence of rods with or without endospores that are negative in the catalase test indicates the presence of clostridia. The culture result must be confirmed by biochemical identification.
7. The test should be considered negative if there are no colonies with the characteristics described above in the sample or if the biochemical identification tests are negative.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. sporogenes ATCC 19404	35-37°C / 48h / AN	growth
C. perfringens ATCC 13124	35-37°C / 48h / AN	growth

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Large volume of culture in hermetically sealed glass bottles may explode due to gas production. Refer to ISO 6461-1 or specific texts for precautions to be taken when incubating tubes. [4]
- The medium is not selective: other sporeforming anaerobes as well *C. butyricum*, lactobacilli and streptococci exhibit good growth.[6]
- Clostridium Broth supplemented with sodium sulphite and ferric citrate: other bacteria can produce sulphide; pasteurisation must be done to remove vegetative forms.[6]
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the prepared medium may be stored at 2-8°C for up to 2 weeks.[6]

REFERENCES

1. Hirsch A, Grinsted, E. Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. J Dairy Res 1954; 21: 101-110.
2. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
3. Gibbs BM, Freame B. Methods for the recovery of clostridia from foods. J Appl Bacteriol 1965; 28:95-111.
4. ISO 6461-1:1986 Water quality — Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) — Part 1: Method by enrichment in a liquid medium.
5. Mead GC. Principles involved in the detection and enumeration of clostridia in foods. Int J Food Microbiol 1992; 17:135-43.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Clostridium Broth	Dehydrated medium	4013042	500 g (13.2 L)

IFU rev 1, 2022/07

CLOSTRIDIUM PERFRINGENS AGAR BASE
KANAMYCIN POLYMYXIN B ANTIMICROBIC SUPPLEMENT
D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and selective supplements

INTENDED USE
For the isolation and enumeration of *Clostridium perfringens* from foods, waters and other materials.

COMPOSITION*
CLOSTRIDIUM PERFRINGENS AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	15 g
Beef extract	5 g
Soy peptone	5 g
Yeast extract	5 g
Sodium metabisulphite	1 g
Ferric (III) ammonium citrate	1 g
Agar	13 g

KANAMYCIN POLYMYXIN B ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 mL OF MEDIUM)

Kanamycin sulphate	6 mg
Polymyxin B	15,000 U.I.

D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 mL OF MEDIUM)

D-cycloserine	200 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Food poisoning caused by *Clostridium perfringens* may occur when foods such as raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices are cooked and held without maintaining adequate heating or refrigeration before serving.[1] The enumeration of *C. perfringens* in food samples plays a key role in the epidemiological investigation of food-borne disease outbreaks and for this purpose various culture media have been proposed since the 1950s.
Clostridium Perfringens Agar Base is prepared according to a modification of Shahidi-Ferguson-perfringens (SFP) agar[2] by the addition of 5 g/L of beef extract to the original formulation and corresponds to the formulation described by USDA-FSIS.[4]
For the isolation and enumeration of *C. perfringens* in food, the most common methods in continental Europe and North America are those using the medium of Harmon et al. (Tryptose Sulfite Cycloserine - TSC - Agar)[5] or Shahidi-Ferguson's medium[2] with kanamycin and polymyxin B (SFP Agar), which have replaced Angelotti's (Sulfite Polymyxin Sulphadiazine Agar) and Marshall's (Tryptone Sulfite Neomycin Agar) media, which were found to be inhibitory for some strains of *C. perfringens*.[2]
TSC Egg Yolk Agar is prepared by adding to Clostridium Perfringens Agar Base, 400 mg/L of D-Cycloserine and Egg Yolk Emulsion enrichment. The Shahidi and Ferguson Perfringens (SFP) medium is prepared by adding kanamycin and polymyxin B and Egg Yolk Emulsion enrichment to the basal medium.
Tryptose, beef extract and soy peptone provide nitrogen, carbon, minerals and amino acids for the microbial growth. The yeast extract is a source of vitamins particularly of the B-group. Ferric ammonium citrate and sodium metabisulfite are indicators of sulphite reduction by *C. perfringens* which produces black colonies. Egg yolk is the substrate to detect lecithinase activity. D-cycloserine or the mixture kanamycin and polymyxin help in the selective isolation of *C. perfringens* by inhibiting accompanying flora.

DIRECTIONS FOR MEDIA PREPARATION
SFP Agar[2]: suspend 22.5 g in 450 mL of cold purified water, heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 50 mL of Egg Yolk Emulsion (REF 42111601) and the contents of one vial of Kanamycin Polymyxin B Antimicrobic Supplement (REF 4240005), reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.
TSC Egg Yolk Agar[4]: suspend 22.5 g in 475 mL of cold purified water, heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 25 mL of Egg Yolk Emulsion (REF 42111601) and the contents of one vial of D-Cycloserine Antimicrobic Supplement (REF 4240002), reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.
TSC Agar and SFP Agar w/o Egg Yolk: prepare the media as described above by omitting the addition of Egg Yolk Emulsion and weighting 22.5 g in 500 mL of water.

PHYSICAL CHARACTERISTICS

Clostridium Perfringens Agar Base	
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Final pH at 20-25 °C	7.6 ± 0.2
D-Cycloserine Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid
Kanamycin Polymyxin B Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid

SPECIMENS
Foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

The presumptive enumeration of *C. perfringens* is performed by spreading the sample on TSC Agar or on SFP Agar with egg yolk and adding a second layer of medium without egg yolk on top of the inoculated layer.

- Inoculate the plates with 0.1 mL of the initial suspension and decimal dilutions of the prepared sample, distributing on the surface with a sterile glass rod spreader. Allow to dry 5-10 minutes.
- Cover the inoculated layer with 10 mL of medium prepared without egg yolk. Allow to solidify.
- Incubate the plates for 20-24 hours at 37°C in an anaerobic atmosphere (5% CO_2, 10% H_2, 85% N_2).

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
For enumeration of *C. perfringens* choose plates containing 20 to 200 typical colonies.
C. perfringens usually produce black or grey to yellow brown colonies as a result of the reduction of sulphite to sulphide which reacts with a ferric salt in the medium, surrounded by an opaque halo of egg yolk precipitation.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C/ 18-24 H / AN	growth, black colonies surrounded by an opaque halo
E. coli ATCC 25922	37°C/ 18-24 H / AN	totally inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- *C. perfringens* colonies may produce an opaque zone in the surrounding medium due to lecithinase activity, but this phenomenon is not a distinguishing feature of all *C. perfringens* strains after overnight incubation. Both lecithinase-positive and lecithinase-negative black colonies must be tested for confirmation.[6]
- Lecithinase-positive facultative anaerobes can grow on SFP Agar to produce completely opaque plates that mask the egg yolk reaction of *C. perfringens*.
- Black colonies must be confirmed as *C. perfringens* by appropriate tests: motility (-), nitrate reduction (+), acid and gas from lactose (+), gelatin liquefaction (+).

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplements
Store the product in the original package at 2-8°C away from direct light.
According to ISO 14189[7] the plated medium with D-cycloserine may be stored at 2-8°C for up to 7 days; the basal medium (without D-cycloserine) may be stored at 2-8°C and used within 4 weeks of preparation.

REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. Shahidi SA, Ferguson AR. New quantitative, qualitative, and confirmatory media for rapid analysis of food for Clostridium perfringens. Appl. Microbiol. 1971; 21:500-506.
3. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
4. United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science. Laboratory Guidebook Notice of Change. MLG Appendix 1.10. Effective Date: 03/07/22.
5. Harmon SM, Kautter DA, Peeler JT. Improved medium for enumeration of Clostridium perfringens. Appl Microbiol 1971; 22:688-92.
6. Hauschild AH, Hilsheimer R. Evaluation and modifications of media for enumeration of Clostridium perfringens. Appl Microbiol 1974; 27:78-82.
7. ISO 14189:2013 Water quality — Enumeration of Clostridium perfringens — Method using membrane filtration

PACKAGING

Product	Type	REF	Pack
Clostridium Perfringens Agar Base	Dehydrated medium	4013072	500 g (11.1 L)
Kanamycin Polymyxin B Antimicrobic Supplement	Freeze-dried supplement	4240005	10 vials, each for 500 mL of medium
D-Cycloserine Antimicrobic Supplement	Freeze-dried supplement	4240002	10 vials, each for 500 mL of medium

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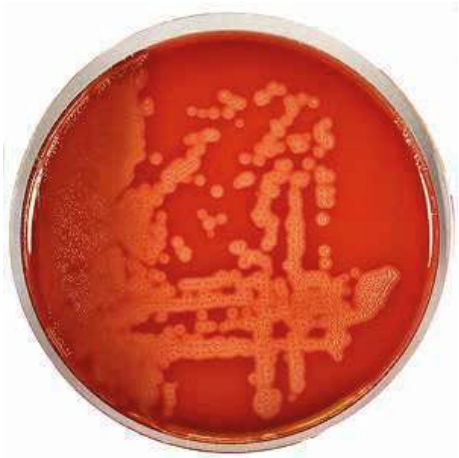
COLUMBIA AGAR BASE
COLUMBIA BLOOD AGAR
Dehydrated culture medium and ready-to-use plates



Columbia Blood Agar:
Group A β-haemolytic *Streptococcus*

INTENDED USE
In vitro diagnostics. Non-selective, general-purpose medium for the isolation, cultivation and haemolytic pattern determination of fastidious and non-fastidious microorganisms, from clinical specimens and other materials.

COMPOSITION *
COLUMBIA AGAR BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize starch	1 g
Sodium chloride	5 g
Agar	12 g

COLUMBIA BLOOD AGAR, READY-TO-USE PLATES
TYPICAL FORMULA

Columbia Agar Base	41 g
Defibrinated sheep blood	50 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Columbia Blood Agar was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones and defibrinated sheep blood into one medium. After 2 years trial, this medium showed remarkably improved growth promoting properties and was found to be superior to blood agar previously used for differentiating β and α haemolytic organisms.[1]
Columbia Agar Base is a non-selective, general-purpose medium, intended for the isolation, cultivation and haemolytic pattern determination of non-fastidious and fastidious microorganisms, such as *Corynebacterium* spp., *Actinomyces* spp., *S. pneumoniae*, *Staphylococcus*, *C. jejuni* from clinical specimens[2,3].
Columbia Blood Agar with 5% sheep blood addition is recommended for purification of colonies and for confirmation tests with incubation at 25°C in aerobic conditions, by ISO 10272 methods for the isolation and enumeration of *Campylobacter* spp. in food.[4]
Columbia Agar Base supplemented with 5-10% (v/v) horse or sheep blood, Campylobacter Growth Supplement (REF 424000X) and a suitable antimicrobial mixture is used for the preparation of plating media for the isolation of *Campylobacter* spp.: Skirrow medium,[5] Blaser Wang medium[6].
Columbia Agar Base supplemented with 5% defibrinated sheep, horse or human blood and a specific antimicrobial mixture is used for the isolation of *Gardnerella vaginalis*. [7,8]
Columbia Agar Base with added 5% defibrinated sheep blood and CNA Antimicrobic Supplement (REF 424000X) is used for the isolation of gram-positive cocci.[1]
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth. The addition of sheep blood enables the determination of haemolytic pattern, as a useful tool for the orientation of bacterial identification.

DIRECTIONS FOR MEDIUM PREPARATION
Columbia Blood Agar: suspend 41 g in 1000 mL of cold purified water; heat to boiling stirring constantly and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 5-7 % of defibrinated sheep or horse blood. Mix well and pour into sterile Petri dishes.
Columbia Agar Base may be used also for the preparation of the following media:
Columbia CNA blood agar: add 5 % sterile defibrinated sheep or horse blood and the content of one vial of CNA Antimicrobic Supplement (REF 4240018); mix well and pour into sterile Petri dishes.
Campylobacter Skirrow medium: to 500 mL of sterilised and pre-cooled base, add 50 mL of defibrinated sheep blood or 25 mL of lysed horse blood, the content of one vial of Campylobacter Growth Supplement (REF 4240021) and the content of one vial of Skirrow Antimicrobic Supplement (REF 4240016); mix well and pour into sterile Petri dishes.
Gardnerella vaginalis agar: to 500ml of sterilised and pre-cooled base, add 25 mL of human, sheep or horse blood and the content of one vial of Gardnerella Selective Supplement (REF 4240019); mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, slightly opalescent
Prepared plates (with animal blood) appearance	red, opaque
Final pH at 20-25 °C	7,3 ± 0,2

SPECIMENS
Columbia Agar Base supplemented with blood and, if necessary, with the suitable antimicrobial mixtures, can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimen types related to specific infections. [10-12] Plates prepared with Columbia Agar Base are not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[10] For the microbiological examination of food consult the ISO standard.[4]

TEST PROCEDURE
Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions, with or without 5 -10% CO_2, and record the results after 24, 48 and, if necessary, 72 hours.

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
Here below are summarized the colony characteristics of some microorganisms which can be isolated on plates prepared with Columbia Agar Base with added 5% defibrinated sheep blood.[13] For other applications of Columbia Agar Base consult the suitable literature.

- The colonies of Group A streptococci typically are about 0.5-1mm in diameter, transparent or translucent, and domed, having a smooth surface and an entire edge. They are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of group B streptococci are typically larger (2-4 mm in diameter) surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or sub-surface beta-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of group A colonies to be of any value in identification.
- Group D streptococcal colonies (*S. bovis*) are somewhat larger than other streptococcal colonies, they are less opaque, raised, and grey to grey-white.
- Pneumococcal colonies are round with entire edges, mucoid and about 1mm in diameter. When the culture has been incubated in CO_2 incubators, the colonies are surrounded by a fairly large zone of alpha-haemolysis.
- The viridans streptococcal colonies vary in size from pinpoint to a size equal to, or larger than, that of group A streptococci. The colonies are usually smaller than those of pneumococci. They may appear mucoidal or translucent or glossy and non-translucent. The colonies may be surrounded by a small zone of alpha-haemolysis (partial destruction of red blood cells) or have no zone of haemolysis.
- Staphylococci colonies are yellow or white with or without the beta-haemolysis zone.
- *Listeria* colonies are surrounded by a small beta-haemolytic zone.

Once colonies have grown on Columbia blood agar plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent members of normal microbiota.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of Columbia Agar Base with addition of 5% defibrinated sheep blood.[14]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 24H / A or CO_2	good growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 24H / A or CO_2	good growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 24H / A or CO_2	good growth
E. coli	ATCC	25922	35-37°C / 24H / A or CO_2	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Due to the carbohydrate (starch) content of Columbia blood agar, β-haemolytic streptococci may exhibit an α-haemolytic reaction around a small clear zone of β-haemolysis or may exhibit weak haemolytic reactions.
- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional media such as selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic models other than expected. *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium[15]; Neisseria, *Mycobacterium*, *Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the self-prepared plates with blood can be stored at +2°C +8°C for 48 hours.[3]

Ready-to-use plates

Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504.
2. Atlas D, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. ISO 10272-1, 10272-2: 2017. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. Part 1:detection, part 2:enumeration.
5. Skirrow MB. Campylobacter Enteritis: A "New" Disease. Br Med J 1977 2(6078):9-11.
6. Blaser MJ,Berkowitz ID, LaForce FM, Cravens J, Reller LB. Wang WLL. Campylobacter enteritis: clinical and epidemiological features. Ann Intern Med. 1979; 91:179-185.
7. Elek SD.The plate virulence test for Diphteria. J Cloin Pathol 1949; 2:250
8. Ison, CA. Dawson SG, Hilton J, Csonka GW, Easmon CSF. Comparison of culture and microscopy in the diagnosis of Gardnerella vaginalis infection. J. Clin. Pathol 1982; 35:550
9. Catlin BV. Gardnerella vaginalis: characteristics, clinical considerations, and controversies. Clin Microbiol Rev 1992; 5:213
10. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
11. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
12. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
13. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington,DC: American Society for Microbiology; 1991.
14. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

15. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999

PACKAGING

Product	Type	REF	Pack
Columbia Agar Base	Dehydrated culture medium	4011362 4011364	500 g (12.2L) 5 kg (122 L
Columbia Blood Agar	Ready-to-use plates	541136	2 x 10 plates ø 90 mm

IFU rev 2, 2022/01

COLUMBIA AGAR EP

Dehydrated culture medium

INTENDED USE

General-purpose medium for the detection of clostridia in non-sterile pharmaceutical products.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	10 g
Peptic digest of meat	5 g
Pancreatic digest of heart	3 g
Yeast extract	5 g
Maize starch	1 g
Sodium Chloride	5 g
Agar	14 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Columbia Blood Agar was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones and defibrinated sheep blood into one medium. After 2 years trial, this medium showed remarkably improved growth promoting properties and was found to be superior to blood agar previously used for differentiating β and α haemolytic organisms.[1]
Columbia Agar EP complies with the formulation given by harmonised microbiological methods of European Pharmacopoeia.[2] The medium is recommended for the procedure of clostridia detection, for the subculture from the Reinforced Medium for Clostridia.
Peptones provide carbon, nitrogen and trace elements for bacterial growth, yeast extract is a source of vitamins, particularly of the B-group B. Sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 43 g in 1000 mL of cold purified water; heat to boiling with frequent agitation. Sterilize by autoclaving at 121°C for 15 minutes. Allow to cool to 47-50°C and add, where necessary, gentamicin sulphate corresponding to 20 mg of gentamicin base and pour into Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, slightly opalescent
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Non sterile pharmaceutical products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

TEST PROCEDURE

1. Prepare a sample using a 1 in 10 dilution (with a minimum total volume of 20 mL) of not less than 2 g or 2 mL of the product to be examined.
2. Divide the sample to 2 portions of at least 10 mL. Heat 1 portion at 80 °C for 10 minutes and cool rapidly. Do not heat the other portion.
3. Use 10 mL or the quantity corresponding to 1 g or 1 mL of the product to be examined of both portions to inoculate suitable amounts of Clostridium Broth (REF 401304).
4. Incubate under anaerobic conditions at 30-35°C for 48 h.
5. After incubation, make subcultures from each container on Columbia Agar EP plates and incubate under anaerobic conditions at 30-35°C for 48-72 h.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic characteristics of the colonies.
The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia. This is confirmed by identification tests.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below is listed the test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. sporogenes	ATCC	19404	30-35°C / 48 h / AN	growth

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

References

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504.
2. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.

Packaging

Product	Type	REF	Pack
Columbia Agar EP	Dehydrated culture medium	4011342	500 g (11.6 L)

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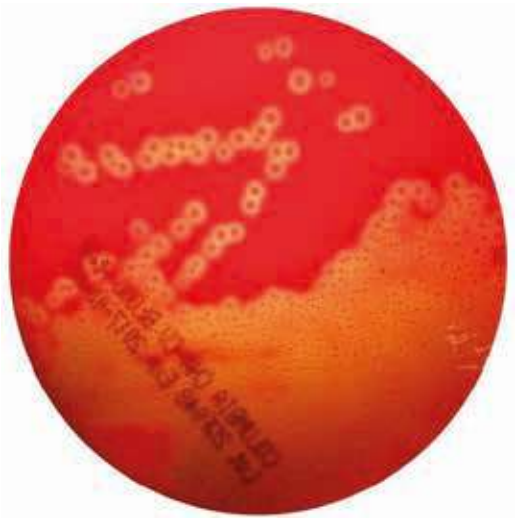
COLUMBIA CNA AGAR BASE
COLUMBIA CNA BLOOD AGAR

Dehydrated culture medium and ready-to-use plates



Columbia CNA Blood Agar:
Group A β-haemolytic *Streptococcus*

Intended use

In vitro diagnostics. Selective medium for the isolation of Gram-positive cocci from clinical and non-clinical specimens containing mixed flora and for the determination of bacterial haemolytic pattern.

Composition *

Columbia CNA Agar Base, dehydrated medium

typical formula (after reconstitution with 1 L of water)

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize starch	1 g
Sodium chloride	5 g
Agar	12 g
Nalidixic acid	15 mg
Colistin	10 mg

Columbia CNA Blood Agar, ready-to-use plates

typical formula

Columbia CNA Agar Base	41 g
Defibrinated sheep blood	50 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Columbia blood agar with 10 mg/L of colistin and 15 mg/L of nalidixic acid was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones, antibiotics and defibrinated sheep blood into one medium for the isolation of Gram-positive cocci. After 2 years trial, this medium showed remarkably improved growth-promoting properties and it was found to be superior to blood agar previously used for differentiating β and α haemolytic organisms.[1]

Columbia CNA Blood Agar is a selective medium intended for the isolation and haemolytic properties determination of Gram-positive cocci (*Staphylococcus* and *Streptococcus*) particularly when Gram-negative bacteria (e.g. *Pseudomonas*, *Proteus*, *Klebsiella*) are present in the specimens and tend to overgrow on conventional blood agar plates.[2,3]

Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth. Colistin, a polypeptide antibiotic of the polymyxin group, and nalidixic acid, a first-generation quinolone, are primarily active against Gram-negative bacteria rendering the medium selective for Gram-positive cocci. The presence of sheep blood enables the determination of haemolytic pattern, as a useful tool for the orientation of bacterial identification.

Directions for medium preparation

Suspend 41 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add 5-7 % sterile defibrinated sheep blood, mix well and pour into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, opalescent
Prepared plates appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

Specimens

Columbia CNA Blood Agar Base supplemented with defibrinated sheep blood can be directly inoculated with clinical specimens collected from various normally non-sterile human sites such as ear, upper respiratory tract, genital tract, pus and exudates.[4,5] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[4]

Test procedure

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate at 35-37°C in aerobic conditions with or without 5 -10% CO_2, and record the results after 18-24 and 48 hours.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.

Here below are summarized the colonies' characteristics of some microorganisms which can be isolated on Columbia CNA Blood Agar plates.[6]

- The colonies of Group A streptococci typically are about 0.5 mm in diameter, transparent or translucent, and domed, having a smooth surface and an entire edge. They are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of group B streptococci are typically larger (1-2 mm in diameter) surrounded by a much smaller zone of complete haemolysis. Some strains do not lyse the blood at all.
- The appearance of surface or sub-surface β-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of group A colonies to be of any value in identification.
- Group D streptococcal colonies (*S. bovis*) are somewhat larger than other streptococcal colonies, they are less opaque, raised, grey to grey-white and non-haemolytic.
- Pneumococcal colonies are round with entire edges, mucoid, and about 0.5-1 mm in diameter. When the culture has been incubated in CO_2 incubators, the colonies are surrounded by a fairly large zone of α-haemolysis.
- The viridans streptococcal colonies vary in size from pinpoint to a size equal to, or larger than, that of group A streptococci. The colonies are usually smaller than those of pneumococci. They may appear mucoidal or translucent or glossy and non-translucent. The colonies may be surrounded by a small zone of α-haemolysis (partial destruction of red blood cells) or have no zone of haemolysis.
- Staphylococci colonies are yellow or white with or without a β-haemolysis zone.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[7]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	growth
P. mirabilis	ATCC	12453	35-37°C / 44-48H / A or CO_2	totally or partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Due to the carbohydrate (starch) content of Columbia CNA Blood Agar, some β-haemolytic streptococci may exhibit an α-haemolytic reaction around a small clear zone of β-haemolysis or may exhibit weak haemolytic reactions.[3]
- The growth and type of haemolysis depend on the metabolic requirements of the organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.
- The colony diameter is generally smaller than that observed on Columbia Blood Agar.
- Some Gram-negative bacteria and yeasts could be resistant to the CNA antibiotic mixture and may not be inhibited on this medium.
- Since some pathogens required carbon dioxide for growing, it is preferable to incubate the plates with 5 -10% CO_2.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Ready-to-use plates

Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504.
2. Atlas D, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
5. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
6. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington,DC: American Society for Microbiology; 1991.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

PACKAGING

Product	Type	REF	Pack
Columbia CNA Blood Agar Base	Dehydrated medium	40113612 40113614	500 g (12.2 L) 5 kg (122 L)
Columbia CNA Blood Agar	Ready-to-use plates	541361	2 x 10 plates ø 90 mm

IFU rev 2, 2022/01

COLUMBIA CNA-CV BLOOD AGAR

Ready-to-use plates

Streptococcus pyogenes on
Columbia CNA-CV Blood Agar

INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of streptococci and enterococci from clinical specimens containing mixed flora and for determination of bacterial haemolytic model.

COMPOSITION - TYPICAL FORMULA *

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize starch	1 g
Sodium chloride	5 g
Agar	12 g
Defibrinated sheep blood	50 mL
Nalidixic acid	15 mg
Colistin	10 mg
Crystal violet	2 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Columbia blood agar with 10 mg/L of colistin and 15 mg/L of nalidixic acid was first described in 1966 by Ellner, Stoessel, Drakeford and Vasi[1] of the Columbia University, who combined meat and casein peptones, antibiotics and defibrinated sheep blood into one medium for the isolation of Gram-positive cocci. Addition of crystal violet has been devised for suppressing the growth of staphylococci.[2]
Columbia CNA-CV Blood Agar is a selective medium intended for the isolation and haemolytic properties determination of streptococci and enterococci, particularly when Gram-negative bacteria (e.g., *Pseudomonas*, *Proteus*, *Klebsiella*) and staphylococci are present in the specimens and tend to overgrow on conventional blood agar plates.[3,4]
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth. Colistin, a polypeptide antibiotic of the polymyxin group, and nalidixic acid, a first-generation quinolone, are primarily active against Gram-negative bacteria, crystal violet suppresses the growth of staphylococci. The presence of sheep blood enables the determination of haemolytic pattern, as a useful tool for the orientation of bacterial identification.

PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Columbia CNA-CV Blood Agar plates can be directly inoculated with clinical specimens collected from various normally non-sterile human sites such as ear, upper respiratory tract, genital tract, pus and exudates.[5,6] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[5]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5-10% CO_2 and record the results after 18-24 and 48 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
Streptococci and enterococci produce growth with β-haemolytic or α-haemolytic or non-haemolytic colonies, that tend to take a bluish colour due to the accumulation of crystal violet. Staphylococci and Gram-negative bacteria are partially or totally inhibited.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	inhibited
P. mirabilis	ATCC	12453	35-37°C / 18-24H / A or CO_2	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Due to the carbohydrate (starch) content of Columbia CNA-CV Blood Agar, some β-haemolytic streptococci may exhibit an α-haemolytic reaction around a small clear zone of β-haemolysis or may exhibit weak haemolytic reactions.[4]
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic models other than expected.
- The colony diameter is generally smaller than that observed on Columbia Blood Agar Sheep.

- Some Gram-negative bacteria and yeasts could be resistant to the CNA antibiotic mixture and may not be inhibited on this medium.
- Some Gram-positive bacteria other than streptococci and enterococci may also grow, depending on their sensitivity to inhibitors.
- Since some pathogens require carbon dioxide for growing, it is preferable to incubate the plates with 5 -10% CO_2.
- Due to the crystal violet content, the appearance of the medium after incubation is darker than Columbia Blood Agar or Columbia CNA Blood Agar.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic or haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504.
2. Fry BA. Basic triphenylmethane dyes and the inhibition of glutamine synthesis by Staphylococcus aureus. J Gen Microbiol 1957; 16: 341-349.
3. Atlas D, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
6. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019

PACKAGING
Product	Type	REF	Pack
Columbia CNA-CV Blood Agar	Ready-to-use plates	541363	2 x 10 plates ø 90 mm

IFU rev 1, 2020/09

CPLM TRICHOMONAS BROTH
TRICHOMONAS CPLM SELECTIVE BROTH
Dehydrated culture medium and ready-to-use tubes

INTENDED USE
For the cultivation of *Trichomonas vaginalis*.

COMPOSITION*
CPLM TRICHOMONAS BROTH, DEHYDRATED MEDIUM

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	20.0 g
Liver extract	12.0 g
Cysteine HCl	1.6 g
Maltose	1.100 g
Agar	1.0 g
Methylene blue	3.0 mg

TRICHOMONAS CPLM SELECTIVE BROTH, READY-TO-USE TUBES

TYPICAL FORMULA

CPLM Trichomonas Broth	37.5 g
Chloramphenicol	0.1 g
Horse serum	50 ml
Purified water	950 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Trichomonas vaginalis, is a flagellate, that lives on the surface of the epithelium of the urogenital tract. It produces trichomoniasis in women, while in men, the infection can be asymptomatic or have characteristics of urethritis, epididymitis, and prostatitis. *Trichomonas vaginalis* infection is the most common non-viral sexually transmitted infection.[1] Worldwide. there are an estimated 250 million cases of *Trichomonas* infection each year, with an overall estimated prevalence of 4,5%.[2]
Culture has greater sensitivity (>80%) than the wet mount method and is considered the gold standard method for the detection of *T. vaginalis*.[2]
CPLM (Cysteine-Peptone-Liver-Maltose Medium) Trichomonas Broth supplemented with horse serum and chloramphenicol is a modification of the STS Medium of Kupferberg *et al*. for the cultivation of *Trichomonas* spp.[3] The classical formula has been modified by the addition of liver extract and horse serum to improve performance.
Tryptone and liver extract provide carbon, nitrogen, vitamins, and minerals to support the growth of *Trichomonas*; maltose is an energy source for microbial growth. Cysteine and agar at low concentration create reducing conditions in the medium that favour the development of *Trichomonas*. Methylene blue is included as an indicator of redox: in the oxidized state it is green in colour, in the reduced state it is colourless. Chloramphenicol, a relatively stable antibiotic added to the medium base, replaces the penicillin and streptomycin recommended for addition to the STS Kupferberg medium; it suppresses the growth of most Gram-positive and Gram-negative bacteria. Horse serum is added to provide useful growth factors for *Trichomonas*.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 35.7 g of CPLM Trichomonas Broth in 950 mL of Ringer's solution or purified water. Bring to the boil with frequent agitation and sterilize by autoclaving at 121 °C for 15 minutes. Cool to 47-50°C and add 50 mL of sterile horse serum and the contents of two vials of Chloramphenicol Antimicrobic Supplement (REF 4240003), reconstituted with 3 mL of a water/ethanol mixture (1:1). Mix well and dispense into sterile tubes using aseptic precautions.

Physical characteristics

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Prepared tubes appearance	yellow with green ring, limpid
Final pH at 20-25 °C	6.5 ± 0.2

Specimens

In women, vaginal secretions are the preferred specimen type for culture, as urine culture is less sensitive. In men, culture specimens require a urethral swab, urine sediment, and/or semen; to improve yield, multiple specimens from men can be used to inoculate a single culture.[4] Specimens must be collected properly and inoculated immediately into the medium. For detailed information, consult appropriate texts.[4-6] Collect specimens before antimicrobial therapy where possible.

Test procedure

Bring to room temperature or preferably to 37°C the required tubes.
Inoculate specimens suspected of containing *Trichomonas* organisms into the medium using swabs containing the specimen or by alternative methods, as appropriate.
Incubate tubes at 35 ± 2°C in an aerobic atmosphere for 2-7 days.

Reading and interpretation

After 48 h of incubation and again daily, aseptically remove a drop of the culture and place it on a slide and cover with a glass coverslip. Examine under 100x-400x magnification. Do not to mix the culture, but remove the material from the bottom of the tube, with a sterile pipette.
A positive culture is defined as visualization of trophozoites with morphology and motility characteristic of *T. vaginalis.*
Negative result is defined as the absence of motile trichomonads after 7 days of incubation.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
T. vaginalis ATCC 30001	35-37 °C / up to 72h / A	motile organism observed
C. albicans ATCC 18804	35-37 °C / up to 72h / A	growth
E. coli ATCC 25922	35-37 °C / up to 72h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- *T. vaginalis* can grow without producing obvious signs of turbidity in the culture medium.
- Culture has a sensitivity of 75%–96% and a specificity of up to 100%.[4,7] A negative results must be viewed cautiously and evaluated in conjunction with clinical symptoms.[2]
- The medium does not contain antifungal agents so yeasts such as *Candida* spp. may grow in the tubes inoculated with the specimens.
- Even if the broth contains chloramphenicol to reduce contamination by vaginal flora, contamination with bacteria may be a major problem. Passage of the cultures after 2–3 days to reduce bacterial contamination may be required to identify *T. vaginalis* definitively.[8]
- Due to the fastidious nature of *T. vaginalis*, the culture will remain viable for a short period of time after reaching the stationary phase.

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use tubes
Store tubes in their original pack at +2°C/+8°C away from direct light.

References

1. McConnaughey M. Life Cycle of Parasites. Reference Module in Biomedical Sciences, 2014.
2. Novak-Weekley SN, Leber AL. Intestinal and urogenital amebae, flagellates and ciliates. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
3. Kupferberg AB, Johnson G, Sprince H. 1948. Nutritional requirements of *Trichomonas vaginalis*. Proc Soc Exp Biol Med 1948;67:304-308.
4. Centers of Disease Control and Prevention, 2015 Sexually Transmitted Diseases Treatment Guidelines. June 4, 2015.
5. Shimizu RY, Garcia LD. Specimen collection, transport, and processing: parasitology. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
6. Hobbs MM et al. Methods for Detection of *Trichomonas vaginalis* in the Male Partners of Infected Women: Implications for Control of Trichomoniasis. J Clin Microbiol. 2006; 44(11): 3994–3999.
7. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. Am J Obstet Gynecol 2009;200: 181–7.
8. Domeika M, Zhurauskaya L,Savicheva A, Frigo N, Sokolovskiy E, Hallén A, Unemo M, Ballard RC. Guidelines for the laboratory diagnosis of trichomoniasis in East European countries. EE SRH Network Journal of the European Academy of Dermatology and Venereology. First published: 02 September 2010.

Packaging

Product	Type	REF	Pack
CPLM Trichomonas Broth	Dehydrated medium	4013312	500 g (14 L)
Trichomonas CPLM Selective Broth	Ready-to-use tubes	5513311	20 x 9 mL glass tubes, 17x125 mm,

IFU rev 4, 2022/05

CRONOBACTER SCREENING BROTH BASE
VANCOMYCIN CSB SUPPLEMENT

Dehydrated culture medium and selective supplement

INTENDED USE
Cronobacter Screening Broth Base, supplemented with vancomycin, is used for the selective enrichment of *Cronobacter* spp. from the samples of the food chain, according to ISO 22964.

COMPOSITION *
CRONOBACTER SCREENING BROTH BASE (4013552)
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Enzymatic digest of animal tissue	10.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Bromocresol purple	0.04 g
Sucrose	10.00 g

VANCOMYCIN CSB SUPPLEMENT (4240057C)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	5 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Cronobacter species (formerly known as *Enterobacter sakazakii*) are Gram-negative rod-shaped, motile pathogenic bacteria of the family *Enterobacteriaceae*. These organisms are regarded as opportunistic pathogens linked with life-threatening infections predominantly in neonates.[1] Clinical syndromes of *Cronobacter* infection include necrotizing enterocolitis (NEC), bacteraemia and meningitis, with case fatality rates ranging from 40-80%.[1,2] The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, water, rice and others.[1,3,4]
Cronobacter Screening Broth Base, supplemented with vancomycin, is a selective enrichment medium used for the procedure of determination of the presence or absence of *Cronobacter* spp. in samples from the food supply chain, according to ISO 22964.[5] It must be used combined with Chromogenic Cronobacter Isolation (CCI) Agar.
The enzymatic digest of animal tissues and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Sucrose is a fermentable carbohydrate while bromocresol purple is a pH indicator: sucrose-fermenting bacteria acidify the medium with a colour change of bromocresol purple from purple to yellow. The selective agent of the medium is vancomycin which inhibits the growth of Gram-positive bacteria.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 14 g in 500 mL of cold purified water. Heat to dissolve with frequent agitation if necessary. Sterilize by autoclaving at 121°C for 15 minutes. Cool below 47°C and add the contents of one vial of Vancomycin CSB Supplement (REF 4240057C) reconstituted with 5 mL of sterile purified water. Mix well and dispense 10 mL into sterile tubes under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	purple, clear
Freeze-dried selective supplement appearance	high, soft pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

SPECIMENS
Food products and ingredients intended for human consumption and the feeding of animals; environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[5]

TEST PROCEDURE
Prepare the test sample in accordance with the relevant part of ISO 6887 dealing with the product concerned.
Add 10 g or 10 mL of sample to 90 mL of Buffered Peptone Water (REF 401278). For inoculum above 10 g preheat the broth to 34-38°C. Incubate the pre-enrichment broth at 36 ± 2 °C for 18 ± 2 hours.
Transfer 0.1 mL of pre-enriched broth into 10 mL of complete Cronobacter Screening Broth.
Incubate the enrichment broth tubes at 41.5 ± 1 °C for 24 ± 2 hours
Streak a loopful of incubated enrichment broth (about 10 µL) on a plate of CCI Agar and incubate upside down at 41.5 ± 1 °C for 24 ± 2 hours.

READING AND INTERPRETATION
Bacterial growth in Cronobacter Screening Broth is evidenced by the development of turbidity in the broth; *Cronobacter* spp. typically turn the medium to yellow.
After incubation of CCI Agar plates, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical *Cronobacter* colonies are small to medium-sized (1 mm to 3 mm) and blue to blue-green in colour.
Colonies of non-typical Gram-negative bacteria may develop on CCI Agar with the following characteristics: white, with or without a grey or black or green centre; some naturally pigmented colonies of non-*Cronobacter* may appear yellow or red.
Perform the confirmation tests on the typical colonies as reported by ISO 22964.[5]

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[5]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
C. sakazakii ATCC 29544+*S. aureus* ATCC 25923	41.5 °C ± 1°C /24 h ± 2 h / A	growth, the medium turns to yellow
C. muytjensis ATCC 51329+*S. aureus* ATCC 25923	41.5 °C ± 1°C /24 h ± 2 h / A	growth, the medium turns to yellow
S. aureus ATCC 25923	41.5 °C ± 1°C /24 h ± 2 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- *Cronobacter* may be present in low numbers in the samples, along with other *Enterobacteriaceae*, such as *E. cloacae*, which may interfere in the determination of the target microorganism.[5]
- The use of large sample sizes can compromise the recovery of stressed *Cronobacter* spp. when interfering microflora are present, such as probiotics [5]

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Freeze-dried supplement

Store the product in the original package at 2-8°C away from direct light.

According to ISO 22964[5] the medium base in tubes or flasks prepared by the user can be stored at +2°C +8°C for up to six months; the tubes complete with vancomycin may be kept at 5 °C ± 3 °C for 1 day.

REFERENCES

1. Yan QQ, Condell O, Power K, Butler F,Tall BD, Fanning S. Cronobacter species (formerly known as Enterobacter sakazakii) in powdered infant formula: a review of our current understanding of the biology of this bacterium, J App Microbiol 2012; 113:1-15
2. Friedemann, M. Epidemiology of invasive neonatal Cronobacter (Enterobacter sakazakii) infections. Eur J Clin Microbiol Infect Dis 2009; 28:1297–1304.
3. Bowen AB, Braden CR. Invasive Enterobacter sakazakii disease in infants. Emerg Infect Dis 2006; 12:1185-89.
4. Healy B, Cooney S, O'Brien S, Iversen C, Whyte P, Nally J, Callanan JJ, Fanning, S (2010) Cronobacter (Enterobacter sakazakii): an opportunistic foodborne pathogen. Foodborne Pathog Dis 2010; 7:339-50.
5. ISO 22964:2017 Microbiology of the food chain — Horizontal method for the detection of Cronobacter spp.

PACKAGING

Product	Type	REF	Pack
Cronobacter Screening Broth Base	Dehydrated medium	4013552	500 g (17.8 L)
Vancomycin CSB Supplement	Freeze-dried supplement	4240057C	10 vials, each for 500 mL of medium base
Cronobacter Screening Broth Base	Ready-to-use tubes	551355	20 x 9 mL

IFU rev 0, 2022/06

Cronobacter Screening Broth: at left a tube with the growth of *Cronobacter sakazakii*. At right an un-inoculated tube.

DECARBOXYLASE MOELLER BASE BROTH

Dehydrated culture medium

Enterobacter aerogenes - from left: uninoculated tube, Moeller arginine (-), Moeller lysine (+), Moeller ornithine (+)

INTENDED USE

In vitro diagnostic. Decarboxylase Moeller Base Broth, when supplemented with amino acids, aids in the differentiation of *Enterobacteriaceae* isolated from clinical and other specimens.

COMPOSITION TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptone	5.000 g
Beef Extract	5.000 g
Pyridoxal	0.005 g
Glucose	0.500 g
Bromocresol Purple	0.010 g
Cresol Red	0.005 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Decarboxylase Moeller Base Broth, devised by Moeller[1] in 1955, is a liquid medium to which amino acids can be added, for the evaluation of Gram-negative enteric bacteria ability to decarboxylate them with the formation of amines with change to alkalinity of the culture medium.[2] The most commonly used amino acids are L-lysine, L-ornithine, L-arginine. Cadaverine is produced from the decarboxylation of lysine, putrescine is produced from ornithine; arginine is first converted into citrulline by the enzyme dihydrolase with removal of an NH_2 group; citrulline is then converted into ornithine and the latter is decarboxylated into putrescine.[3] The amines that are formed in these enzymatic reactions increase the pH to alkalinity, with a colour change of the indicator system bromocresol purple and cresol red. Glucose, included in the medium is fermented by *Enterobacteriaceae* with the production, during the first hours of incubation, of an acidic environment, necessary for the full performance of the decarboxylase enzyme. When the medium containing amino acids is inoculated with a glucose fermenting and decarboxylase positive strain, it turns first to yellow for the production of acids from the fermentation of glucose, then to purple for the production of amines. The positive test for the decarboxylase is therefore indicated by the formation of a purple colour, the negative test by the presence of a yellow colour. Pyridoxal is a coenzyme that activates the decarboxylase enzyme; the peptone and the meat extract provide nitrogen, carbon and trace elements necessary for bacterial growth. Since the peptones can be oxidized and deaminated with production of ammonia, to avoid false positives, it is necessary to create anaerobic conditions for performing the test, covering the medium with mineral oil. Together with the test tubes containing amino acids, inoculate a test tube of basal medium without amino acids: if the latter turns to alkalinity with purple colour formation, the test is invalidated.[2]
The medium is intended for the differentiation of *Enterobacteriaceae* colonies isolated from clinical or other specimens. The production of ornithine decarboxylase is particularly useful for differentiating *Klebsiella* from *Enterobacter*: *Klebsiella* spp. are non-motile and do not produce ornithine decarboxylase, while most *Enterobacter* spp. are motile and usually produce the enzyme.[4]
Positive lysine decarboxylase species include *K. pneumoniae, K. oxytoca, E. aerogenes, S. marcescens*, S. Typhi, *E. tarda, M. morganii, V. cholerae, V. parahemolyticus, P. shigelloides*; the species that decompose arginine include *E. cloacae, C. sakazakii, V. fluvialis, P. shigelloides, S. aureus, E. faecalis, E. faecium*.[3]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 10.5 g in 1000 mL of cold purified water. Add 10 g of the chosen amino acid in L form (final concentration 1% w/v) or 20 g/L of the DL form (final concentration 2% w/v). Mix well and, if necessary, heat to dissolve. If required, especially when L-ornithine is added, readjust the pH with 1N NaOH. Distribute 4-5 mL into screw-cap tubes and sterilise at 121°C for 15 minutes. Also prepare tubes omitting the addition of amino acids (control tubes).

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, limpid or slightly opalescent
Final pH at 20-25 °C	6.0 ± 0.2 (after amino acid addition)

SPECIMENS

The specimens consist of bacteria strains isolated from clinical specimens or other samples, purified on appropriate medium (e.g. Tryptic Soy Agar or Blood Agar).

TEST PROCEDURE

With an inoculating needle or loop, transfer one colony into the tube with the amino acid and mix well; a tube with Decarboxylase Moeller Base Broth without amino acid is included as a control and inoculated with the test organism.
To all the inoculated tubes, with and without amino acids, add 2 mL of sterile mineral oil (about 1 cm) to the surface.
Incubate the tubes, with the caps tightened, at 35-37°C for up to 4 days with daily observation.

READING AND INTERPRETATION

After 18-24 h, 48 h, 72 h and 96 h of incubation, observe the presence of growth (turbidity) and the colour change of the medium.
Positive reaction: the medium initially turns yellow due to the fermentation of glucose and then turns purple due to the formation of amines.
Negative reaction: the medium is turbid with a light-yellow colour (glucose fermentation).
Test tube without amino acids: the medium is light yellow in colour (glucose fermentation); if this test tube shows a purple colour, the test is invalidated.
The following table summarizes the reactive models on Moeller Medium with added amino acids.

Microorganism	Lysine	Ornithine	Arginine
Escherichia coli	+	*var*	*var*
Enterobacter cloacae	-	+	+
Enterobacter aerogenes	+	+	-
Edwardsiella tarda	+	+	-
Salmonella typhi	+	-	*var*
Citrobacter freundii	-	*var*	*var*
Proteus vulgaris	-	-	-
Proteus mirabilis	-	+	-
Shigella dysenteriae	-	-	-
Shigella sonnei	-	+	-
Serratia liquefaciens	*var*	-	-
Serratia marcescens	+	+	-
Klebsiella pneumoniae	-	+	-

+: positive (alkaline reaction, purple colour); -: negative reaction (yellow colour); var: variable reaction

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS		
P. vulgaris ATCC 9484	35-37°C ° / 44-48H /A	- (yellow)	- (yellow)	- (yellow)
S. Enteritidis ATCC 13076	35-37°C ° / 44-48H /A	+ (purple)	+ (purple)	+ (purple)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Non-fermenting bacteria don't ferment glucose in anaerobic conditions of the test. However, they decarboxylate the amino-acid when the medium is overlaid with mineral oil. Since the glucose is not fermented, they don't turn the medium to yellow at any time. Due to decarboxylation, the alkalinity makes the tube deep purple;[3] this can be checked by comparing with uninoculated tube. Some non-fermenting strains can give a delayed reaction and an absent or very slight colour change of the medium and, for this reason, Moeller's medium is not always considered satisfactory for glucose non-fermenting strains.[2,5]
- The lysine decarboxylation test does not measure the amount of intracellular enzyme, rather they indicated whether the amount of amines produced is sufficient to raise the pH of the culture medium. The change in growth conditions (concentrations of glucose, lysine and amino acids other than lysine) can significantly influence the activity of the enzyme lysine decarboxylase in coliforms.[6]
- With the tubes held vertically during incubation, the decarboxylase test may show two layers of different colours; shake the tube gently before attempting to make the interpretation.[2]
- Sometimes there may be problems of interpretation of the positive result due to the formation of an indistinct yellowish-purple colour. If this occurs, always compare with an uninoculated control tube. Any trace of purple colour, after at least 24 hours of incubation, must be interpreted as a positive test.[2]
- In some cases, after a particularly prolonged incubation, an indistinct colour or even a discolouration of the medium may appear due to the destruction of bromocresol purple.[2] This discolouration or indistinct colour occurs frequently with *P. mirabilis* and *P. vulgaris*.[1]
- *Salmonella* Gallinarum gives a delayed positive ornithine decarboxylation reaction requiring 5-6 days of incubation due to a poor bacterial permeability to the amino acid.[2]
- Many *E. coli* strains, including adonitol fermenting strains, exhibit a delayed ornithine decarboxylation reaction.[2]
- The amino acid decarboxylation is one of the tests necessary for the identification of *Enterobacteriaceae*. The results of the decarboxylation tests must be interpreted together with other tests for a correct identification of the strains. Therefore, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks.

REFERENCES

1. Moeller V. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta Pathol Microbiol Scand 1955; 36(2):158-72.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Rao S. Amino acid metabolism tests. www.microrao.com
4. Farmer *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology,7th ed. American Society for Microbiology, Washington,DC. 1999
5. Barrow, G.I., Feltham R.K.A. Bacterial characters and characterization. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 1999.
6. Piluscki RW, Claytn NW, Cabelli VJ, Cohen PS. Limitations of the Moeller Lysine and Ornithine Decarboxylase Tests. App Environ Microbiol. 1979; 37(2): 254-260.

PACKAGING

Product	Type	REF	Pack
Decarboxylase Moeller Base Broth	Dehydrated medium	4013662	500 g (47.6 L)

IFU rev 2, 2020/06

DEOXYRIBONUCLEASE TEST MEDIUM

Dehydrated culture medium

DNAse Test Agar - from left:
S. aureus DNAse+, *K. pneumoniae* DNAse-

INTENDED USE

In vitro diagnostic. For the differentiation of microorganisms by the ability to produce deoxyribonuclease enzyme.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	20 g
Deoxyribonucleic acid	2 g
Sodium chloride	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Deoxyribonuclease Test Medium, prepared according to the formula of Jeffries, Holtman and Guse,[1] is a differential medium to test the ability of an organism to produce deoxyribonuclease (DNase) enzyme. The DNase test should be used in conjunction with other tests for the identification of *S. aureus* (+)[2] and is an aid in the differentiation and identification of non-pigmented *Serratia* strains (+) from *Klebsiella-Enterobacter* (-).[3]
Cunningham, Catlin and Garilhe[4] in 1956 demonstrated that coagulase positive, mannitol fermenters and chromogenic *S. aureus* strains, produce a calcium-dependent, thermostable, deoxyribonuclease capable of hydrolysing the 5'-phosphodiester bonds of DNA. Weckman and Caltin[5] in a study with 87 staphylococcal strains of clinical origin showed that the deoxyribonuclease activity is well correlated with the production of coagulase and the coagulase test can be accompanied by the DNase test for the detection of pathogenic staphylococci.
Tryptose provides carbon and nitrogen for growth; sodium chloride maintains the osmotic balance; deoxyribonucleic acid enables the detection of deoxyribonuclease that depolymerizes DNA.
The depolymerization of the DNA may be detected by adding a hydrochloric acid solution to the plates and observing clear zones around the colonies. In the absence of DNase activity, the reagent reacts with the polymerized DNA, resulting in the formation of a cloudy precipitate.
The medium can be supplemented with mannitol, a pH indicator and dyes to obtain additional differential information or to avoid the use of hydrochloric acid to detect the DNase.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	whitish, opalescent
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Deoxyribonuclease Test Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

TEST PROCEDURE

Use a heavy inoculum and draw a line 3-4cm long from the rim to the centre of the DNase Test Agar plate.
Incubate aerobically at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

After incubation flood the plate to a depth of a few millimetres of 1N HCl and leave the plate to stand for a few minutes to allow the reagent to absorb into the medium. Decant excess hydrochloric acid and then examine against a dark background. Polymerized DNA is precipitated and produces a white cloudy area in the agar because of the reaction of HCl with DNA.
Positive result: colonies or growth are surrounded by clear zones.
Negative result: no zone of clearing or cloudy precipitate around colony/growth and throughout DNase test agar plate.

DISCRETIONARY ADDITIVES

Deoxyribonuclease Test Medium may be supplemented with several compounds for obtaining additional differential information or to avoid the use of hydrochloric acid for detecting the production of DNase. The modified medium as described below can be incubated as previously described.
1. DNase Test Agar W/ mannitol (Coobe[6]): before sterilization, add 10 g/L of mannitol and 0.025 g/L of phenol red or bromothymol blue to the base medium; the modified medium allows the detection of mannitol fermentation (yellow colonies with yellow halo).
2. DNase Test Agar W/toluidine blue (Schreir[7]): add to the base medium, before sterilization, blue toluidine 0.1 g/L; the modified medium is blue in colour because of the formation of complexes with polymerized DNA and allows the detection of the production of DNase without the addition of HCl (DNase +: pink zone around the growth, DNase -: the medium remains blue). The medium with toluidine is inhibitory for Gram positive bacteria therefore it is suitable for the detection of the DNase of *Enterobacteriaceae* only.
3. DNase Test Agar W/methyl green (Smith[8]): add to the base medium, after sterilization at 47-50°C, 10 mL/L of a 0.5% solution of methyl green in water, repeatedly extracted with chloroform; the modified medium is green in colour because of the formation of complexes with polymerized DNA and allows the detection of the production of DNase without the addition of hydrochloric acid (DNase +: colourless zone around the growth, DNase -: the medium remains green).

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24H / A	clear zones around the colonies (DNase +)
K. pneumoniae ATCC 27736	35-37°C / 18-24H / A	no zone of clearing (DNase -)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The detection of DNase enzyme is not a sufficient to speciate an organism; additional biochemical and serological tests must be performed.
- Other microorganisms such as *B. bronchiseptica*, *P. vulgaris, P. mirabilis, C. diphtheriae, Clostridium septicum, E. coli, P. aeruginosa, A. hydrophila, Vibrio, Bacillus, Streptococcus*, are either positive or variable for DNase production. [3]
- The addition of 1N HCl to the plate inactivates microbial growth and the plates cannot be further incubated or used for other diagnostic tests.
- Both *S. aureus* and *S. epidermidis* produce an extracellular DNase, however the quantity produced by *S. aureus* is much higher.[3]
- Some MRSA strains do not give positive DNase test results and some strains of coagulase-negative staphylococci such as *Staphylococcus capitis* may give weak reactions. [9]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C +8°C for up to four weeks.[3]

REFERENCES

1. Jeffries CD, Holtman DF, Guse DG. Rapid method for determining the activity of microorganisms on nucleic acids. J Bacteriol 1957; 73: 590.
2. Public Health England. UK Standards for Microbiology Investigations. Deoxyribonuclease test. Test Procedure TP 12, 09.18.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Cunningham L, Catlin BW, de Garilhe MP. A deoxyribonuclease Micrococcus pyogenes. J Am Chem Soc,1956; 78:4642-4645.
5. Weckman BG, Catlin BW Deoxyribonuclease activity of micrococci from clinical sources. J. Bacteriol 1957; 73:747-753.
6. Coobe ER. The rapid recognition of Staphylococcus aureus: deoxyribonuclease and coagulase test in correlation with sensitivities and other properties. Ulster Med J 1968; 37:146-149.
7. Schreir JB. Modification of deoxyribonuclease test medium for rapid identification of Serratia marcescens. Amer J Clin Path 1969;51:711-716.
8. Smith PB, Hancock GA, Rhoden DL. Improved medium for detecting deoxyribonuclease-producing bacteria. *Appl Microbiol* 1969; 18: 991-994.
9. Deoxyribonuclease (DNase) Test: Principle, Procedure and results - Learn Microbiology Online.

PACKAGING

Product	Type	REF	Pack
Deoxyribonuclease Test Medium	Dehydrated culture medium	4013682	500 g (11.9 L)

IFU rev 2, 2022/03

DERMATOPHYTE SELECTIVE MEDIUM - DTM - (TAPLIN)
DERMATOPHYTE ANTIMICROBIC SUPPLEMENT
Dehydrated culture medium, selective supplement and ready-to-use plates

Trichophyton mentagrophytes on DTM

INTENDED USE

In vitro diagnostics. Selective and differential medium base, selective supplement and ready-to-use plates for the detection of dermatophytes from cutaneous specimens.

COMPOSITION *

DERMATOPHYTE SELECTIVE MEDIUM-DTM-(TAPLIN)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Soy peptone	11.0 g
Glucose	10.0 g
Phenol red	0.2 g
Cycloheximide	0.5 g
Gentamicin sulphate	0.1 g
Agar	15.0 g

DERMATOPHYTE ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Chlortetracycline HCl	50 mg

DERMATOPHYTE SELECTIVE MEDIUM-DTM, READY-TO-USE PLATES
TYPICAL FORMULA

Dermatophyte Selective Medium-DTM-(Taplin)	36.8 g
Chlortetracycline HCl	100 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

The dermatophyte fungi are classified in three genera: *Epidermophyton* spp., *Microsporum* spp. and *Trichophyton* spp. The most common dermatophyte infections are *tinea pedis* (athlete's foot), *tinea unguium* (nail infection) in adults and *tinea capitis* (scalp ringworm) in children.[1]
Dermatophyte Test Medium or DTM has been formulated by Taplin, Zaias and Rebbell[2] in 1969; the dehydrated medium Dermatophyte Selective Medium and the supplement Dermatophyte Antimicrobic Supplement are prepared according to the formula of Taplin *et al.* and are intended for selective isolation and differentiation of dermatophyte fungi responsible for lesions of the skin, nails, hair.[1]
Soy peptone provide the nutrients for microbial growth. Glucose is a source of carbon and energy for enhancing dermatophytes growth. Phenol red is a pH indicator, used to detect acid/alkaline production and to differentiate dermatophytes that cultivate with a change to red of the medium because of the production of basic metabolites. The antimicrobials included in the medium base and in the supplement partially suppress the growth of bacteria and fungi: cycloheximide inhibits most saprophytic moulds, gentamicin inhibits most Gram-negative and some Gram-positive bacteria, chlortetracycline has a bacteriostatic activity against a wide range of microorganisms including Gram-positive and Gram-negative.
The medium allows the diagnosis of dermatophytes after at least 48 hours of incubation.
Allen[3] reported an accuracy of 97% in the identification of dermatophytes with the DTM medium; several authors[4-7] reported that DTM is an effective and convenient medium for confirming dermatophyte infections in Laboratory and in-office.

Directions for medium preparation

Suspend 18.4 g in 500 mL of cold purified water, heat to boiling with agitation and sterilize by autoclaving at 115°C for 10 minutes. Cool to 47-50°C and aseptically add the contents of one vial of Dermatophyte Antimicrobic Supplement (REF 4240024) reconstituted with 5 mL of sterile purified water, under aseptic conditions. Mix well and distribute into sterile Petri dishes or into sterile screw cap bottle/tubes and cool in a slanted position.

Physical characteristics

Dermatophyte Selective Medium	
Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates/tubes appearance	orange, limpid
Final pH at 20-25 °C	5.5 ± 0.2
Dermatophyte Antimicrobic Supplement	
Freeze-dried supplement appearance	high, soft yellow pastille
Reconstituted supplement appearance	yellow, colourless

Specimens

DTM is intended for the examination of cutaneous specimens such as nails, hair, skin.[1] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.[1]

Test procedure

Allow plates/flasks or tubes to come to room temperature.
Press cutaneous specimens by gently pressing lightly the samples onto the agar surface.
Incubate aerobically, at 23-27°C for 4-7 days.
Negative cultures can be reported after 7 days, but plates should be re-incubated for a further week and examined before discarding at two weeks.[1]

Reading and interpretation

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Dermatophytes produce alkaline metabolites which elevate the pH of the medium inducing a colour change of phenol red from orange to red. Examine the medium for evidence of white or light pinkish aerial growth and of a pink to red colour in medium.
For fast-growing dermatophytes, the red colour appears after 48 hours of incubation; for slow-growing dermatophytes, 3 to 7 days of incubation are required. When there are small colonies, the red colour remains limited to the area around the colony; when the growth is confluent and conspicuous, the indicator changes over the entire plate or flask or tube.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
T. mentagrophytes ATCC 28185	23-27°C / 94-96h / A	growth, the medium turns red-violet
C. albicans ATCC 18804	23-27°C / 94-96h / A	good partially inhibited
A. brasiliensis ATCC 9642	23-27°C / 94-96h / A	good partially inhibited
E. coli ATCC 25922	23-27°C / 94-96h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Saprophytes may redden the medium if specimen material is heavy contaminated but they can be recognized by their dark green or black hyphae; dermatophytes exhibit white aerial hyphae.[8]
- Disregard any colour after 10 days of incubation; it may be due to growth of contaminants.[8]
- A medium containing cycloheximide should not be used when infection with a non-dermatophyte mould is likely or suspected.[1]
- Even if the microbial colonies on the medium are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Selective supplement
Store the product in the original package at +2°C /+8°C away from direct light.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 2 weeks.

References

1. Public Health England. Investigation of dermatological specimens for superficial mycoses. SMI B 39, Issue no: 3.1, 2016.

2. Taplin D, Zaias N, Rebbell G, Blank H. Isolation and recognition of dermatophytes on a new medium (DTM) Arch Derm 1969; 99:203-209.
3. Allen AM, Drewry RA, Weaver RE. Evaluation of Two New Color Indicator Media for Diagnosis of Dermatophytosis. Arch Dermatol. 1970;102(1):68–70
4. Elewski BE, Leyden J, Rinaldi MG, Atillasoy E. Office practice-based confirmation of onychomycosis: a US nationwide prospective survey. Arch Intern Med. 2002;162(18):2133-2138.
5. Jennings MB, Rinaldi MG. Confirmation of dermatophytes in nail specimens using in-office dermatophyte test medium cultures. Insights from a multispecialty survey. J Am Podiatr Med Assoc. 2003;93(3):195-202.
6. Rahman MA, Chowdhury OA, Debnath MR, et al. Comparison among Different Culture Media for the Detection of Dermatophytes. Mymensingh Med J. 2018;27(3):626-630.
7. Rich P, Harkless LB, Atillasoy ES. Dermatophyte test medium culture for evaluating toenail infections in patients with diabetes. Diabetes Care. 2003;26(5):1480-1484.
8. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

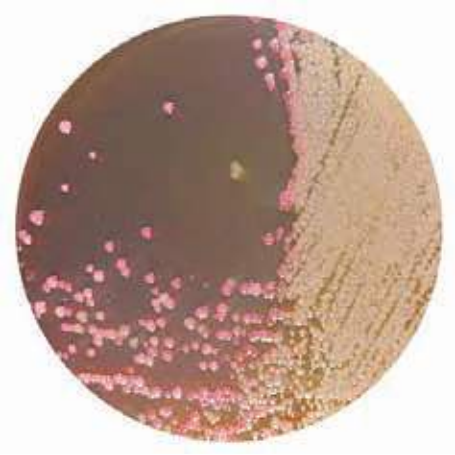
PACKAGING

Product	Type	REF	Pack
Dermatophyte Selective Medium -DTM- (Taplin)	Dehydrated medium	40136912	500 g (13,6 L)
Dermatophyte Antimicrobic Supplement	Freeze-dried supplement	4240024	10 vials, each for 500 mL of medium
Dermatophyte Selective Medium (DTM)	Ready-to-use plates	541369	2 x 10 plates ø 90 mm

IFU rev 3, 2022/04

DESOXYCHOLATE AGAR

Dehydrated and ready-to-use culture medium



Desoxycholate Agar: *K. pneumoniae* (pink-red colonies), *S.* Enteritidis (cream colonies)

INTENDED USE

In vitro diagnostic. Moderately selective medium for the isolation and differentiation of Gram-negative enteric bacteria from clinical specimens and for the enumeration of coliforms in dairy products.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE FLASKS

Peptocomplex	10.000 g
Lactose	10.000 g
Sodium chloride	5.000 g
Potassium phosphate bibasic	2.000 g
Ferric citrate	1.000 g
Sodium citrate	1.000 g
Sodium deoxycholate	1.000 g
Neutral red	0.033 g
Agar	15.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Desoxycholate Agar, prepared on the basis of the formulation described by Leifson[1] in 1935, is intended for the isolation and differentiation of Gram negative enteric bacteria from a variety of clinical samples[2] and for the enumeration of coliforms in dairy products[3].
Peptocomplex provides nitrogen, carbon and trace elements for microbial growth; potassium phosphate acts as a buffer system, sodium chloride maintains the osmotic balance; the moderate selectivity of the medium is due to the presence of sodium deoxycholate, sodium citrate and iron citrate which allow a good growth of Gram negative bacteria and a partial to total inhibition of Gram positive bacteria. Lactose is included into the medium as a fermentable carbohydrate, neutral red as a pH indicator: coliforms ferment lactose producing an acidification of the medium around the colony highlighted by the precipitation of sodium deoxycholate and the development of a red colour; bacteria that do not ferment lactose (including enteric pathogens *Salmonella* and *Shigella*) do not acidify the medium and cultivate with colourless colonies.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 45 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, cool to 47-50 °C, mix well and pour into sterile Petri dishes. Do not sterilize in autoclave and do not overheat.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige to pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Desoxycholate Agar is intended for the bacteriological processing of clinical specimens such as faeces in which detect Gram negative enteric bacteria. Good laboratory practices for collection, transport and storage of the specimens should be applied. For food samples, refer to the applicable international standards.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Clinical specimens[4]
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate in aerobic conditions at 35-37°C for 18-24 hours.
Enumeration of coliform bacteria in dairy products[3]
- Introduce 1-4 ml of sample (or decimal dilutions of the sample) into sterile Petri dishes.
- Add 10-20 ml of medium cooled to 47-50°C and mix well the medium with the inoculum.
- Allow the medium to solidify and pour a surface covering layer of 3-4 ml of uninoculated medium.
- Incubate aerobically at 35-37°C for 18-24 hours.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Gram negative bacteria grow with different characteristics depending on their ability to ferment lactose and to induce the pH indicator changes.
Lactose non-fermenting Gram-negative enteric bacteria: colourless colonies
Lactose fermenting Gram negative enteric bacteria (coliforms): red colonies sometimes surrounded by a red-pink opaque zone (precipitation of sodium deoxycholate).

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, red-violet colonies
S. Enteritidis ATCC 13076	35-37°C / 18-24 H / A	growth, colourless colonies
E. faecalis ATCC 29212	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Aerobic or facultative anaerobic Gram-negative bacteria other than *Enterobacteriaceae* (e.g., *Pseudomonas*, *Aeromonas*) may grow on the medium with colourless colonies.
- The medium does not differentiate non-fermenting lactose bacteria such as *Proteus* spp. from *Salmonella* and *Shigella*. It is advised to screen the colonies by flooding the plate with one drop of MUCAP reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C_8 esterase enzyme, typical of *Salmonella* spp.[10]
- Desoxycholate Agar does not contain an indicator system for the production of hydrogen sulphide therefore *Salmonella* colonies do not have a black centre.
- At pH above 7.5 the medium loses some of its inhibitory properties towards Gram positive bacteria.[1,4]
- With overheating there may be a decrease in the gelling power of the agar due to its hydrolysis by iron citrate and sodium citrate.[1,4]
- It is advisable to use a freshly prepared medium.[4]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C +8°C for up to one weeks.[4]
Ready-to-use medium in flasks
Store flasks in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Leifson E. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J Pathol Bacteriol 1935; 40: 581-599.
2. Atlas D, Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.335.
3. American Public Health Association (1978) `Standard Methods for the Examination of Dairy Products. 14th ed. 1978; New York: APHA Inc, pp. 58-59.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
Desoxycholate Agar	Dehydrated medium	4013702	500 g (11.1L)
Desoxycholate Agar	Ready-to-use flasks	5113702	6 x 100 mL

IFU rev 2, 2022/03

DESOXYCHOLATE CITRATE AGAR

Dehydrated culture medium

Salmonella Typhimurium
on Desoxycholate Citrate Agar

INTENDED USE

In vitro diagnostic. Selective and differential medium for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella,* from clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Peptone	5.00 g
Beef extract	5.00 g
Lactose	10.00 g
Sodium citrate	5.00 g
Ferric ammonium citrate	1.00 g
Sodium deoxycholate	2.50 g
Sodium thiosulphate	5.00 g
Neutral red	0.03 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Desoxycholate Citrate Agar, prepared on the basis of a modification of the formula described by Leifson[1] in 1935, is intended for the isolation and differentiation of Gram-negative enteric pathogens (*Salmonella* and *Shigella*) from faecal specimens.
Several formulas have been proposed, based on the original media of Leifson[1], Haynes[2], Hajna and Damon[3] with different concentrations of sodium citrate, ferric citrate or ferric ammonium citrate and sodium deoxycholate, presence or absence of sucrose and sodium thiosulfate, slightly different concentrations of neutral red. Desoxycholate Citrate Agar (Leifson) has more selectivity characteristics than Desoxycholate Agar (REF 401370) and is less selective than Haynes' medium.[4]
Peptone and beef extract provide carbon, nitrogen and trace elements for bacterial growth; the selectivity of the medium is due to the presence of sodium deoxycholate, sodium citrate and ferric citrate, which allow a good growth of Gram-negative bacteria, a partial inhibition of coliforms and a total inhibition of Gram-positive bacteria. Lactose is included as a fermentable carbohydrate, neutral red as a pH indicator; bacteria that do not ferment lactose (including enteric pathogens *Salmonella* and *Shigella*) do not acidify the medium and cultivate with colourless colonies. The coliform strains, that are able to grow in the presence of selective compounds, ferment lactose producing an acidification of the medium around the colonies, the precipitation of sodium deoxycholate and the development of a red colour. *Salmonella* spp. produce thiosulphate reductase that causes the release of a sulphide molecule from the sodium thiosulfate present in the medium; this sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 48.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Cool to 47-50 °C, mix well and pour into sterile Petri dishes. Do not sterilize in autoclave and do not overheat.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Medium appearance	red, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Desoxycholate Citrate Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swab. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using the enrichment step in Selenite Broth followed by subculture on Desoxycholate Citrate Agar and on a second plating medium.[5]
For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised, followed by subculture in two different selective media: Desoxycholate Citrate Agar and a second less selective medium (e.g., Mac Conkey Agar).[5]
Incubate inoculated Desoxycholate Citrate Agar plates with the specimen or with specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours. If there is no microbial growth or doubts in reading the black colony centre, incubate for an additional 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Lactose non-fermenting Gram-negative enteric bacteria: colourless colonies with or without black centre. *Salmonella* colonies generally have a light black centre, *Shigella* colonies do not have a black centre.
Lactose fermenting Gram negative enteric bacteria (coliforms): red colonies sometimes surrounded by a red-pink opaque zone.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, red colonies with light black centre
S. flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, colourless colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, red colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- If pH is raised above 7.5 many Gram-positive bacteria may exhibit growth.[4]
- With overheating there may be an increased degree of inhibition and a decrease in the gelling power of the agar due to its hydrolysis by ferric citrate and sodium citrate.[1,4]
- It is recommended to use freshly prepared medium.[4]
- Do not remelt the medium once it has been prepared as this can reduce its productivity characteristics towards target strains.[6]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella* and/or *Shigella*, with lower selectivity such as Mac Conkey Agar or Levine EMB Blue Agar and with higher selectivity such as SS Agar or, if S. Typhi is suspected, Bismuth Sulphite Agar, should be used; other media for the isolation of other enteric pathogens should be inoculated with the specimen.[5]
- Non-enteric, lactose non-fermenting organisms such as *Pseudomonas* and *Aeromonas* may grow.
- Surface colonies of Lactose non-fermenting bacteria often absorb a little colour (pinkish) from the medium and organisms may be mistaken for coliforms.[4]
- Some strains of *Proteus* spp. may not be completely inhibited and colonies may resemble *Salmonella*. It is advised to screen the colonies by adding one drop of MUCAP reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C_8 esterase enzyme, typical of *Salmonella* spp.[7]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C +8°C for up to one weeks.[4]

REFERENCES

1. Leifson E. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J Pathol Bacteriol 1935; 40: 581-599.
2. Hynes M. The isolation of intestinal pathogens. J Pathol Bacteriol 1942; 54:193-207.
3. Hajna AA, Damon SR, New enrichment and plating medium for the isolation of Salmonella and Shigella organisms. App Microbiol. 1956; 4:341.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.685.
6. Liang C, Fung DYC. Performance of Some Heat-Sensitive Differential Agars Prepared and Melted by Microwave Energy. J Food Prot. 1988 Jul; 51(7):577-578.
7. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526.

PACKAGING

Product	Type	REF	Pack
Desoxycholate Citrate Agar	Dehydrated medium	4013752	500 g (10.3 L)

IFU rev 2, 2022/03

DESOXYCHOLATE LACTOSE AGAR

Dehydrated culture medium

INTENDED USE

Slightly selective medium for the isolation and differentiation of Gram-negative enteric bacteria and for the enumeration of coliforms from water, wastewater, milk and dairy products.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10.000 g
Lactose	10.000 g
Sodium chloride	5.000 g
Sodium citrate	2.000 g
Sodium deoxycholate	0.500 g
Neutral red	0.033 g
Agar	15.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Desoxycholate Lactose Agar is prepared on the basis of a modification of the formulation described by Leifson[1] in 1935 by lowering the concentration of sodium deoxycholate. Desoxycholate Lactose Agar was recommended by APHA[2,3] in pour plate procedures for isolation and enumeration of coliforms in milk, water and other specimens. The medium is no longer included in recent editions of these manuals. Peptocomplex provides nitrogen, carbon and trace elements for microbial growth; sodium chloride maintains the osmotic balance; the slight selectivity is due to the presence of sodium deoxycholate and sodium citrate which allow a good growth of Gram-negative bacteria and a partial inhibition of Gram-positive bacteria. Lactose is included into the medium as a fermentable carbohydrate, neutral red as a pH indicator: coliforms ferment lactose producing an acidification of the medium and exhibiting colonies with red colour, often surrounded by a pink-red opaque halo due to the precipitation of sodium deoxycholate. Bacteria that do not ferment lactose (including enteric pathogens *Salmonella* and *Shigella*) do not acidify the medium and cultivate with colourless colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and boil for 1 minute for dissolve the medium completely. Cool to 47-50 °C, mix well and pour into sterile Petri dishes. Do not sterilize in autoclave and do not overheat.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige to pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

Desoxycholate Lactose Agar is intended for the bacteriological processing of water, wastewater, milk, dairy products and other samples of sanitary significance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Enumeration of coliforms in water and dairy products:[4]

- Introduce 1-4 mL of sample (and/or decimal dilutions of the sample) into sterile Petri dishes.
- Add 10-20 mL of medium cooled to 44-47°C and mix well the medium with the inoculum.
- Allow the medium to solidify and pour a surface covering layer of 3-4 mL of uninoculated medium.
- Incubate aerobically at 35-37°C for 18-24 hours. If negative after 24 hours, re-incubate additional 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Gram negative bacteria grow with different characteristics depending on their ability to ferment lactose and to induce the pH indicator changes.
Lactose fermenting Gram-negative enteric bacteria (coliforms): pink or bright red lenticular colonies sometimes surrounded by a red-pink opaque zone (precipitation of sodium deoxycholate).
Lactose non-fermenting Gram-negative enteric bacteria: colourless colonies

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, red-violet colonies surrounded by a red-pink opaque zone
S. Enteritidis ATCC 13076	35-37°C / 18-24 H / A	growth, colourless colonies
S. aureus ATCC 25922	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Aerobic or facultative anaerobic Gram-negative bacteria other than *Enterobacteriaceae* (e.g. *Pseudomonas*, *Aeromonas*) may grow on the medium with colourless colonies.
- Desoxycholate Lactose Agar does not contain an indicator system for the production of hydrogen sulphide therefore *Salmonella* colonies do not have a black centre.
- At pH above 7.5 the medium loses some of its inhibitory properties towards Gram positive bacteria.[1,4]
- With overheating there may be a decrease in the gelling power of the agar due to its hydrolysis by sodium citrate.[1,4]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the self-prepared plates can be stored at +2°C /+8°C for up to one week.[4]

REFERENCES

1. Leifson E. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J Pathol Bacteriol 1935; 40: 581-599.
2. American Public Health Association. Standard methods for the examination of water and wastewater, 11th ed. 1960.
3. American Public Health Association. Standard Methods for the Examination of Dairy Products. 14th ed. 1967
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Desoxycholate Lactose Agar	Dehydrated medium	4013802	500 g (11.7 L)

IFU rev 2, 2022/07

DEXTROSE AGAR

Dehydrated culture medium

INTENDED USE

For cultivating a wide variety of microorganisms with or without added blood.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	3 g
Tryptose	10 g
Glucose	10 g
Sodium chloride	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Dextrose Agar is based on the formulation proposed by Norton[1] in 1932 as a basal medium containing 0.5-1% dextrose with 5% defibrinated blood for the isolation of many fastidious bacteria.
Dextrose Agar can be used as it is, with the addition of 5% of sterile defibrinated blood for the preparation of blood agar, or heated to 80°C for the preparation of chocolate agar.
The medium is not suitable for demonstrating the haemolytic properties of microorganisms, due to the high glucose content.
Tryptose and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Glucose is a source of carbon and energy and its high concentration allows the production of early, abundant organism growth. Agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 43 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, if required add the suitable enrichment, mix well and distribute intro sterile Petri dishes

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, clear
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of varying morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24h / A	growth
E. coli ATCC 25922	35-37°C / 18-24h / A	growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Norton. 1932. J. Lab. Clin. Med. 17:558.

PACKAGING

Product	Type	REF	Pack
Dextrose Agar	Dehydrated medium	4013852	500 g (11.6 L)

IFU rev 1, 2022/07

DEXTROSE BROTH

Dehydrated culture medium

INTENDED USE

For cultivating a wide variety of microorganisms and for detection of gas production from glucose fermentation.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	3 g
Tryptose	10 g
Glucose	5 g
Sodium chloride	5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Dextrose Broth is a modification of Dextrose Agar proposed by Norton[1] in 1932, prepared without agar and with a reduced concentration of glucose. Dextrose Broth is a high nutritious liquid medium that allows the production of early, abundant organism growth even with a small inoculum. It may be used also for detecting gas formation from enteric bacilli through dextrose fermentation.
The addition of 0.1-0.2% agar to the Dextrose Broth promotes anaerobic growth and dispersion of the reducing substances and CO_2 formed in the environment. The low concentration of agar is suitable for aerobic growth in the upper clear zone, and microaerobic and anaerobic growth in the lower, flocculating agar zones.
Tryptose and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Glucose is a source of carbon and energy.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 23 g in 1000 mL of cold purified water. Heat to dissolve with frequent agitation, distribute into suitable containers and sterilise by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, clear
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.
For the detection of gas production from glucose fermentation by enteric bacilli, inoculate the fermentation tubes and incubate at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of a turbidly. Gas formation can be observed as bubbles production accumulated into Durham tubes.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24h / A	growth
E. coli ATCC 25922	35-37°C / 18-24h / A	growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place

REFERENCES

1. Norton. 1932. J. Lab. Clin. Med. 17:558.

PACKAGING

Product	Type	REF	Pack
Dextrose Broth	Dehydrated medium	4013862	500 g (21.7 L)

IFU rev 1, 2022/07

DG18 AGAR BASE
DG18 AGAR
Dehydrated and ready-to-use culture medium, selective supplement

INTENDED USE
For the enumeration of yeasts and moulds in foods and animal feeding stuffs with water activity less than or equal to 0.95 (ISO 21527-2).

COMPOSITION*
DG18 AGAR BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	5 g
D-glucose	10 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate	0.5 g
Dichloran (2,6-dichloro-4-nitroaniline)	0.002 g
Agar	13.5 g

CHLORAMPHENICOL ANTIMICROBIC SUPPLEMENT
VIAL CONTENTS FOR 500 ML OF MEDIUM BASE

Chloramphenicol	50 mg

DG 18 AGAR, READY-TO-USE PLATES
TYPICAL FORMULA

DG 18 Agar Base	30 g
Glycerol	220 g
Chloramphenicol	0.1 g
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
DG18 Agar is a low water activity (a_w) medium, devised by Hocking and Pitt for enumeration of xerophilic fungi from low-moisture foods.[1] It is recommended by ISO 21527-2 and by FDA-BAM for the enumeration of viable osmophilic yeasts and xerophilic moulds in products intended for human consumption or feeding the animals, having a water activity less than or equal to 0.95, by means of the colony count technique.[2,3]
Glycerol in the medium reduces the water activity from 0.999 to 0.95. Pitt and Hocking[4] showed that glycerol is a suitable solute for the cultivation of a range of xerophilic fungi: it is less inhibitory than NaCl to some species, produces transparent media, and is more readily handled than sugars are at high concentrations. Dichloran (2,6-dichloro-4-nitroaniline) has been shown to inhibit spreading of mucoraceous fungi and to limit colony diameters of other genera in a fungal enumeration medium for foods.[5] The enzymatic digest of casein provides nitrogen, carbon, minerals and amino acids for the microbial growth. Glucose is a source of carbon and energy. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate enhances the mycological growth. The selective properties of the medium are increased by the presence of chloramphenicol, a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 15 g in 500 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and add 110 g (220 g/L) of Glycerol anhydrous (REF 421015). Reconstitute one vial of Chloramphenicol Antimicrobic Supplement (REF 4240003) with 3 mL of a mixture of sterile purified water-ethanol (1:1) and add the content to DG18 Agar Base (100 mg/L). Sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C, mix well and distribute 15 mL amounts into sterile Petri dishes. Avoid exposure of the medium to light.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	beige, clear to slightly opalescent
Final pH at 20-25 °C	5.6 ± 0.2

Products intended for human consumption or feeding the animals having a water activity less than or equal to 0.95 such as dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereals products, flours, nuts, spices and condiments, etc. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE
The working procedure described here is taken from ISO 21527-2.[2]
1. On to one DG18 Agar plate, using a fresh sterile pipette, transfer 0.1 mL of the test sample if liquid, or 0.1 mL of the initial suspension in the case of other products.
2. Repeat this operation with subsequent dilutions, using a new sterile pipette for each decimal dilution.
3. To facilitate enumeration of low populations of yeasts and moulds, volume up to 0.3 mL of 10^{-1} dilution of sample, or of test sample if liquid, can be spread on to three plates
4. Spread the liquid over the surface of the agar with a sterile spreader, until the liquid is completely absorbed into the medium.
5. Incubate aerobically the inoculated plates in an upright position at 25 ± 1°C for 5 to 7 days.

READING AND INTERPRETATION
After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
If necessary, carry out an examination with a binocular magnifier or with a microscope in order to distinguish between cells of yeasts or moulds and bacteria from colonies.
Read the plates after 2 days, 5 days and 7 days of incubation. If the presence of *Xeromyces bisporus* is suspected, incubate the plates for 10 days.
Select the dishes containing less than 150 colonies/propagules/germs and count these colonies/propagules/germs,
Report as number of colonies/propagules/germs per gram of food.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[2]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25 ± 1°C/ 5 days/A	growth
Wallemia sebi ATCC 42694	25 ± 1°C/ 5 days/A	growth
Aspergillus restrictus ATCC 42693	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Eurotium rubrum ATCC 42690	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25 ± 1°C/ 5 days/A	inhibited
Bacillus subtilis ATCC 6633	25 ± 1°C/ 5 days/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Avoid exposure of the medium to light, since cytotoxic breakdown products can result in underestimation of mycoflora in samples.[2]
- Where bacterial overgrowth may be a problem, chloramphenicol (50 mg/L) and chlortetracycline (50 mg/L) are recommended.[2]
- DG 18 Agar and the procedure taken from ISO 21527-2 do not apply to dehydrated products with water activity less than or equal to 0.60 and do not allow the enumeration of mould spores.[2]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[2]
- Non-linearity of counts from dilution plating often occurs, i.e. 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[2]
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[2]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that identification testing be performed on isolates, from pure culture.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place

According Baird RM et al. the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 7 days.[7]

Ready-to-use plates and selective supplement

Store in their original pack at 2-8°C away from direct light.

REFERENCES

1. Hocking, A.D., and Pitt,J.L. (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Enviornm. Microbiol 39,488-492.
2. ISO 21527-2:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity less than or equal to 0,95.
3. FDA-BAM Chapter 18: Yeasts, Molds and Mycotoxins. Content current as of: 10/31/2017.
4. Pitt, J. I., and A. D. Hocking. 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. J. Gen. Microbiol. 101:35-40.
5. King, A. D., A. D. Hocking, and J. I. Pitt. 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbiol. 37:959-964.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 216-218.

PACKAGING

Product	Type	REF	Pack
DG 18 Agar Base	Dehydrated medium	4013942	500 g (16.6 L)
Chloramphenicol Antimicrobic Supplement	Freeze-dried supplement	4240003	10 vials, each for 500 mL of medium
DG 18 Agar	Ready-to-use plates	541394	2 x 10 plates ø 90 mm

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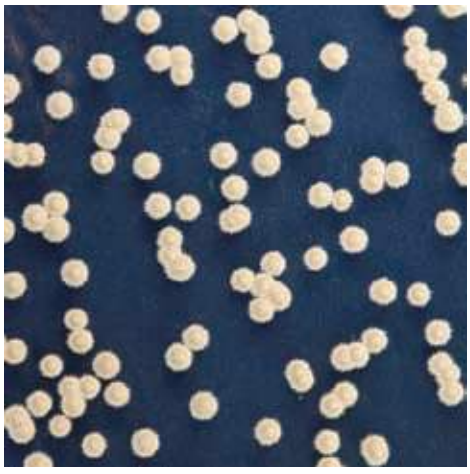

DG 18 Agar: *Wallemia sebi*

DG18 CHLORAMPHENICOL AGAR

Dehydrated culture medium

INTENDED USE

Culture medium completed with chloramphenicol for the enumeration of yeasts and moulds in foods and animal feeding stuffs with water activity less than or equal to 0.95 (ISO 21527-2).

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	5 g
D-glucose	10 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate	0.5 g
Dichloran (2,6-dichloro-4-nitroaniline)	0.002 g
Chloramphenicol	0.1 g
Agar	13.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

DG18 (dichloran 18% mass fraction glycerol) Chloramphenicol Agar is a low water activity medium, devised by Hocking and Pitt for enumeration of xerophilic fungi from low-moisture foods.[1] It is recommended by ISO 21527-2 and by FDA-BAM for the enumeration of viable osmophilic yeasts and xerophilic moulds in products intended for human consumption or feeding the animals, having a water activity less than or equal to 0.95, by means of the colony count technique.[2,3]

Glycerol in the medium reduces the water activity from 0.999 to 0.95. Pitt and Hocking[4] showed that glycerol is a suitable solute for the cultivation of a range of xerophilic fungi: it is less inhibitory than NaCl to some species, produces transparent media, and is more readily handled than sugars are at high concentrations. Dichloran (2,6-dichloro-4-nitroaniline) has been shown to inhibit spreading of mucoraceous fungi and to limit colony diameters of other genera in a fungal enumeration medium for foods.[5] The enzymatic digest of casein provides nitrogen, carbon, minerals and amino acids for the microbial growth. Glucose is a source of carbon and energy. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate enhances the mycological growth. The selective properties of the medium are increased by the presence of chloramphenicol already included in the dehydrated medium: it is a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30.1 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and add 220 g of Glycerol anhydrous (REF 421015). Sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C, mix well and distribute 15 mL amounts into sterile Petri dishes. Avoid exposure of the medium to light.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	beige, clear to slightly opalescent
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Products intended for human consumption or feeding the animals having a water activity less than or equal to 0.95 such as dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereals products, flours, nuts, spices and condiments, etc. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

The working procedure described here is taken from ISO 21527-2.[2]

1. On to one DG18 Chloramphenicol Agar plate, using a fresh sterile pipette, transfer 0.1 mL of the test sample if liquid, or 0.1 mL of the initial suspension in the case of other products.
2. Repeat this operation with subsequent dilutions, using a new sterile pipette for each decimal dilution.
3. To facilitate enumeration of low populations of yeasts and moulds, volume up to 0.3 mL of 10^{-1} dilution of sample, or of test sample if liquid, can be spread on to three plates
4. Spread the liquid over the surface of the agar with a sterile spreader, until the liquid is completely absorbed into the medium.
5. Incubate aerobically the inoculated plates in an upright position at 25 ± 1°C for 5 to 7 days.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.

If necessary, carry out an examination with a binocular magnifier or with a microscope in order to distinguish between cells of yeasts or moulds and bacteria from colonies.

Read the plates after 2 days, 5 days and 7 days of incubation. If the presence of *Xeromyces bisporus* is suspected, incubate the plates for 10 days.

Select the dishes containing less than 150 colonies/propagules/germs and count these colonies/propagules/germs,

Report as number of colonies/propagules/germs per gram of food.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[2]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25 ± 1°C/ 5 days/A	growth
Wallemia sebi ATCC 42694	25 ± 1°C/ 5 days/A	growth
Aspergillus restrictus ATCC 42693	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Eurotium rubrum ATCC 42690	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25 ± 1°C/ 5 days/A	inhibited
Bacillus subtilis ATCC 6633	25 ± 1°C/ 5 days/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Avoid exposure of the medium to light, since cytotoxic breakdown products can result in underestimation of mycoflora in samples.[2]
- DG 18 Chloramphenicol Agar and the procedure taken from ISO 21527-2 do not apply to dehydrated products with water activity less than or equal to 0,60 and do not allow the enumeration of mould spores.[2]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[2]
- Non-linearity of counts from dilution plating often occurs, i.e. 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[2]
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[2]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that identification testing be performed on isolates, from pure culture.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According Baird RM *et al.* the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 7 days.[6]

REFERENCES

1. Hocking, A.D.,and Pitt,J.L. (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Enviornm. Microbiol 39,488-492.
2. ISO 21527-2:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity less than or equal to 0,95.
3. FDA-BAM Chapter 18: Yeasts, Molds and Mycotoxins. Content current as of: 10/31/2017.
4. Pitt, J. I., and A. D. Hocking. 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. J. Gen. Microbiol. 101:35-40.
5. King, A. D., A. D. Hocking, and J. I. Pitt. 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbiol. 37:959-964.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 216-218.

PACKAGING

Product	Type	REF	Pack
DG 18 Chloramphenicol Agar	Dehydrated medium	401394C2	500 g (16.6 L)

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DG18 Chloramphenicol Agar colonies of *Eurotium rubrum*

DIFFERENTIAL CLOSTRIDIAL AGAR

Dehydrated culture medium

INTENDED USE

Basic medium to which iron (III) ammonium citrate and sodium sulphite solutions are added, for enumeration of sulphite-reducing clostridia spores in dried foodstuffs.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	5.0 g
Enzymatic digest of meat	5.0 g
Meat extract	8.0 g
Yeast extract	1.0 g
Starch	1.0 g
Glucose	1.0 g
L-cysteine HCl	0.5 g
Resazurin	2.0 mg
Agar	20.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Differential Clostridial Agar has been formulated by Weenk et al.[1] for the enumeration of spores of mesophilic clostridia in dried foods. They established that the sulphite activity and the ferrous ion should be rigorously standardised and the basal medium should be free of added acetate and lactate.
Differential Clostridial Agar is included by APHA[2] in the list of commonly used media for the isolation and enumeration of clostridia in foodstuffs.
The medium is very rich and permits the growth of most clostridia, and many other anaerobes and facultative anaerobes. Pancreatic digest of casein, enzymatic digest of meat and meat extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group and glucose is a source of carbon and energy. L-cysteine is a reducing agent and favours the growth of anaerobes. Starch helps to detoxify metabolic by-products. Sodium sulphite and ferric citrate are added to the medium and act as indicators: sulphite reducing clostridia produce sulphide from sulphite, which results in the formation of black medium. The redox indicator resazurin is used to monitor anaerobiosis. Agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 45-48°C and add 5 mL of Solution A and 1 mL of Solution B.
Solution A (freshly prepared): 1 g of ferric (III) ammonium citrate in 5 mL of purified water, sterilized in the autoclave at 121°C for 15 minutes or sterilised by filtration.
Solution B: 2.5 g of sodium sulfite anhydrous in 10 mL of purified water, sterilised by filtration. May be stored for no more than one month.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid or slightly opalescent
Final pH at 20-25 °C	7.6 ± 0.2

SPECIMENS

Foodstuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

TEST PROCEDURE

Prepare samples or heated samples and dilutions and inoculate duplicate Petri dishes with 1 mL of each of the appropriate dilutions of the food material. Pour 15 mL of melted medium and mix well the inoculum with the medium.
Let the medium solidify and overlay the plates with a layer of the same medium. Incubate 2-5 days at 30- 35 °C under anaerobic conditions.
Weenk *et al*. developed an alternate procedure by using spiral plating with Differential Clostridial Agar and a DCA overlay.[1,2]

READING AND INTERPRETATION

Clostridia reduce sulphite to sulphide and the iron sulphide produced causes the culture medium to turn black.
Count the black colonies on the plates containing between 15 and 150 characteristic colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	30°C / 72h / AN	growth with black colonies
C. sporogenes ATCC 19404	30°C / 72h / AN	growth with black colonies

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Some bacilli might mimic clostridia under the conditions of the procedure.[1]
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place. According to Curtis *et al.* basal medium may be stored at +2/+8 °C for 2 weeks whereas the complete medium should be used the same day (do not remelt).[3]

References

1. Weenk GH, Van den Brink JA, Struijk CB, Mossel DA. Modified methods for the enumeration of spores of mesophilic Clostridium species in dried foods. Int J Food Microbiol 27(2-3):185-200.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
3. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:201-3.

Packaging

Product	Type	REF	Pack
Differential Clostridial Agar	Dehydrated medium	4013122	500 g (12 L)

IFU rev 1, 2023/02

DOUBLE MODIFIED LYSINE IRON AGAR (DMLIA)
DMLIA NOVOBIOCIN SUPPLEMENT

Dehydrated culture medium, selective supplement and ready-to-use plates

Salmonella Derby on DMLIA

Intended use

For selective and differential isolation of *Salmonella* from foods.

Composition*

Double Modified Lysine Iron Agar (DMLIA) – dehydrated medium
Typical formula after reconstitution with 1 L of water

Peptone	5.00 g
Yeast extract	3.00 g
Glucose	1.00 g
L-Lysine HCl	10.00 g
Ferric ammonium citrate	0.80 g
Sodium thiosulphate	6.80 g
Bile salts n° 3	1.50 g
Lactose	10.00 g
Sucrose	10.00 g
Bromocresol purple	0.02 g
Agar	15.00 g

DMLIA Novobiocin Supplement
Vial contents for 500 mL of medium

Novobiocin	7.5 mg

Double Modified Lysine Iron Agar DMLIA – ready-to-use plates
Typical formula

Double Modified Lysine Iron Agar	63.12 g
Novobiocin	15.00 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Double Modified Lysine Iron Agar (DMLIA), prepared by adding 15 mg/L novobiocin to the base medium, corresponds to the formula of the selective and differential medium described by Rappold and Bolderdijk[1] in 1979 and recommended by the USDA[2-4] for the isolation of H_2S-positive and -negative strains of *Salmonella* in a wide variety of foodstuffs.
The dehydrated medium consists mainly of Lysine Iron Agar supplemented with bile salts, lactose, sucrose and additional amounts of sodium thiosulphate and ferric ammonium citrate.
Peptone provides nitrogen, carbon, minerals for bacterial growth; yeast extract is a source of vitamins, particularly of the B-group; glucose, lactose and sucrose are fermentable carbohydrates; bromocresol purple is a pH indicator, yellow at pH below 5.2 and purple at pH above pH 6.8; ferric ammonium citrate and sodium thiosulphate are the indicator system for the formation and detection of hydrogen sulphide. Lysine is included as a substrate for detection of lysine decarboxylase: when lysine is decarboxylated it is converted in cadaverine causing an alkaline reaction (the medium remains purple). Bile salts and novobiocin (included in the selective supplement) are the inhibitory agents, active mostly against Gram-positive bacteria but also against a few Gram-negative bacteria.
Salmonella spp. decarboxylates lysine and, being lactose/sucrose positive, induce an alkaline reaction in the medium with the development of colonies with different shades of purple (mauve colonies); the formation of hydrogen sulphide is indicated by the formation of colonies with a black centre.

Directions for medium preparation

Suspend 31.56 g in 500 mL of cold purified water. Heat to boiling with frequent stirring to dissolve completely. Do not autoclave. Cool to approximately 45-50°C and add the contents of one vial of DMLIA Novobiocin Supplement (REF 4240029) reconstituted with 5 mL of sterile purified water. Mix well and distribute 15-20 mL into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	purple, slightly opalescent
Freeze-dried selective supplement	short, dense, white pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	6.7 ± 0.2

Specimens

Meat, poultry, pasteurized eggs and siluriformes (fish) products and environmental sponges. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to the applicable International Standard.[2]

Test procedure
Transfer a loopful of growth from the selective enrichment broths by streaking onto four quadrants of a Double Modified Lysine Iron Agar (DMLIA) plate to obtain isolated colonies.
Incubate at 35 ± 2 °C for 18-24 hours. If there is no growth or no typical colonies, re-incubate for a further 18-24 hours.
For analytical details refer to the cited USDA document.[2]

Reading and interpretation
After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Consider mauve-coloured colonies with or without a black centre as typical for *Salmonella*.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[5]

Control strains	Incubation T°/ t - ATM	Expected results
Salmonella sp. H_2S positive	35°C / 24h / A	good growth, mauve-coloured colonies with a black centre
Salmonella sp. H_2S negative	35°C / 24h / A	good growth, mauve-coloured colonies without a black centre

A: aerobic incubation

Limitations of the method
- Lactose-positive *Salmonella* variants have been described with an incidence rate of less than 1%.[5,6] These salmonellae may grow with yellowish colonies on DMLIA plate.
- Complete identification of the colonies must be carried out by biochemical, immunological, molecular or mass spectrometric techniques, after purification of the colonies by subculture on appropriate medium. For analytical details refer to the cited USDA document.[2]

Storage conditions
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place
Freeze-dried supplement
Store the product in the original package at 2-8°C away from direct light.
According to USDA document the self-prepared plates can be stored at +2°C +8°C for up to 3 weeks.[3]
Ready-to-use plates
Store plates in their original pack at 2-8°C away from direct light.

References

1. Rappold H, Bolderdijk R. Modified lysine iron agar for isolation of Salmonella from food. Appl Environ Microbiol 1979; 38(1):162-3.
2. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science. Laboratory Guidebook, Notice of Change: Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Eggs and Siluriformes (Fish) Products and Environmental Sponges. MLG 1.11, Effective Date: 08/16/21.
3. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science. Laboratory Guidebook, Notice of Change: Media and Reagents. MLG Appendix 1.10, Effective Date: 03/07/22.
4. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science Laboratory Guidebook, Notice of Change: Flow Chart Specific for FSIS Laboratory Isolation and Identification of Salmonella. Chapter MLG 4.0, , Effective Date 02/24/20.
5. Ewing W H. Edwards and Ewing's identification of the Enterobacteriaceae. 4th ed. New York, N.Y: Elsevier Science Publishing Co., Inc.; 1986. Differentiation of Enterobacteriaceae by biochemical reactions; pp. 47–72.
6. Patrick L. McDonough, Sang J. Shin, Donald H. Lein. Diagnostic and Public Health Dilemma of Lactose-Fermenting Salmonella enterica Serotype Typhimurium in Cattle in the Northeastern United States. J Clin Microbiol 2000; 38(3): 1221–1226.

Packaging
Product	Type	REF	Pack
Double Modified Lysine Iron Agar (DMLIA)	Dehydrated medium	4013252	500 g (7.9 L)
DMLIA Novobiocin Supplement	Freeze-dried supplement	4240029	10 vials, each for 500 mL of medium base
Double Modified Lysine Iron Agar (DMLIA)	Ready-to-use plates	541325	2 x 10 plates ø 90 mm

IFU rev 0, 2022/07

DRBC AGAR BASE
Dehydrated culture medium, selective supplement

Intended use
For the enumeration of yeasts and moulds in foods and animal feeding stuffs with water activity greater than 0.95 (ISO 21527-1)

Composition *
DRBC Agar Base, dehydrated medium
typical formula * (after reconstitution with 1 L of water)

Enzymatic digest of animal and plant tissues	5 g
D-glucose	10 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate	0.5 g
Dichloran (2,6-dichloro-4-nitroaniline)	0.002 g
Rose bengal	0.025 g
Agar	15 g

Chloramphenicol Antimicrobic Supplement
Vial contents for 500 ml of medium base

Chloramphenicol	50 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
DRBC (Dichloran-Rose Bengal Chloramphenicol Agar) is a modification of Rose-Bengal-Chloramphenicol Agar (RBC) from Jarvis[1], devised by King et al.[2] It is recommended by ISO 21527-1[3] for the enumeration of viable yeasts and moulds in products intended for human consumption

or feeding of animals, having a water activity greater than 0.95 and by FDA-BAM[4] for analysing sample containing "spreader" moulds (e.g., *Mucor*, *Rhizopus*, etc.).[4]Dichloran (2,6-dichloro-4-nitroaniline), in combination with rose bengal, has been shown to inhibit spreading of mucoraceous fungi and to limit colony diameters of other genera in a fungal enumeration medium for foods.[5] The enzymatic digest of animal and plant tissues provides nitrogen, carbon, minerals and amino acids for the microbial growth. Glucose is a source of carbon and energy. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate enhances the microbial growth. The selective properties of the medium are increased by the presence of chloramphenicol, a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria.

Directions for medium preparation

Suspend 15.8 g in 500 mL of cold purified water and heat to boiling dissolve completely. Reconstitute one vial of Chloramphenicol Antimicrobic Supplement (REF 4240003) with 3 mL of a mixture of sterile purified water-ethanol (1:1) and add the content to DRBC Agar Base (100 mg/L). Sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C mix well and distribute 15 mL amounts into sterile Petri dishes. Avoid exposure of the medium to light.

Physical characteristics

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, clear to slightly opalescent
Final pH at 20-25 °C	5.6 ± 0.2

Specimens

Products intended for human consumption or feeding the animals having a water activity greater than 0.95 such as eggs, meat, dairy products (except milk powder), fruits, vegetables, fresh pastes, etc. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

Test procedure

The working procedure described here is taken from ISO 21527-1.[3]

1. On to one DRBC Agar plate, using a fresh sterile pipette, transfer 0.1 mL of the test sample if liquid, or 0.1 mL of the initial suspension in the case of other products.
2. Repeat this operation with subsequent dilutions, using a new sterile pipette for each decimal dilution.
3. To facilitate enumeration of low populations of yeasts and moulds, volume up to 0.3 mL of 10^{-1} dilution of sample, or of test sample if liquid, can be spread on to three plates
4. Spread the liquid over the surface of the agar with a sterile spreader, until the liquid is completely absorbed into the medium.
5. Incubate aerobically the inoculated plates in an upright position at 25 ± 1°C for 5 days.

Reading and interpretation

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies. If necessary, carry out an examination with a binocular magnifier or with a microscope in order to distinguish between cells of yeasts or moulds and bacteria from colonies. Read the plates between 2 days and 5 days of incubation. Select the dishes containing less than 150 colonies/propagules/germs and count these colonies/propagules/germs. Report as number of colonies/propagules/germs per gram of food.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

Control strains	Incubation T°/ t - ATM	Expected results
Saccharomyces cerevisiae ATCC 9763	25 ± 1°C/ 5 days/A	growth
Candida albicans ATCC 10231	25 ± 1°C/ 5 days/A	growth
Aspergillus brasiliensis ATCC 16404	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Mucor racemosus ATCC 42647	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25 ± 1°C/ 5 days/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Media containing rose bengal are light-sensitive; relatively short exposure to light will result in the formation of inhibitory compounds.[5]
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[3]
- Where bacterial overgrowth may be a problem, chloramphenicol (50 mg/L) and chlortetracycline (50 mg/L) are recommended.[3]
- DRBC Agar and the procedure taken from ISO 21527-1 do not allow the enumeration of mould spores and are not suitable for enumeration of heat-resistant fungi, such as *Byssochlamys fulva* or *Byssochlamys nivea*, in canned or bottled fruit and vegetables.[3]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[3]
- Non-linearity of counts from dilution plating often occurs, i.e. 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[3]

Storage conditions

Dehydrated medium: store at +10°C /+30°C away from direct light in a dry place. According to Baird RM *et al.* the self-prepared plates may be stored at +2/+8°C for 7 days.[6]
Freeze-dried supplement: store the product in the original package at 2-8°C away from direct light.

References

1. Jarvis B. Comparison of an improved rose-bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J Appl Bacteriol 1973; 36: 723-727.
2. King DA, Hocking AD, Pitt JI. Dichloran-rose Bengal medium for enumeration and isolation of moulds from foods. Appl Environm Microbiol 1979; 37: 959-964.
3. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. Chapter 18: Yeasts, Molds and Mycotoxins. Content current as of: 10/31/2017.

5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Media M183: Dichloran rose bengal chloramphenicol (DRBC) agar. Content current as of: 10/16/2017.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:195-6.

PACKAGING

Product	Type	REF	Pack
DRBC Agar Base	Dehydrated medium	4013932	500 g (15.8 L)
Chloramphenicol Antimicrobic Supplement	Freeze-dried supplement	4240003	10 vials, each for 500 mL of medium

IFU rev 2, 2022/07

DRBC CHLORAMPHENICOL AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

Culture medium complete with chloramphenicol for the enumeration of yeasts and moulds in foods and animal feeding stuffs with water activity greater than 0.95 (ISO 21527-1)

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Enzymatic digest of animal and plant tissues	5 g
D-glucose	10 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate	0.5 g
Dichloran (2,6-dichloro-4-nitroaniline)	0.002 g
Rose bengal	0.025 g
Chloramphenicol	0.1 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

DRBC (Dichloran-Rose Bengal Chloramphenicol) Agar is a modification of Rose-Bengal-Chloramphenicol Agar (RBC) from Jarvis[1], devised by King et al.[2] It is recommended by ISO 21527-1[3] for the enumeration of viable yeasts and moulds in products, intended for human consumption or feeding of animals, having a water activity greater than 0.95 and by FDA_BAM[4] for analysing sample containing "spreader" moulds (e.g. *Mucor*, *Rhizopus*, etc.).[4]
Dichloran (2,6-dichloro-4-nitroaniline), in combination with rose bengal, has been shown to inhibit spreading of mucoraceous fungi and to limit colony diameters of other genera in a fungal enumeration medium for foods.[5] The enzymatic digest of animal and plant tissues provides nitrogen, carbon, minerals and amino acids for the microbial growth. Glucose is a source of carbon and energy. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate enhances the microbial growth. The selective properties of the medium are increased by the presence of chloramphenicol, already present in the dehydrated medium; it is a broad-spectrum antibiotic which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 31.6 g in 1000 ml of cold purified water and heat to boiling with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C mix well and distribute 15 mL amounts into sterile Petri dishes. Avoid exposure of the medium to light.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, clear to slightly opalescent
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Products intended for human consumption or feeding the animals having a water activity greater than 0.95 such as eggs, meat, dairy products (except milk powder), fruits, vegetables, fresh pastes, etc. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

The working procedure described here is taken from ISO 21527-1.[3]

1. On to one DRBC Agar plate, using a fresh sterile pipette, transfer 0.1 mL of the test sample if liquid, or 0.1 mL of the initial suspension in the case of other products.
2. Repeat this operation with subsequent dilutions, using a new sterile pipette for each decimal dilution.
3. To facilitate enumeration of low populations of yeasts and moulds, volume up to 0.3 mL of 10^{-1} dilution of sample, or of test sample if liquid, can be spread on to three plates
4. Spread the liquid over the surface of the agar with a sterile spreader, until the liquid is completely absorbed into the medium.
5. Incubate aerobically the inoculated plates in an upright position at 25 ± 1°C for 5 days.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies. If necessary, carry out an examination with a binocular magnifier or with a microscope in order to distinguish between cells of yeasts or moulds and bacteria from colonies. Read the plates between 2 days and 5 days of incubation. Select the dishes containing less than 150 colonies/propagules/germs and count these colonies/propagules/germs. Report as number of colonies/propagules/germs per gram of food.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25 ± 1°C/ 5 days/A	growth
Candida albicans ATCC 10231	25 ± 1°C/ 5 days/A	growth
Aspergillus brasiliensis ATCC 16404	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Mucor racemosus ATCC 42647	25 ± 1°C/ 5 days/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25 ± 1°C/ 5 days/A	inhibited
Bacillus subtilis ATCC 6633	25 ± 1°C/ 5 days/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Media containing rose bengal are light-sensitive; relatively short exposure to light will result in the formation of inhibitory compounds.[5]
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[3]
- Where bacterial overgrowth may be a problem, chloramphenicol (50 mg/L) and chlortetracycline (50 mg/L) are recommended.[3]
- DRBC Agar and the procedure taken from ISO 21527-1 do not allow the enumeration of mould spores and are not suitable for enumeration of heat-resistant fungi, such as *Byssochlamys fulva* or *Byssochlamys nivea*, in canned or bottled fruit and vegetables.[3]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[3]
- Non-linearity of counts from dilution plating often occurs, i.e. 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[3]

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place. According to Baird RM et al. the self-prepared plates may be stored at +2/+8°C for 7 days.[6]

Ready-to-use plates

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Jarvis B. Comparison of an improved rose-bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J Appl Bacteriol 1973; 36: 723-727.
2. King DA, Hocking AD, Pitt JI. Dichloran-rose Bengal medium for enumeration and isolation of moulds from foods. Appl Environm Microbiol 1979; 37: 959-964.
3. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. Chapter 18: Yeasts, Molds and Mycotoxins. Content current as of: 10/31/2017.
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Media M183: Dichloran rose bengal chloramphenicol (DRBC) agar. Content current as of: 10/16/2017.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:195-6.

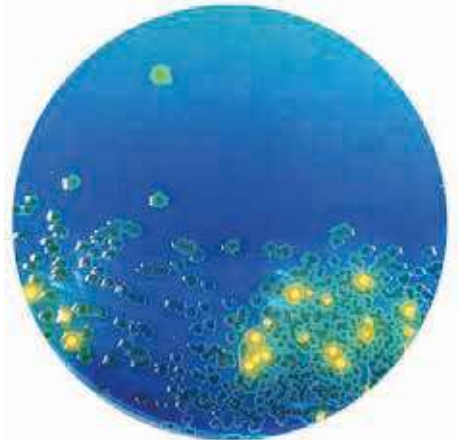
PACKAGING

Product	Type	REF	Pack
DRBC Chloramphenicol Agar	Dehydrated medium	401393C2	500 g (15.8 L)
DRBC Agar	Ready-to-use plates	541393	2 x 10 plates ø 90 mm

IFU rev 2, 2022/07

DRIGALSKI LACTOSE AGAR

Dehydrated culture medium



Drigalski Lactose Agar: *E. coli* (yellow colonies), S. Enteritidis (greenish colonies)

INTENDED USE

In vitro diagnostic. Selective and differential medium for the isolation of *Enterobacteriaceae* and other Gram-negative bacilli from clinical and non-clinical specimens.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	15 g
Beef extract	3 g
Yeast extract	3 g
Sodium deoxycholate	1 g
Sodium thiosulphate	1 g
Lactose	15 g
Agar	13 g
Crystal violet	5 mg
Bromothymol blue	80 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Wilhelm von Drigalski and Heinrich Conradi in 1902 discovered the inhibitory activity of the crystal violet on the growth of Gram-positive bacteria and developed a selective and differential medium for the isolation of *B.typhi*. Drigalski-Conradi medium, modified several times over the years, is the formulation on which the current Drigalski Lactose Agar is based, still widely used as a selective and differential medium for the isolation of *Enterobacteriaceae* and other non-fermenting Gram-negative bacteria from clinical specimens such as urine, faeces, and other biological materials.[1]

The use of Drigalski Lactose Agar, with the addition of cephalosporins, has been proposed for the isolation of extended spectrum beta-lactamase-producing enterobacteria,[3] and, supplemented with carbapenems, for the isolation of carbapenemase-producing enterobacteria[4].

Peptone, meat extract and yeast extract provide nitrogen, carbon, minerals and vitamins for the microbial growth The selective compounds of the medium are sodium deoxycholate, crystal violet and sodium thiosulphate which have an inhibitory activity against Gram positive bacteria; lactose is present in the medium as a fermentable carbohydrate: lactose-fermenting bacteria acidify the medium with a colour change of bromothymol blue from blue-green to yellow.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 51 g in 1000 mL of cold purified water. Heat to boiling with agitation and sterilize by autoclaving at 115°C for 20 minutes. Cool to 47-50 °C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	green, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Drigalski Lactose Agar is intended for the bacteriological processing of clinical specimens such as urine, faeces, and other biological materials. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic conditions at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Gram negative bacteria grow with different characteristics depending on their ability to ferment lactose and to induce the pH indicator changes.

E. coli, Klebsiella, Citrobacter, Enterobacter ferment lactose with acid production and grow with yellow or yellow-green colonies with, often, an opaque yellow halo.

Salmonella, Shigella, Proteus, Alkaligenes, Pseudomonas do not ferment lactose and grow with gray to green-blue colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, yellow colonies
S. Enteritidis NCTC 5188	35-37°C / 18-24 H / A	growth, blue-green colonies
S. aureus ATCC 25923	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection, NCTC: National Type Culture Collection of the UK Health Protection Agency

LIMITATIONS OF THE METHOD

- On this medium, some *Proteus* strains may grow with swarming colonies.
- Incubations prolonged beyond 24 hours can induce a medium change to alkalinity of the lactose-positive strains.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Dupeyron CM, Guillerman GA, Leluan GJ. Rapid diagnosis of gram negative urinary infections: identification and antimicrobial susceptibility testing in 24 hours. J Clin Pathol 198; 39:208-211.
2. Grohs P, Tillecovidin B, Caumont-Prim A, et al. Comparison of Five Media for Detection of Extended-Spectrum Beta-Lactamase by Use of the Wasp Instrument for Automated Specimen Processing. J Clin Microbiol 2013; 51: 2713–2716.
3. Vidal-Navarro L, Pfeiffer C, Bouziges N, Sotto A, Lavigne JP. Faecal Carriage of Multidrug-Resistant Gram-negative Bacilli During a Non-Outbreak Situation in a French University Hospital. J Antimicrob Chemother 2010; 65: 2455-8.
4. Nordmann P, Girlich D, Poirel L. Detection of Carbapenemase Producers in Enterobacteriaceae by Use of a Novel Screening Medium. J Clin Microbiol 2012; 50: 2761–2766.
5. Gokul Yaratha, MD, Sarah Perloff, DO, Kinesh Changala, MBBS. Lactose vs non-lactose fermenting E. coli: Epidemiology, Clinical Outcomes, and Resistance. Open Forum Infect Dis 2017; V4 (Suppl 1)

PACKAGING

Product	Type	REF	Pack
Drigalski Lactose Agar	Dehydrated medium	4013302	500 g (9.8L)

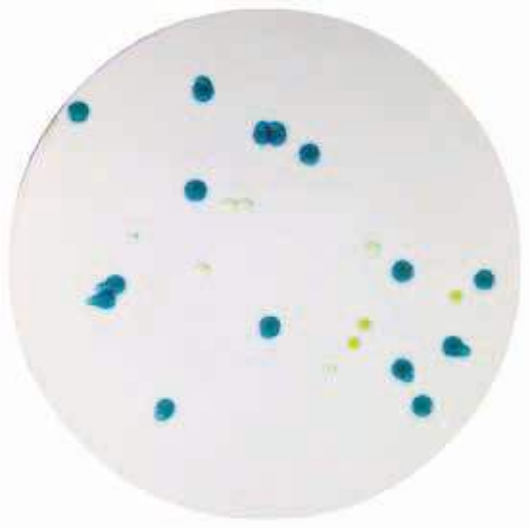
IFU rev 2, 2022/02

ChromArt

E.C.O.A.GAR

(ENTEROCOCCUS CHROMOGENIC OTTAVIANI & AGOSTI AGAR)

Dehydrated culture medium, selective supplement and ready-to use plates



E.C.O.A.gar:
colonies of Enterococcus sp. (blue)
and contaminants

INTENDED USE

For the isolation, enumeration and presumptive confirmation of enterococci in water, milk, food.

COMPOSITION*

E.C.O.A.GAR, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Peptones	**28.0 g**
Sodium chloride	5.0 g
Glucose	1.0 g
Emulsifying agents	5.7 g
Phosphate buffer	5.0 g
Agar	15.0 g
Chromogenic substrates	180.0 mg
Selective compounds	26.0 mg

KANAMYCIN SELECTIVE SUPPLEMENT
VIAL CONTENTS FOR 500 mL OF MEDIUM

Kanamycin sulphate	10 mg

E.C.O.A.GAR, READY-TO-USE PLATES
TYPICAL FORMULA

E.C.O.A.gar, dehydrated medium	60 g
Kanamycin sulphate	20 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

E.C.O.A.gar is a selective chromogenic medium for the isolation, enumeration, and presumptive confirmation of enterococci in water, milk, food, and other materials of sanitary interest. It is prepared according to the formulation developed by F. Ottaviani and M. Agosti.[1] Selectivity of the medium is achieved with a mixture of antimicrobial compounds included in the base medium and with the addition, after sterilization, of kanamycin sulphate.
The medium contains no sodium azide, is not classified as hazardous, and therefore requires no special precautions for use and disposal.
The differential characteristics of the medium are due to a mixture of chromogenic compounds for the determination of specific enterococcal enzymes. Compared with conventional KAA and KF media, E.C.O.A.gar exhibits higher specificity and sensitivity for the detection of *Enterococcus* strains lacking specific enzyme complexes (e.g., for the for hydrolysis of esculin) and that do not form typical colonies: specifically *Enterococcus avium* strain FAIR-E101, *Enterococcus faecium* strains FAIR-E 102, E130, E 131, and E-338, *Enterococcus hirae* strain FAIR-E 174, and *Enterococcus malodoratus* strains FAIR-E168 and E169, all from the BCCM/LMG Bacteria Collection of the University of Ghent (B)[1].

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of Kanamycin Selective Supplement (REF 4240055) reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	very pale violet, clear
Freeze-dried selective supplement	low, dense, white pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

SPECIMENS

Water, milk, food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

TEST PROCEDURE

The medium can be used in accordance with standard laboratory procedures, inoculated by spread-plate or poured plate or membrane filtration techniques and incubated at 37°C for 24 hours.
The E.C.O.A.gar medium provides the laboratory with the following advantages:

- higher enterococcal recovery than traditional KAA and KF media due to the higher differential power;
- greater safety for operators, limited environmental impact, and easier management of laboratory waste due to the absence of sodium azide.

READING AND INTERPRETATION

After incubation, observe bacterial growth, recording any specific morphological and colour characteristics of the colonies.
Enterococci grow blue-green colonies.
Rare colonies of non-*Enterococcus* strains, resistant to antimicrobials in the culture medium, grow with colourless, violet-grey or magenta-red colonies or with natural pigmentation.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. faecalis	ATCC	29212	37°C / 24H / A	good growth, blue colonies
E. faecium	ATCC	19434	37°C / 24H / A	good growth, blue colonies
E. coli	ATCC	25922	37°C / 24H / A	inhibited
S. aureus	ATCC	25923	37°C / 24H / A	inhibited

LIMITATIONS OF THE METHOD

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place.
Freeze-dried supplement and ready-to-use plates
Store the products in the original package at 2-8°C away from direct light.

REFERENCE

1. Ottaviani F, Agosti M. (2000) Personal communication

PACKAGING

Product	Type	REF	Pack
E.C.O.A.gar	Dehydrated medium	4014302	500 g (8.3 L)
Kanamycin Selective Supplement	Freeze-dried supplement	4240055	10 vials, each for 500 mL of medium base
E.C.O.A.gar	Ready-to-use plates	491430	3 x 10 plates ø 55 mm

IFU rev 1, 2022/08

EC BROTH

Dehydrated and ready-to-use culture medium

INTENDED USE

Selective medium for the confirmatory phase in procedures for detection and enumeration of *Escherichia coli* and faecal coliforms in foodstuffs and waters.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Tryptone	20.0 g
Lactose	5.0 g
Dipotassium hydrogen phosphate	4.0 g
Potassium dihydrogen phosphate	1.5 g
Sodium chloride	5.0 g
Bile Salts n°3	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Escherichia coli (EC) medium was first introduced by Hajna and Perry for improved selective detection of coliform bacteria and presumptive detection of *E. coli* in water, foods, shellfish, milk and other materials.[1,2]
EC Broth is recommended:

- by ISO 7251[3] for the confirmatory MPN test in the procedure for detection and enumeration of *E. coli* in foodstuffs,
- by FDA-BAM[4] for the confirmatory MPN test for faecal (thermotolerant) coliforms and *E. coli* in foods, seawater and shellfish,
- by APHA[5] for the confirmatory test in water samples.

The EC Broth tubes are inoculated with broth from positive presumptive tubes of Lauryl Sulphate Broth and incubated at 44°C (for *E. coli* according to ISO 7252)[3] or at 44.5 ± 0.2°C (for thermotolerant coliforms and *E. coli* according to FDA-BAM and APHA)[4,5], for 24 ± 2 h. If gas is produced, the test is positive, indicating the presence of thermotolerant coliforms or *E. coli*.
Tryptone provides nitrogen, carbon and minerals for microbial growth; lactose is a fermentable carbohydrate. Phosphates act as buffer system and sodium chloride maintains the osmotic balance. Bile salts n° 3, inhibit the growth of Gram-positive bacteria, especially bacilli and enterococci.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37 g in 1000 mL of cold purified water; heat slightly to completely dissolve the powder, mix well and distribute 10 mL into test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. The Durham tubes shall not contain air bubbles after sterilization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	light yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.[3] Foods, seawater and shellfish.[4] For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

For the confirmatory test of *E. coli* / faecal coliforms proceed as following:
From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth (REF 401580) showing opacity, cloudiness or any visible gas, inoculate with a sampling loop a tube of EC Broth.
Incubate the tubes at 44 °C ± 1°C[3] (or 44.5 ± 0.2°C)[4] for 24 h ± 2 h.
If, at this stage, there is no visible gas in the EC broth, extend the incubation up to a total of 48 h ± 2 h. For live shellfish, the total incubation time shall be limited to 24 h ± 2 h.[3]

READING AND INTERPRETATION

The medium becomes turbid when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.
Consider as positive for faecal coliform bacteria the EC Broth tubes with the production of gas.[4]
Consider as positive for *E. coli* bacteria the EC Broth tubes with the production of gas, and which at 44°C produce indole from tryptophan.[3]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	44°C/24-48 H/A	growth, with gas production
P. aeruginosa ATCC 27853	44°C/24-48 H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to FDA-BAM, the prepared EC Broth can be stored in the refrigerator for up to 1 month in screw cap tubes.
Ready-to-use tubes
Store tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Hajna AA, Perry CA. Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am J Public Health 1943; 33:550-556.
2. Perry CA, Hajna AA. Further evaluation of EC medium for the isolation of coliform bacteria and Escherichia coli. Am. J. Public Health 1944; 34:735-738.
3. ISO 7251:2005 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique
4. FDA-BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Content current as of:10/09/2020
5. APHA Standard Methods for the Examination of Water and Wastewater, 23rd ed. 2017.

PACKAGING

Product	Type	REF	Pack
EC Broth	Dehydrated medium	4014252	500 g (13.5 L)
EC Broth	Ready-to-use tubes	551425	20 x 10 mL with Durham tubes

IFU rev 2, 2022/07

EC BROTH MUG

Dehydrated culture medium

INTENDED USE

Selective medium for the confirmatory phase of procedures for detection and enumeration of *Escherichia coli* in foodstuffs and waters.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	20.00 g
Lactose	5.00 g
Dipotassium hydrogen phosphate	4.00 g
Potassium dihydrogen phosphate	1.50 g
Sodium chloride	5.00 g
Bile salts n° 3	1.50 g
Tryptophan	1.00 g
4-methylumbelliferone beta-D-glucuronide (MUG)	0.05 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Escherichia coli (EC) medium was first introduced by Hajna and Perry for improved selective detection of coliform bacteria and presumptive detection of *E. coli* in water, foods, shellfish, milk and other materials.[1,2] Feng and Hartman[3] developed EC medium with 4-methylumbelliferyl-β-D-glucuronide (MUG) for rapid screening of *E. coli* detection. Moberg[4] reported that a MUG concentration of 50 µg/mL provided the same intensity of blue fluorescence as the 100 µg/mL MUG levels. Koburger and Miller[5] recommended EC broth with MUG to test contamination in shellfish. Approved by the U.S. Environmental Protection Agency[6], EC-MUG is an effective and rapid method for detection and verification of *E. coli* in food, water, and environmental samples.[7]
EC Broth MUG is recommended by FDA-BAM[8] for the confirmatory MPN test for *E. coli* in shellfish meats and by APHA[9] for the confirmatory test of *E. coli* or thermotolerant coliforms and *E. coli* in water samples.
The EC Broth MUG tubes are inoculated with broth from positive presumptive tubes of Lauryl Sulphate Broth and incubated at 44.5 ± 0.2°C for 24 ± 2 h. If fluorescence is produced, the test is positive, indicating the presence of *E. coli*. The presence of thermotolerant coliforms and *E. coli* can be determined simultaneously by including a Durham tube in EC Broth test tubes.[9] Tryptone provides nitrogen, carbon and minerals for microbial growth; lactose is a fermentable carbohydrate. Phosphates are used as buffering agents to control the pH in the medium. Sodium

chloride maintains the osmotic balance. Bile salts n° 3, inhibit the development of Gram-positive bacteria, especially bacilli and enterococci, while at the same time promoting *E. coli* growth. MUG is cleaved by β-D-glucuronidase produced by *E. coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be determined directly by using a long-wave ultraviolet light (Wood's lamp). Tryptophan is added to the medium for improving the performance of rapid direct indole test into the EC Broth MUG tubes.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 38 g in 1000 mL of cold purified water; heat slightly to completely dissolve the powder, mix well and distribute 10 mL into test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. The Durham tubes shall not contain air bubbles after sterilization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	light yellow, limpid
Final pH at 20-25 °C	6.9 ± 0.2

SPECIMENS

Foods, waters, seawater and shellfish. Refer to applicable International Standards and regulations for the collection of samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

For the confirmatory test of *E. coli* proceed as following[8]:

1. From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth (REF 401580) showing opacity, cloudiness or any visible gas inoculate with a sampling loop a tube of EC Broth MUG.
2. Incubate the tubes at 44.5 ± 0.2°C for 24 h ± 2 h.
3. If required, perform the indole test by adding few drops of Kovac's Reagent (REF 19171000) to the EC Broth MUG tubes.

For partitioning of *E. coli* from MF Total Coliform proceed as following[9]:

1. Remove membrane containing total coliform colonies from the medium (e.g. LES Endo Agar) and carefully curl and insert into a tube of EC Broth MUG. Alternatively remove the colonies with a swab and inoculate a tube of EC Broth MUG or, if quantification is required, inoculate individual colonies into the broth.
2. Within 30 minutes immerse all test tubes in a water bath incubator at 44.5 ± 0.2°C for 24 h ± 2 h

READING AND INTERPRETATION

Presence of growth (turbidity) and a bright blue fluorescence under a long-wave (366 nm) UV light (with or without the production of gas) are considered confirmatory for the presence of *E. coli*.[7]
Indole test: appearance of distinct red colour in upper layer is positive test.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	44°C/24H/A	growth, with fluorescence under Wood's lamp
E. aerogenes ATCC 13048	44°C/24H/A	growth, without fluorescence under Wood's lamp
P. aeruginosa ATCC 27853	44°C/24 H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive and fluorescent under Wood's Lamp; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[10-12]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains[11].
- Up to 10% of *E. coli* have been reported to be slow or non-lactose fermenting but should be MUG-positive.[8,13]
- Since the incubation temperature is critical, the use of submerged waterproofed culture is recommended or the use of an incubator that is documented to hold the temperature at 44.5°C± 0.2°C throughout the chamber over a 24 hours period.[9]

STORAGE CONDITIONS

Store at +2°C /+8°C away from direct light in a dry place.
According to FDA-BAM, the prepared EC Broth MUG can be stored in the refrigerator for up to 1 month in screw cap tubes.[8]

REFERENCES

1. Hajna AA, Perry CA. Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am J Public Health 1943; 33:550-556.
2. Perry CA, Hajna AA. Further evaluation of EC medium for the isolation of coliform bacteria and Escherichia coli. Am. J. Public Health 1944; 34:735-738.
3. Feng, P. C. S., and P. A. Hartman. 1982. Fluorogenic assay for immediate confirmation of Escherichia coli. Appl. Environ. Microbiol. 43:1320–1329.
4. Moberg, L. J. 1985. Fluorogenic assay for rapid detection of Escherichia coli in food. Appl. Environ. Microbiol. 50:1383–1387.
5. Koburger, J. A., and M. L. Miller. 1985. Evaluation of a fluorogenic MPN procedure for determining Escherichia coli in oysters. J. Food Prot. 48:244–245
6. United States Environmental Protection Agency, Office of Water. 1991. Test methods for Escherichia coli in drinking water. EC medium with Mug tube procedure and Nutrient agar with Mug membrane filter procedure. U.S. Environmental Protection Agency, Washington, DC. http://nepis.epa.gov/
7. Cheeptham N, Lal A. Use of EC-MUG Media to Confirm Escherichia coli Contamination in Water. ASM Protocol 23 August 2010.
8. FDA-BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Content current as of:10/09/2020
9. APHA Standard Methods for the Examination of Water and Wastewater, 23rd ed. 2017.
10. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.'
11. Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 48:285-288
12. Kaluzewski S, D Tomczuk D. Evaluation of the Usefulness of Tests for Productionof Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
13. Gokul Yaratha, MD, Sarah Perloff, DO, Kinesh Changala, MBBS. Lactose vs non-lactose fermenting E. coli: Epidemiology, Clinical Outcomes, and Resistance. Open Forum Infect Dis 2017; V4 (Suppl 1)

PACKAGING

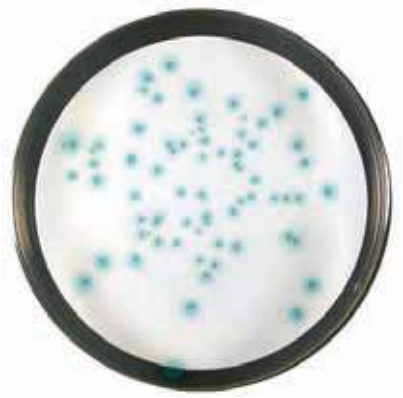
Product	Type	REF	Pack
EC Broth MUG	Dehydrated medium	4014262	500 g (13.2 L)

IFU rev 1, 2022/07

ChromArt

EC X-GLUC AGAR (CHROMOGENIC E. COLI)

Dehydrated and ready-to-use culture medium



EC X-GLUC Agar:
E.coli on a membrane filter

INTENDED USE

Chromogenic medium for the enumeration of *Escherichia coli*

COMPOSITION – TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE PLATES AND FLASKS

Tryptone	20.00 g
Yeast extract	5.00 g
Bile salts n. 3	1.50 g
Disodium hydrogen phosphate	5.00 g
Potassium dihydrogen phosphate	1.50 g
Sodium chloride	5.00 g
5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-GLUC)	0.06 g
Tryptophan	1.00 g
Agar	12.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

EC X-GLUC Agar (Chromogenic E. coli), is a selective and differential medium for the enumeration and identification of *Escherichia coli*. The medium is included in the UNICHIM Standard No. 1185[1] for the detection of *E. coli* by the MF technique in water and in the review of methods for water ISSN:1125-2464[2].

Tryptone provides nitrogen, carbon, amino acids and minerals for the microbial growth, yeast extract is a source of vitamins, particularly of group B. Sodium chloride maintains the osmotic balance while phosphates buffer the medium. Bile salts n° 3 act as a selective agent, inhibiting the growth of Gram-positive bacteria. Detection of *E. coli* is based on the ability of β-D-glucuronidase to cleave the substrate X-glucuronide with the formation of blue-green colonies.

Colonies cultured on EC X-GLUC Agar can be tested directly for indole by depositing a drop of Kovacs' reagent (REF 19171000) and observing for the red colour change of the reagent.

Natali et al.[3] evaluated EC X-GLUC Agar with microbial strains isolated from water samples and concluded that EC X-GLUC Agar gives better results than Levine EMB Agar and MacConkey Agar MUG in the detection of *E. coli*.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 51 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates and flasks appearance	beige, slightly opalescent
Final pH of complete medium (at 20-25°C)	7.0 ± 0.2

SPECIMENS

Water and food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

TEST PROCEDURE

Membrane filtration method

Filter 100 mL (or other volumes, e.g., 250 mL for bottled water) of the sample using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and, preferentially, with grid lines. The minimum volume for filtration is 10 mL of sample or dilutions thereof to ensure even distribution of the bacteria on the membrane filter.

After filtration place the membrane filter on the EC X-GLUC Agar, ensuring that no air is trapped underneath, invert petri dish, and incubate at 44 ± 0.5 °C for 21-24 h.

Pour-plate method

Pour 1 mL of the decimal dilutions of the sample into the plates. Add about 15 mL of pre-cooled EC X-GLUC Agar. Mix well the inoculum with the medium. Incubate at 44 ± 0.5 °C for 21-24 h.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Count all blue-green colonies (positive for β-D-glucuronidase) confirmed by indole test (+) as *E. coli*.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922:	44°C / 24 ore /A	growth with blue-green colonies, indole positive
E. aerogenes ATCC 13048	37°C / 24 ore /A	growth with colourless colonies, indole negative
E. faecalis ATCC 19433	37°C / 24 ore /A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia, Enterobacter* and *Yersinia* strains (1-5%).[4-6]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[6,7]
- In addition to expressing β-D-glucuronidase, *E. coli* is able to produce indole from tryptophan. Therefore, in case of any doubt of *E. coli* colonies on the primary agar medium, indole test may be used as an additional confirmation.[7]

STORAGE CONDITIONS

Dehydrated medium

Store at +2°C /+8°C away from direct light in a dry place.

Ready-to-use plates and flasks

Store in their original pack at 2-8°C away from direct light.

REFERENCES

1. Unichim n° 1185: 2000.
2. Bonadonna L. *Escherichia coli* nelle acque significato sanitario e metodologie di analisi. ISSN:1125-2464, 2001
3. Natali, P., Neri, A. Rossi, P., Ferrari, M. (1999) Biologi Italiani, n° 10/99, 20-22
4. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
5. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 1984; 48:285-288
6. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Production of Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
7. ISO 9308-1:2014 Water quality - Enumeration of Escherichia coli and coliform bacteria - Part 1: Membrane filtration method for waters with low bacterial background flora.

PACKAGING

Product	Type	REF	Pack
EC X-GLUC Agar	Dehydrated medium	4019682	500 g (9.8 L)
EC X-GLUC Agar	Ready-to-use plates	497102	3 x 10 plates ø 55 mm
EC X-GLUC Agar	Ready-to-use flasks	5119672	6 x 100 mL

IFU rev 4, 2022/08

EDWARDS AESCULIN MEDIUM

Dehydrated culture medium

INTENDED USE

A selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci associated to bovine mastitis.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	10.00 g
Peptocomplex	10.00 g
Sodium chloride	5.00 g
Aesculin	1.00 g
Thallous sulphate	0.33 g
Agar	15.00 g
Crystal violet	1.30 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Bovine mastitis is an inflammatory response of the udder tissue in the mammary gland due to physical trauma or microorganism infections.[1] It is considered the most common disease leading to economic loss in dairy industries due to reduced yield and poor quality of milk.[1,2]

Many bacterial species have been identified as causative agents for bovine mastitis and among them, streptococci and in particular *Streptococcus agalactiae* play a key role. *S. agalactiae* is a Gram-positive pathogen known to cause bovine mastitis before it was appreciated as pathogenic in humans.[3] It can be transmitted via milking machine and through oro-faecal route, particularly through contaminated drinking water.[1]

In 1933, Edwards[4] used an esculin blood agar containing crystal violet and sodium azide to isolate mastitis streptococci. A similar medium containing thallous acetate was used by McKenzie[5] to isolate the causative agent of mastitis.

In Edwards Aesculin Medium crystal violet and thallium sulphate are the selective agents that suppress the growth of a wide variety of Gram-negative and Gram-positive bacteria with the exception of streptococci. Essential factors for microbial growth are provided by beef extract and peptocomplex. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Aesculin differentiates esculin-positive organisms (group D streptococci growing with black colonies) from esculin-negative organisms (*S. agalactiae* growing with blue to colourless colonies). Supplementation with blood provides additional growth factors and differentiates streptococci on the base of haemolytic pattern.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.3 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and aseptically add 5-7% of sterile defibrinated sheep blood. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-violet, fine, homogeneous, free-flowing powder
Solution appearance	violet, limpid
Prepared plates appearance	red, opaque
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Milk samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

TEST PROCEDURE

Inoculate the centrifuged deposits of milk samples and streak with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Incubate at 35-37°C for 24-48 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
S. agalactiae grows with blue to colourless, typically beta haemolytic, colonies.
Group D streptococci grows with black to brown (aesculinase positive) non-haemolytic colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. agalactiae ATCC13813	35-37°C / 18-24h / A	growth, blue to colourless β-haemolytic colonies
E. faecalis ATCC 29212	35-37°C / 18-24h / A	growth, black to brown non-haemolytic colonies
S. aureus ATCC 25923	35-37°C / 18-24h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Wei Nee Cheng, Sung Gu Han. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. As-Austral J Anim Sci. 2020 Nov; 33(11): 1699–1713.
2. Gomes F, Henriques M. Control of bovine mastitis: old and recent therapeutic approaches. Curr Microbiol. 2016; 72:377–82.
3. Morven S. Edwards, Carol J. Baker, in Infectious Diseases of the Fetus and Newborn (Seventh Edition), 2011.
4. Edwards SJ. J. Comp. Pathol. Therap. 1933; 46:211-217.
5. McKenzie DA. Vet. Rec. 1941; 53: 473-480.

PACKAGING

Product	Type	REF	Pack
Edwards Aesculin Medium	Dehydrated medium	4014312	500 g (12.1 L)

IFU rev 1, 2022/08

ENTEROBACTERIACEAE (EE) BROTH MOSSEL

Dehydrated culture medium

INTENDED USE

For the detection and enumeration of *Enterobacteriaceae* in foods.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.000 g
Glucose	5.000 g
Disodium hydrogen phosphate	6.450 g
Potassium dihydrogen phosphate	2.000 g
Oxgall	20.000 g
Brilliant green	0.014 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Enterobacteriaceae can be injured in food-processing procedures and are usually considered by food manufacturers as hygiene indicators and thus used to monitor the effectiveness of preventive measures taken. Enterobacteriaceae Enrichment (EE) Broth Mossel is a modification of brilliant green bile lactose broth designed by Mossel, Visser and Cornelissen[1], which in turn is a modification of MacConkey's liquid medium.
The medium is prepared according to the formulation described in the withdrawn ISO 21528:2004 Standard.[2]
EE Broth Mossel can be used for the selective enrichment, detection and the enumeration with the MPN technique of *Enterobacteriaceae,* mainly when the target microorganisms are expected to be in low number and need resuscitation.
The medium contains brilliant green and bile as the inhibitory agents for Gram-positive bacteria, glucose as the main energy source and peptone which provides the essential factors for growth. Phosphates are the buffering agents to control the pH in the medium and the inhibition of growth in earlier stages of enrichment and auto sterilisation at the end.[3]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 43.5 g in 1000 mL of cold purified water. Heat to dissolve completely and dispense 100 mL portions in 250 mL flasks (or 10 mL in tubes) and autoclave at 121°C for 5 minutes. Cool rapidly in cold running tap water. If necessary, prepare the medium in double concentration by weighing 87 g/L.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	green, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

TEST PROCEDURE

General procedure

Take a sample (x g or x mL) depending on the sensitivity required and add 9x mL of Buffered Peptone Water (REF 401278) and homogenise.
Transfer the appropriate volume to a sterile container according to the required detection limit. Incubate at 37 °C for 18 h ± 2 h.
Transfer 1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of EE Broth Mossel.
Incubate the inoculated broth under aerobic conditions at 37 °C for 22 ± 2 hours.
Using a loop, streak the surface of a dish containing Violet Red Bile Glucose Agar (REF 402188) with the incubated enrichment medium and incubate the dish at 37 °C for 24 h ± 2 h hours.

Enumeration of *Enterobacteriaceae* using the MPN technique

Inoculate single and double concentration EE Broth tubes in triplicate with 1 mL and 10 mL of sample and its decimal dilutions respectively. Incubate at 37°C for 22 ± 2 hours.
Streak a loopful from each tube on Violet Red Bile Glucose Agar plates and incubate at 37°C for 24 h ± 2 hours.

Presence-absence procedure

- Inoculate 1 g or 1 mL of suitably diluted feed into a bottle containing 10 mL Tryptic Soy Broth (REF 402155) and incubate at 20-25°C for 2 hours, shaking the bottle every 15 minutes for 30 seconds.
- Add 10 mL of prepared EE Broth at double concentration and incubate at 37°C for 22 ± 2 hours.
- Mix well and observe for turbidity due to bacterial growth.
- From the growth-positive bottles, transfer a loopful onto a Violet Red Bile Glucose Agar plate and incubate at 37°C for 24 h ± 2 h hours.

READING AND INTERPRETATION

Turbidity with some change of colour towards yellowish-green provides presumptive evidence of the presence of *Enterobacteriaceae*.
Typical *Enterobacteriaceae* colonies on Violet Red Bile Glucose (VRBG) Agar are pink to red or purple (with or without precipitation haloes).

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below some test strains useful for the quality control of the medium.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 8739	37°/ 24 H-A	growth with gas
E. faecalis ATCC 19433	37°/ 24 H-A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- This medium is heat sensitive, avoid overheating.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to Baird RM *et al.* the tubed broth can be stored in screw-capped containers at +2°C / +8°C for 1 month.[3]

REFERENCES

1. Mossel DAA, Visser M, Cornelissen AMR. The Examination of Foods for Enterobacteriaceae using a Test of the Type Generally Adopted for the Detection of Salmonellae. J Appl Bacteriol 1963; 26:444.
2. ISO 21528-1:2004 - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part 1: Detection and enumeration by MPN technique with pre-enrichment.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:216-217.

PACKAGING

Product	Type	REF	Pack
EE Broth Mossel	Dehydrated medium	4014662	500 g (11.5 L)

IFU rev 4, 2022/08

ENDO AGAR

Dehydrated culture medium

INTENDED USE

For the detection and differentiation of coliforms and other of Gram-negative enteric bacteria.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.0 g
Lactose	10.0 g
Dipotassium hydrogen phosphate	3.5 g
Sodium sulphite	2.5 g
Pararosanilin (basic fuchsin)	0.4 g
Agar	11.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Endo Agar was originally devised by Endo[1] for the isolation of the typhoid bacillus. Many modifications of this medium have been done over the years. Endo Agar has been recommended as a slightly selective medium for confirmation of the presumptive test for members of the coliform group and has been an important culture medium for the microbiological examination of water and wastewater, dairy products and foods;[2-4] however, Standard Methods for the examination of these materials now recommend alternative formulations.
Endo Agar is today used for the differentiation of lactose fermenting and non-lactose fermenting intestinal organisms, particularly during confirmation of the presumptive test for coliforms and, in some areas, for the isolation and differentiation of *Enterobacteriaceae.*
Essential growth factors are provided by tryptone which is a source of nitrogen, carbon and minerals. Dipotassium phosphate is used as buffering agent to control the pH in the medium. A partial inhibition of Gram-positive bacteria is achieved without the traditional use of bile salts but with the inclusion of the combination sodium sulphite/acid fuchsin. Sodium sulphite in the medium also has the function of decolourising acid fuchsin as it occurs in Schiff's reagent. Lactose-fermenting bacteria produce acetaldehyde from lactose which releases the fuchsin from the colourless fuchsin-sulphite compound and colours the colonies red; when the reaction is rapid and very intense (e.g in the case of *E. coli*), the fuchsin crystallises out and produces a metallic sheen to the colonies. Non-lactose fermenting organisms produce colourless colonies against the pink background of the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well for resuspending the precipitate and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder with small dark particles
Solution appearance	pink-orange, opalescent with small dark particles
Prepared medium appearance	pink rose to tan rose trace orange, slightly opalescent with small dark particles
Final pH at 20-25 °C	7.5 ± 0.2

SPECIMENS

Water, wastewater, food, environmental samples. Consult the appropriate references for sample collection, storage and preparation.

TEST PROCEDURE

Streak the sample suspension or the growth obtained in a selective broth (e.g. Brilliant Green Bile Broth) on Endo Agar plate.
Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical *E. coli* colonies are pink to red with golden metallic sheen
Typical coliforms other than *E. coli* colonies are pink to red.
Typical non-lactose fermenters colonies are colourless against the pink background of the medium

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C/24H-A	good growth, pink-red colonies with metallic sheen
S. Enteritidis ATCC 13076	37°C/24H-A	good growth, colourless colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- Endo Agar is not a highly selective medium and some yeasts and some Gram-positive bacteria such as enterococci or staphylococci may grow.
- On Endo Agar, swarming of *Proteus* is not inhibited.
- Avoid exposure of the medium to light, as it may lead to photooxidation and decrease the productivity of the medium.
- Overheating of the medium must be avoided, as it may destroy the productivity of the medium.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place. On exposure to oxygen the plated Endo Agar gradually becomes red due to the oxidation of sulphite and can thus no longer be used.

REFERENCES

1. Endo S. Über ein Verfahren zum Nachweis der Typhus bacillen. Centr f Bakt 1904; 35:109-110.
2. ICMSF. Microorganisms in Foods: their significance and Methods of Enumeration, 2nd ed 1978

3. APHA. Standard Methods for the Examination of Dairy Products, 13th ed 1972
4. APHA. Standard Methods for the Examination of Water and Wastewater, 20th ed 1998

PACKAGING

Product	Type	REF	Pack
Endo Agar	Dehydrated medium	4014602	500 g (12.1 L)

IFU rev 2, 2022/08

ChromArt

ENTEROBACTER SAKAZAKII ISOLATION AGAR (ESIA)

Dehydrated and ready-to-use culture medium

ESIA: blue-green colonies: *Cronobacter sakazakii*; white colonies: *Salmonella* Enteritidis.

INTENDED USE

Chromogenic selective medium for the detection of *Cronobacter (Enterobacter) sakazakii* in foods.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Peptone	7.00 g
Yeast extract	3.00 g
Sodium chloride	5.00 g
Sodium deoxycholate	0.60 g
Crystal violet	0.002 g
X α-glucoside	0.150 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Cronobacter species (formerly known as *Enterobacter sakazakii*) are Gram-negative rod-shaped, motile pathogenic bacteria of the family *Enterobacteriaceae*. These organisms are regarded as opportunistic pathogens linked with life-threatening infections predominantly in neonates.[1] Clinical syndromes of *Cronobacter* infection include necrotizing enterocolitis (NEC), bacteraemia and meningitis, with case fatality rates ranging from 40-80%.[1,2] The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, water, rice and others.[1,3,4]
Enterobacter Sakazakii Isolation Agar (ESIA) is a selective medium for Gram-negative bacteria, containing a chromogenic compound for the differentiation of *Cronobacter sakazakii* that cultivates with blue-green colonies.
The use of Buffered Peptone Water as a non-selective enrichment, mLST Broth (401476) as a selective enrichment and the ESIA isolation medium allow the specific detection of *C. sakazakii* in food samples especially in milk powder and powdered infant formula. The ESIA medium and work procedure described below are in accordance with the withdrawn standard ISO/TS 22964:2006[4], replaced by ISO Standard 22964:2017.[5]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale violet, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale violet, clear
Final pH of complete medium (at 20-25°C)	7.0 ± 0.2

SPECIMENS

Milk powder, powdered infant formula and environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

TEST PROCEDURE

1. Prepare the initial sample suspension (primary dilution) by adding x g of the test sample to 9 times x ml of Buffered Peptone Water Casein (REF 401278C or 401278): e.g., 25 g + 225 mL of Buffered Peptone Water Casein or 10 g + 90 mL of Buffered Peptone Water Casein.
2. Incubate at 37 ± 1°C for 18 ± 2 hours.
3. After incubation of the inoculated pre-enrichment medium, transfer 0.1 ml of the obtained culture into 10 mL mLST Broth (REF 401476).
4. Incubate at 44 ± 0.5°C for 24 ± 2 hours.
5. After incubation, streak a 10 µl loopful from the mLST broth onto the surface of the ESIA plate and incubate at 44 ± 1°C for 24 ± 2 hours.

READING AND INTERPRETATION

After incubation, observe bacterial growth, recording each specific morphological and chromatic characteristic of the colonies.
Presumptive positive result for *C. sakazakii*: presence of blue to green colonies, 1 to 3 mm in diameter.
Negative result for *C. sakazakii*: absence of typical blue-green colonies or presence of mauve-violet colonies.
Confirm colonies with biochemical tests recommended by ISO 22964.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.
Productivity: *Cronobacter sakazakii* good growth, green to blue colonies
Specificity: *Enterobacter gergovieae* scanty growth, mauve colonies
Selectivity: *S. aureus*, inhibited
Incubation at 44°C for 24 hours.

LIMITATIONS OF THE METHOD

- Some coliforms grow on ESIA with violet colonies, easily distinguishable from the blue colonies of *C. sakazakii*.

STORAGE CONDITIONS

Dehydrated medium
Store at +2°C /+8°C away from direct light in a dry place
According to ISO 22964:2006, the self-prepared plates may be kept at 0 °C to 5 °C for up to 14 days.[4]
Ready-to-use plates
Store plates in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Carol Iversen et al., The taxonomy of Enterobacter sakazakii: proposal of a new genus Cronobacter gen. nov. and descriptions of Cronobacter sakazakii comb. nov. Cronobacter sakazakii subsp. sakazakii, comb. nov., Cronobacter sakazakii subsp. malonaticus subsp. nov., Cronobacter turicensis sp. nov., Cronobacter muytjensii sp. nov., Cronobacter dublinensis sp. nov. and Cronobacter genomospecies BMC Evol Biol. 2007; 7: 64.
2. Simmons BP et al. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of powdered infant formula. Infect Control Hosp Epidemiol 1989; 10: 398.
3. Van Acker J et al. Outbreack of necrotizing enterocolitis associated with *E.sakazakii* in powdered milk formula. J Clin Microbiol 2001; 39:293-297.
4. ISO/TS 22964:2006. Milk and milk products – Detection of *Enterobacter sakazakii*
5. ISO 22964:2017. Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

PACKAGING

Product	Type	REF	Pack
Enterobacter Sakazakii Isolation Agar (ESIA)	Dehydrated medium	4014782	500 g (16.2 L)
Enterobacter Sakazakii Isolation Agar (ESIA)	Ready-to-use plates	541478	2 x 10 plates ø 90 mm

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ENTEROBACTERIA ENRICHMENT BROTH MOSSEL EP

Dehydrated culture medium

INTENDED USE

For the detection and enumeration of bile-tolerant Gram-negative bacteria in pharmaceutical products.

COMPOSITION - TYPICAL FORMULA ^ (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of gelatin	10.000 g
Glucose anhydrous	4.500 g *
Bile Salts	3.055 g **
Potassium dihydrogen phosphate	2.000 g
Disodium hydrogen phosphate anhydrous	6.400 g ***
Brilliant green	0.015 g

^The formula may be adjusted and/or supplemented to meet the required performances criteria.
* Equivalent to 5.0 g di glucose monohydrate
** Equivalent to 20 g di dehydrated ox bile
*** Equivalent to 8 g di disodium hydrogen phosphate dihydrate

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Enterobacteria Enrichment Broth Mossel EP is a modification of brilliant green bile lactose broth designed by Mossel, Visser and Cornelissen[1], which in turn is a modification of MacConkey's liquid medium. Enterobacteria Enrichment Broth Mossel EP is recommended as an enrichment medium for bile-tolerant Gram-negative bacteria in the microbiological examination of pharmaceutical products. It can be used as test for absence or quantitative test.[2] The medium contains brilliant green and bile as the inhibitory agents for Gram-positive bacteria, glucose as the main energy source and pancreatic digest of gelatin which provides the essential factors for growth. Phosphates are the buffering agents to control the pH in the medium and the inhibition of growth in earlier stages of enrichment and auto sterilisation at the end.[3]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 26 g in 1000 mL of cold purified water. Mix thoroughly to completely dissolve the powder. Dispense 100 mL portions in 250 mL flasks (or 10 mL in tubes). Heat the medium at 100°C using free flowing steam for 30 minutes only. Cool rapidly in cold running tap water. Do not autoclave.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	green, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Non-sterile pharmaceutical products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

Test procedure
Prepare the sample suspension in Tryptic Soy Broth (REF 402155) using at least 1 g or 1 mL of sample.
Incubate this suspension at 20°C - 25°C for 2-5 hours to ensure revitalisation but not multiplication of bacteria.

a- Test for absence

Inoculate a quantity of the initial suspension into Enterobacteria Enrichment Broth Mossel EP to ensure an inoculum of 1 g of sample and incubate at 30°C - 35°C for 24-48 hours
Subculture on plates of VRBG Agar EP (REF 402189) and incubate 30°C -35°C for 18-24 hours.

b- Quantitative test

Inoculate suitable quantities of Enterobacteria Enrichment Broth Mossel EP with the initial suspension and/or dilution of sample containing respectively 1 g, 0.1 g, 0.01 g and 0.001 g of the product to be examined. Incubate at 30°C - 35°C for 24-48 hours.
Subculture each of the cultures on a plate of VRBG Agar EP (REF 402189) and incubate 30°C -35°C for 18-24 hours

Reading and interpretation
Turbidity in the medium with some change of colour towards yellowish-green provides presumptive evidence of the presence of bile-tolerant Gram-negative bacteria.
Typical glucose fermenters colonies on VRBG Agar EP are pink to red or purple (with or without precipitation haloes).
Typical non-glucose fermenters colonies on VRBG Agar EP are transparent and colourless.
Test for absence: according to European Pharmacopoeia the product complies with the test if there is no growth of colonies on VRBG Agar EP plates.
Quantitative test: refer to European Pharmacopoeia for the interpretation criteria of probable number of bacteria per g or mL of product.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below some test strains useful for the quality control of the medium.

Control strains	Incubation T°/ t - ATM	Expected results
E. coli ATCC 8739	30-35°/ 24 H-A	growth
P. aeruginosa ATCC 9027	30-35°/ 24 H-A	growth
S. aureus ATCC 6538	30-35°/ 48 H-A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- This medium is heat sensitive, avoid overheating.

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place.
According to Baird RM, the self-prepared tubes or bottles can be stored at +2°C +8°C in in screw-capped containers for up to four weeks.[3]

References

1. Mossel DAA, Visser M, Cornelissen AMR. The Examination of Foods for Enterobacteriaceae using a Test of the Type Generally Adopted for the Detection of Salmonellae. J Appl Bacteriol 1963; 26:444.
2. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:216-217.

Packaging
Product	Type	REF	Pack
Enterobacteria Enrichment Broth Mossel EP	Dehydrated medium	4014672	500 g (19 L)

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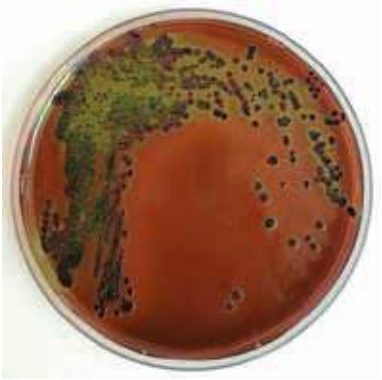
EO. ME. BLUE AGAR WITH LACTOSE AND SUCROSE

Dehydrated culture medium



Eo.Me.Blue Agar with Lactose and Sucrose: colonies of *E. coli*

Intended use
For the isolation of *Enterobacteriaceae* and for the differentiation of lactose/sucrose-fermenting microorganisms.

Composition -typical formula * (after reconstitution with 1 L of water)
Tryptone	10.000 g
Sodium chloride	5.000 g
Lactose	5.000 g
Sucrose	5.000 g
Dipotassium hydrogen phosphate	2.000 g
Eosin yellow	0.400 g
Methylene blue	0.065 g
Agar	15.000 g

* The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
Eo.Me.Blue Agar with Lactose and Sucrose is prepared on the basis of the formulation described by Holt-Harris & Teague in 1916.[1]
Compared to Levine's modified formula[2], this medium contains sucrose in addition to lactose which is fermented by certain enteric bacteria more readily than lactose. Eo.Me.Blue Agar with Lactose and Sucrose is a versatile, moderately selective medium for the isolation and differentiation of *Enterobacteriaceae* based on the fermentation of lactose and sucrose, from a variety of specimens.

The simultaneous presence of lactose and sucrose allows to differentiate lactose and sucrose-negative pathogens from lactose positive coliforms and lactose-negative, sucrose-positive flora (e.g., *Proteus vulgaris, Citrobacter, Aeromonas hydrophila*)
Peptone provides nitrogen, carbon, minerals for microbial growth; eosin yellow and methylene blue have a slight inhibitory activity towards Gram-positive microorganisms; the optimal ratio between the contents of the two dyes is required for the differentiation of lactose/sucrose-fermenting enteric bacteria from non-lactose/sucrose fermenters.
The phosphate buffer allows the differentiation between *E. coli* and *E. aerogenes*. *E. coli* causes considerable acidification of the medium even in the presence of a buffer system, whereas *E. aerogenes*, being only slightly fermenting, causes less acidification. The lowering of pH during *E. coli* growth, causes the formation of amidic bonds between the eosin and the methylene blue, which manifests as a metallic purple coloration of the colonies.

Directions for medium preparation
Suspend 42.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to about 50°C and before distributing to plates, gently shake the flask to disperse the flocculent precipitate that is formed during sterilisation. Flocculent precipitate should not be removed.

Physical characteristics
Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder
Solution appearance	violet with metallic sheen, flocculent, hazy
Prepared plates appearance	violet, limpid or slightly hazy
Final pH at 20-25 °C	7.2 ± 0.2

Specimens
Eo.Me.Blue Agar with Lactose and Sucrose is intended for the bacteriological processing a variety of specimens on which detect *Enterobacteriaceae*. Good laboratory practices for collection, transport and storage of the specimens should be applied.

Test procedure
Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate in aerobic conditions at 35-37°C for 18-24 hours.

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
E. coli colonies are 2-3 mm in diameter, slightly raised, concave, rarely convex; they are violet-cyclamen with a darker centre that extends for about 3/4 of the diameter, with greenish metallic sheen.
E. aerogenes colonies are convex with a diameter of about 4-6 mm, pink to lavender in colour, with a darker centre smaller than that observed with *E. coli*; they are normally free of greenish metallic sheen.
The colonies of sucrose fermenters such as *Proteus* spp. are violet colonies and the medium may exhibit metallic sheen.
The colonies of non-lactose/sucrose fermenters (*Salmonella, Shigella*) are transparent, amber or pink or colourless.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, violet-cyclamen colonies with a darker centre with metallic sheen
E. aerogenes ATCC 13048	35-37°C / 18-24 H / A	growth, dark pink colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 H / A	growth, colourless or whitish colonies
E. faecalis ATCC 19433	35-37°C / 18-24 H / A	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- Eo.Me.Blue Agar with Lactose and Sucrose is only moderately selective; some staphylococci, streptococci and yeasts grow exhibiting small, pinpoint colonies.[3]
- Some strains of *Salmonella* and *Shigella* will not grow on the medium.[3]
- Store prepared medium in the dark at 2-8°C; the photosensitive dyes in the medium may inhibit growth of certain bacteria, mainly *Proteus*, if stored in light.[4]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, perform the suitable tests on isolates from pure culture for complete identification.

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored in the dark at 2-8°C for 6-8 weeks.[3]

References

1. Holt-Harris JE, Teague O. A new culture medium for the isolation of Bacillus typhosus from stools. J Inf Dis 1916; 18:596-600
2. Levine M. Differentiation of B coli and B aerogenes on a simplified eosin-methylene blue agar J Inf Dis 1918; 23:43-47
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Girolami RL Stamm JM (1976) Inhibitory Effect of Light on Growth-Supporting Properties of Eosin Methylene Blue Agar. Appl Environ Microbiol 1976;31: 141–142

Packaging
Product	Type	REF	Pack
Eo.Me.Blue Agar with Lactose and Sucrose	Dehydrated medium	40145012	500 g (11.7 L)

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EP NEUTRALISING DILUENT: SEE PHARMACOPOEIA DILUENT

ETHYL VIOLET AZIDE (EVA) BROTH – LITSKY

Dehydrated and ready-to-use culture medium

Ethyl Violet Azide Broth; from left: uninoculated tube and tube with *Enterococcus faecalis*.

INTENDED USE

Selective medium for the detection of enterococci in water and other samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Tryptose	20.00 g
Sodium chloride	5.00 g
Glucose	5.00 g
Dipotassium hydrogen phosphate	2.70 g
Potassium dihydrogen phosphate	2.70 g
Sodium azide	0.40 g
Ethyl violet	0.83 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Enterococci are considered a better indicator of sewage contamination than *Escherichia coli* as they are more resistant to chlorine.
Litsky et al.[1,2] developed a selective medium containing ethyl violet and sodium azide for the specific growth of enterococci from pure cultures or from glucose azide broth that showed growth when inoculated with sewage-contaminated water. They also devised a new test for enterococci, wherein glucose azide broth was used as a presumptive medium and ethyl violet azide broth as a confirmatory medium.
EVA Broth has been proposed, in conjunction with Azide Dextrose Broth, for enumeration of enterococci by MPN technique[3-6].
A similar procedure is included in the APAT, IRSA-CNR guidelines for the detection of faecal streptococci/enterococci in water using the MPN method.[6]
Tryptose provides nitrogen, amino acids and trace elements for microbial growth; sodium azide limits the growth of Gram-negative bacteria by blocking the enzyme cytochrome oxidase, and ethyl violet inhibits Gram-positive bacilli and Gram-positive cocci except enterococci. Glucose is a fermentable carbohydrate and a source of carbon and energy; phosphates are used as buffering agents to control the pH in the medium and sodium chloride contributes to maintaining the osmotic balance.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.8 g in 1000 mL of cold purified water. Heat gently to dissolve, distribute 10 mL into tubes and sterilise by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Prepared tubes appearance	amber, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Water, food, milk, animal feeding stuffs, environmental samples in the area of food production and food handling, and other samples. Refer to applicable international standards and regulations for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

For the analysis, it is necessary to determine the volume according to the type and quality of water to be examined. For waste water or water of low quality, it is generally necessary to analysed decimal dilutions of the sample, whereas for treated water smaller dilutions and in any case different aliquots can be analysed.

1. Inoculate a series of tubes of Azide Dextrose Broth (REF 401105-551105) with appropriate graduated quantities of a 100 mL sample. Use sample volumes of 10 mL or less. The strength of the broth will be proportional to the sample size.
2. Incubate at 35-37°C for 24 ± 2 hours and observe for microbial growth (turbidity of broth); if no turbidity is observed, continue incubation for a further 24 hours.
3. Remove 1 mL of broth culture from the positive tubes and inoculate into the corresponding tubes containing Ethyl Violet Azide Broth for confirmation testing. Incubate the tubes at 35-37 °C for 24+24 (±3) hours.

READING AND INTERPRETATION

Consider tubes with turbidity accompanied by a violet-grey deposit at the bottom of the tube as positive for enterococci.
After confirmation tests, apply MPN tables for estimating the number of enterococci per volumetric unit of sample.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 19433	37°C /48H-A	good growth with violet-grey deposit at the bottom of the tube
E. faecium ATCC 19434	37°C /48H-A	good growth with violet-grey deposit at the bottom of the tube
E. coli ATCC 25922	37°C /48H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The MPN method with Azide Dextrose Broth/Ethyl Violet Azide Broth is not applicable to salt water as specified by APHA, for which the membrane filter technique is recommended.
- Since some Gram-positive bacilli and cocci other than faecal streptococci grow in Azide Dextrose Broth, a confirmation test in Ethyl Violet Azide Broth or other suitable medium is required.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place

According to MacFaddin, the prepared Ethyl Violet Azide Broth can be stored in the refrigerator for up to 6-8 weeks in screw cap tubes.[3]

Ready-to-use tubes

Store tubes in their original pack at +2°C / +8°C away from direct light.

REFERENCES

1. Litsky W, Mallmann WL, Fifield CW. A new medium for the detection of enterococci in water. Am. J Pub Health 1953; 43:873
2. Litsky W, Mallmann WL, Fifield CW. Comparison of the most probable number of Escherichia coli and enterococci in rivers waters. Am J Public Health 1955;45.1049.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
4. APHA Standard Methods for the Examination of Water and Wastewater 14th ed. Washington, DC: American Public Health Association, 1975
5. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D.C. 1976
6. WHO Examination of water for pollution control. Part III: Biological, Bacteriological and Virological Examination., ed. Oxford. Pergamon Press, World Health Organization.1982
7. APAT, IRSA-CNR Manuali e Linee Guida 29/2003 Metodi analitici per le acque. Cap 3, 7040

PACKAGING

Product	Type	REF	Pack
Ethyl Violet Azide (EVA) Broth - Litsky	Dehydrated medium	4014852	500 g (14 L)
Ethyl Violet Azide Broth	Ready-to-use tubes	551485	20 x 10 mL

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EUGON BROTH
EUGON LT SUPPLEMENT
EUGON LT SUP BROTH

Dehydrated and ready-to-use medium, supplement

INTENDED USE

For the enumeration of microorganisms in cosmetics.

COMPOSITIONS - TYPICAL FORMULAS *

EUGON BROTH DEHYDRATED MEDIUM (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	15.0 g
Soy peptone	5.0 g
Sodium chloride	4.0 g
Glucose	5.5 g
Sodium sulphite	0.2 g
L-cystine	0.7 g

EUGON LT SUPPLEMENT

SUPPLEMENT – FLASK CONTENT (100 ML)

Sodium laureth sulphate	1.56 g
Egg lecithin	1.00 g
Polysorbate 80	15.00 g
Purified water	85.00 mL

EUGON LT SUPP BROTH

READY-TO-USE FLASKS AND TUBES

Tryptone	15.00 g
Soy peptone	5.00 g
Sodium chloride	4.00 g
Glucose	5.50 g
Sodium laureth sulphate	1.56 g
Egg lecithin	1.00 g
Polysorbate 80	15.00 g
Sodium sulphite	0.20 g
L-cystine	0.70 g
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Eugon LT SUP Broth is recommended for the detection and enumeration of aerobic mesophilic bacteria, yeasts and moulds, for the neutralization of preservatives in cosmetic products and for the dispersion of the sample into the liquid medium.

Eugon LT SUP Broth complies with the requirements of the following standards: ISO 16212, ISO 17516, ISO 18415, ISO 18416, ISO 21149, ISO 21150, ISO 22717 and ISO 22718.[1-8]

Eugon LT SUP Broth is prepared with the medium base Eugon Broth supplemented with egg lecithin, sodium laureth sulphate and polysorbate 80.

Tryptone and soy peptone are sources of nitrogen, carbon and vitamins for the microbial growth. Glucose provides carbon and is source of energy. Lecithin and polysorbate 80 are included as neutralising of antimicrobial agents such as phenyl derivatives, aldehydes and quaternary ammonium salts. Sodium laureth sulphate assures a good dispersion of the cosmetics into the liquid medium.

DIRECTIONS FOR MEDIA PREPARATION

PREPARATION OF EUGON LT SUP BROTH: Suspend 30.4 g in 900 mL of cold purified water. Heat to boiling stirring constantly and add the content of one flask of Eugon LT Supplement (100 mL). Distribute in tubes or bottles and sterilize by autoclaving at 121°C for 15 minutes. After autoclaving, gently shake the medium to mix the phases that may have formed.

PREPARATION OF MEDIUM FOR GENERAL PURPOSES: Suspend 30.4 g in 1000 mL of cold purified water. Heat to boiling stirring constantly, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Appearance of liquid supplement	pale yellow, limpid
Solution and prepared medium appearance	yellow, opalescent with a light precipitate
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

The specimens consist of cosmetic products. Refer to the current Standards for sample collection and preparation. The medium is not intended for microbiological examination of clinical specimens.

TEST PROCEDURE

Allow tubes and flasks to come to room temperature.
For the detection of microorganisms by enrichment, prepare the initial suspension of the sample (generally 1:10) by adding at least 1 g or 1 mL of the well-mixed product under test into 9 mL of Eugon LT SUP Broth and, if necessary, prepare the decimal dilutions of the initial suspension using the same broth.
Incubate the initial suspension and the decimal dilutions at 32.5°C ± 2.5°C for a minimum of 20 hours and observe the presence of growth. Using a sterile loop, streak an aliquot of the incubated Eugon LT SUP Broth on the surface of a Petri dish with the specific medium for the target organism.
For the enumeration of microorganisms dilute the sample in the broth, generally by 1:10 and perform the successive dilutions in the broth, if necessary. Within 45 minutes, subculture onto the agar intended for the enumeration of the target microorganisms (Tryptic Soy Agar REF 542150 or Sabouraud Dextrose Chloramphenicol Agar REF 542006).
The user is responsible for choosing the appropriate agar media for detection and enumeration of microorganisms, the incubation temperature and time according to the intended use and to the applied ISO Standard.[1-8]

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of turbidity into the broth. For the interpretation of the results obtained on the isolation media refer to the instructions for use of the specific agar medium.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory.

LIMITATIONS OF THE METHOD

Because of the wide variety of samples examined, it is the user's responsibility to validate this medium for its specific application. The neutralization of the antimicrobial properties of the sample must be verified and validated.

STORAGE CONDITIONS

Dehydrated medium
Store at 10-30°C away from direct light in a dry place.
Supplement
Store in the original pack at 2-8°C away from direct light.
Ready-to-use tubes and flasks
Store tubes and flasks in their original pack at 2-25°C away from direct light.

REFERENCES

1. ISO 17516 - Cosmetics - Microbiology - Microbiological limits.
2. ISO 18415:2017/Amd 1:2022 Cosmetics — Microbiology — Detection of specified and non-specified microorganisms — Amendment 1
3. ISO 21149:2017/Amd 1:2022 Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria — Amendment 1
4. ISO 21150:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Escherichia coli — Amendment 1
5. ISO 22717:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa — Amendment 1
6. ISO 22718:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Staphylococcus aureus — Amendment 1
7. ISO 18416:2015/Amd 1:2022 Cosmetics — Microbiology — Detection of Candida albicans — Amendment 1
8. ISO 16212:2017/Amd 1:2022 Cosmetics — Microbiology — Enumeration of yeast and mould — Amendment 1

PACKAGING

Product	Type	REF	Pack
Eugon Broth	Dehydrated medium	4016432 4016434	500 g (16.4 L) 5 kg (164 L)
Eugon LT Supplement	Liquid supplement	421540	6 x 100 mL
Eugon LT SUP Broth	Ready-to-use tubes	551583	20 x 9 mL glass tubes, 17x125 mm
Eugon LT SUP Broth	Ready-to-use flasks	5115832	6 x 90 mL

IFU rev 1, 2021/07

FERMENTATION BROTH BASE
FERMENTATION BROTH RHAMNOSE
FERMENTATION BROTH XYLOSE
Dehydrated and ready-to-use culture medium

INTENDED USE
Supplemented with carbohydrates, Fermentation Broth Base is used for the determination of fermentation reactions of microorganisms.

COMPOSITION - TYPICAL FORMULAS *
FERMENTATION BROTH BASE (DEHYDRATED MEDIUM)
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.0 g
Beef extract	1.0 g
Sodium chloride	5.0 g
Bromocresol purple	0.02 g

FERMENTATION BROTH RHAMNOSE (READY-TO-USE TUBES)

Peptone	10.0 g
Beef extract	1.0 g
Sodium chloride	5.0 g
Bromocresol purple	0.02 g
L-rhamnose	5.00 g
Purified water	1000 mL

FERMENTATION BROTH XYLOSE (READY-TO-USE TUBES)

Peptone	10.0 g
Beef extract	1.0 g
Sodium chloride	5.0 g
Bromocresol purple	0.02 g
D-xylose	5.00 g
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Carbohydrate fermentation tests detect the ability of microorganisms to ferment a specific carbohydrate. Fermentation patterns can be used to differentiate among bacterial groups or species.
Fermentation Broth Base is formulated as recommended by ISO 11290[1,2] (Carbohydrate Utilization Broth) and by FDA-BAM[3] (Purple Carbohydrate Fermentation Broth Base).
ISO 11290 recommends the medium supplemented with L-rhamnose and D-xylose in the confirmation procedure of *Listeria monocytogenes*.
FDA-BAM recommends the medium supplemented with dulcitol, lactose, sucrose in the confirmation procedure of *Salmonella*[4] and supplemented with dextrose, esculin, maltose, rhamnose, mannitol, and xylose in the confirmation procedure of *Listeria monocytogenes*[5].
The basal medium contains a peptone with a low carbohydrates content and beef extract which are sources of nitrogen, carbon and minerals for bacterial growth. Sodium chloride maintains the osmotic balance. Bromocresol purple is a pH indicator: when Fermentation Broth Base is prepared with a supplemented carbohydrate, most of the end products of its fermentation are organic acids, which produce a colour change of the pH indicator from purple to yellow; if the test is negative, a catabolic attack of peptones will occur with the formation of ammonia, the alkalinisation of the medium and a colour change of indicator to darker purple. A Durham tube can be inserted into the test tube to record gas production: if gas is produced during the fermentation reaction, it is collected in the inverted Durham tube.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 16 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Dispense the medium into tubes of suitable capacity to obtain portions appropriate for the test. Sterilize for 15 min in the autoclave at 121 °C.
Prepare a filter sterilized 5% solution of the suitable carbohydrate (e.g., 5 g of L-rhamnose or D-xylose in 100 mL of purified water).
For each carbohydrate, add aseptically x mL of carbohydrate solution to 9x mL of the Fermentation Broth Base (e.g. 2.7 mL of Fermentation Broth Base + 0.3 mL of carbohydrate solution or 4.5 mL of Fermentation Broth Base + 0.5 mL of carbohydrate solution).
Dispense the medium with Durham fermentation tubes, if gas formation is to be recorded.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	pale violet, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS
Pure culture of bacterial strains under examination.

TEST PROCEDURE
Using a loop, aseptically inoculate each tube with a pure culture of the strain under examination obtained from a non-selective agar (e.g Tryptic Soy Yeast Extract Agar REF 402166).
Swirl the tube gently to mix contents and incubate at 37°C for 24 h to 48 h.
Prolonged incubation may be required to be considered a negative result.[6]
Inoculate and incubate also a tube without the addition of carbohydrates (control tube).

READING AND INTERPRETATION
Positive reactions (acid formation) are indicated by a yellow colour which occur mostly within 24 h to 48 h for microvolumes tubes, and up to 5 days for macro volumes tubes.

Positive reaction (carbohydrate degradation): the medium is turbid, turns yellow and the formation of gas bubbles can be observed, if Durham tubes are present.
Negative reaction: the medium is turbid and remains purple or changes to deep purple.
No yellow colour should appear in the control tube.
After a positive reaction has been observed, discard the tube; by prolonging the incubation, an inversion of the reaction may be observed.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below the test strains useful for the quality control of the medium supplemented with rhamnose.

Control strains	Incubation T°/ t / ATM	Expected results
L. monocytogenes ATCC 13932	37°/ 24-48 H / A	the medium turns to yellow
L. ivanovii ATCC 19119	37°/ 24-48 H / A	the medium doesn't turn to yellow

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection.

Limitations of the method

- There exist rare strains of *L. monocytogenes* which do not ferment L-rhamnose.[1]
- Carbohydrate fermentation is one of the tests used to identify pure bacterial cultures. For complete identification, other suitable tests must be carried out.

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use medium in tubes
Store tubes in their original pack at +2°C / +8°C away from direct light.

References

1. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method
2. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.
3. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, M130.
4. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 5: Salmonella, March 2022.
5. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods, April 2022.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

Packaging

Product	Type	REF	Pack
Fermentation Broth Base	Dehydrated medium	4014882	500 g (31.2 L)
Fermentation Broth Rhamnose	Ready-to-use tubes	521488R	24 x 3 mL
Fermentation Broth Xylose	Ready-to-use tubes	521488X	24 x 3 mL

IFU rev 1, 2023/01

FRASER BROTH BASE
FRASER SELECTIVE SUPPLEMENT
FRASER HALF SELECTIVE SUPPLEMENT

Dehydrated culture medium and selective supplements

Intended use

With the addition of selective supplements, Fraser Broth Base is used for primary and secondary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain (ISO 11290-1) and for sample preparation in the enumeration procedure (ISO 11290-2).

Composition *

Fraser Broth Base, dehydrated medium
typical formula after reconstitution with 1 L of water

Enzymatic digest of animal tissue	5.00 g
Enzymatic digest of casein	5.00 g
Meat extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Disodium hydrogen phosphate dihydrate°	12.00 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Lithium chloride	3.00 g

Fraser Half Selective Supplement
(Vial contents for 225 mL of medium)

Ferric ammonium citrate	112.50 mg
Nalidixic acid	2.25 mg
Acriflavine HCl	2.81 mg

Fraser Selective Supplement
(Vial contents for 500 mL of medium)

Ferric ammonium citrate	250.0 mg
Nalidixic acid	10.0 mg
Acriflavine HCl	12.5 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.
° Equivalent to 9.6 g of disodium hydrogen phosphate anhydrous

Principle of the method and explanation of the procedure

Although improved control measures since the 1990s have significantly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meat and meat products, it remains a significant cause of foodborne illness.[1]
Identification traditionally involves culture methods based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.[2]

ISO,[3,4] FDA,[5] USDA-FSIS[6] protocols differ in the recommended culture media but they all involve one or more enrichment steps followed by plating into one or two selective isolation media.
Fraser Broth was developed by Judy A. Fraser and William H. Sperberby[7] by a modification of the USDA secondary enrichment broth through the addition of lithium chloride and ferric ammonium citrate. The efficacy of Fraser Broth was documented by testing a wide range of food and environmental samples from food processing facilities.
Fraser Broth Base contains all the basic ingredients with the exception of ferric ammonium citrate, acriflavine and nalidixic acid which are contained in selective supplements that enable the two complete media Half-Fraser Broth and Fraser Broth to be prepared.
Half-Fraser Broth and Fraser Broth are used for primary and secondary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain according to ISO 11290-1.[3] Half-Fraser Broth may be used for sample preparation in the enumeration procedure according to ISO 11290-2.[4]
Peptones and yeast extract provide nitrogen, carbon, vitamins particularly of the B-group and trace elements for microbial growth; phosphates are used as buffering agents to control the pH in the medium. Selectivity is provided by the presence of nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine which inhibits many Gram-positive bacteria; lithium chloride and the high salt (NaCl) tolerance of *Listeria* are used to inhibit growth of enterococci. Half-Fraser Broth contains half the concentrations of acriflavine and nalidixic acid compared to Fraser Broth. Esculin is hydrolysed to glucose and aesculetin (6-7-dihydroxycoumarin): aesculetin reacts with the iron salts in the medium, giving it a brown-black colour. Since all *Listeria* spp. hydrolyse esculin, cultures which do not blacken can be considered to be *Listeria*-free.[7]

DIRECTIONS FOR MEDIA PREPARATION

HALF-FRASER BROTH

Suspend 12.91 g of Fraser Broth Base in 225 mL of cold purified water. Heat to boiling to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Cool to room temperature add the contents of one vial of Fraser Half Selective Supplement (code 4240044) reconstituted with 3 mL of ethanol/ sterile purified water (1:1).

FRASER BROTH

Suspend 28.7 g of Fraser Broth Base in 500 mL of cold purified water. Heat to boiling to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Cool to room temperature add the contents of one vial of Fraser Selective Supplement (code 4240043) reconstituted with 5 mL of ethanol/ sterile purified water (1:1). Mix well and pour into sterile tubes or flasks under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes and flasks appearance	yellow-brown, limpid
Freeze-dried selective supplements	low, fragile yellow ochre tablets; yellow ochre opalescent solutions after reconstitution
Final pH of complete media (at 20-25°C)	7.2 ± 0.2

SPECIMENS

Foods, animal deeding stuffs, food chain and environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[3,4]

TEST PROCEDURE

Detection of *Listeria monocytogenes* and *Listeria* spp. (ISO 11290-1)[3]

1. In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 mL of Half-Fraser Broth, to obtain a tenfold dilution, and homogenize.
2. Incubate the primary enrichment medium at 30 °C ± 1°C for 25 h ± 1 h.
3. Transfer 0.1 mL of the culture to a tube or bottle containing 10 mL of secondary enrichment medium (Fraser Broth) and incubate for 24 h ± 2 h at 37 °C ± 1°C. In the case of *Listeria* spp. other than *Listeria monocytogenes* detection, additional 24 h incubation can allow for recovery of more species.
4. From the primary enrichment culture inoculate, by means of a loop, the surface of the first selective plating medium, Agar Listeria according to Ottaviani and Agosti (ALOA) (REF 401605), to obtain well-separated colonies. Proceed in the same way with the second selective plating-out medium of choice (e.g., PALCAM or Oxford Agar, REF 401604 or 401600).
5. From the secondary enrichment medium, repeat the procedure with the two selective plating-out media.
6. Incubate ALOA plates at 37°C ± 1°C for 24 ± 2 hours; if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
7. Incubate the second plating out medium according to the instructions for use
8. Examine the dishes for the presence of presumptive colonies of *L. monocytogenes* or *Listeria* spp.

Notes
It is possible to store at 5 °C the pre-enriched sample after incubation before transfer to Fraser broth for a maximum of 72 h.
Half-Fraser broth and Fraser broth can be refrigerated at 5 °C before isolation on selective agar for a maximum of 72.
After incubation, ALOA plates can be refrigerated at 5 °C for a maximum of 48 h before reading.

Enumeration of *Listeria monocytogenes* and of *Listeria* spp. (ISO 11290-2)[4]

1. Prepare a sample suspension in Buffered Peptone Water or other suitable enrichment broth according to ISO 6887 (all parts); in case both detection and enumeration are performed according to parts 1 and 2 of ISO 11290, the sample suspension may be made in half-Fraser broth (with or without the addition of the selective supplement).
2. Inoculate 0.1 mL of the sample suspension and 0.1 mL of further decimal dilutions onto 90 mm plates of ALOA medium.
3. For samples with suspected low number of target-strains, inoculate 1 mL of the sample suspension and 1 mL of further decimal dilutions onto 140 mm plates of ALOA medium.
4. Examine after incubation at 37°C for 24 ± 2 hours and, if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
5. Count *L. monocytogenes* colonies and *Listeria* spp. colonies in plates with less than 150 colonies (90 mm diameter plates) or 360 colonies (140 mm plates) according to the section "reading and interpretation".

READING AND INTERPRETATION

After incubation, typically *Listeria* spp. produce a blackening of the two enrichment broths.
After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
With ALOA plates, consider as presumptive *L. monocytogenes* the blue-green colonies surrounded by an opaque halo; consider as presumptive *Listeria* spp. the blue-green colonies with or without opaque halo.
Second plating-out medium: examine for the presence of typical colonies according to the characteristics of the chosen medium.
Confirm typical colonies by the methods and tests indicated in ISO 11290-1 or ISO 11290-2, after purification of the colonies in Tryptic Soy Yeast Extract Agar (REF 402166).

The mandatory confirmatory tests for *L. monocytogenes*, according to ISO 11290 and using ALOA medium, are the following: β-haemolysis (+), carbohydrate utilization (L-rhamnose +; D-xylose -). Optional confirmatory tests for *L. monocytogenes* are: catalase (+), mobility at 25°C (+), CAMP test (+). The mandatory confirmatory tests for *Listeria* spp. are: microscopic examination, catalase (+); optional tests are: VP (+), mobility at 25°C (+).
Miniaturized galleries for the biochemical identification of *Listeria monocytogenes* may be used (Listeria Mono Confirm Test REF 193000)

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

Control strains		Incubation T°/ t / ATM	Expected results
L. monocytogenes +	ATCC 13932	30 or 37°C / 24h A	> 10 typical colonies after subculture on ALOA
E. faecalis +	ATCC 29212		
E. coli	ATCC 25922		
L. monocytogenes +	NCTC 7973	30 or 37°C / 24h A	> 10 typical colonies after subculture on ALOA
E. faecalis +	ATCC 29212		
E. coli	ATCC 25922		
E. faecalis	ATCC 29212	30 or 37°C / 24h A	< 100 colonies after subculture on TSA
E. coli	ATCC 25922	30 or 37°C / 24h A	totally inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Culture: 30°C for Half-Fraser Broth, 37°C for Fraser Broth

Limitations of the method

- Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.
- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried selective supplements
Store the product in the original package at +2°C / +8°C away from direct light.

References

1. Buchanana RL *et al*. A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments Food Control Volume 75, May 2017, Pages 1-13
2. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. FEMS Microbiol Rev. 2005 Nov;29(5):851-75
3. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
4. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.
5. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods, April 2022.
6. USDA-FSIS. Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat, Siluriformes (Fish) and Egg Products, and Environmental Samples. MLG 8.13, 10/01/2021
7. Fraser JA, Sperber WH. Rapid Detection of Listeria spp. in Food and Environmental Samples by Esculin Hydrolysis. J Food Prot 1988 Oct;51(10):762-765.

Packaging

Product	Type	REF	Pack
Fraser Broth Base	Dehydrated medium	4014952 4014954	500 g (8.7 L) 5 kg (87 L)
Fraser Selective Supplement	Freeze-dried supplement	4240043	10 vials, each for 500 mL of medium
Fraser Half Selective Supplement	Freeze-dried supplement	4240044	10 vials, each for 225 mL of medium

IFU rev 3, 2022/07

Fraser Broth: uninoculated tube on the left and tube inoculated with *L. monocytogenes* on the right

GARDNERELLA SELECTIVE AGAR

Ready-to-use plates

Gardnerella Selective Agar: *Gardnerella vaginalis*

INTENDED USE

In vitro diagnostic device. Selective medium with defibrinated sheep blood for the isolation of *Gardnerella vaginalis* from vaginal discharge.

COMPOSITION - TYPICAL FORMULA *

Peptocomplex	10 g
Tryptose	10 g
Peptone	3 g
Maize starch	1 g
Sodium Chloride	5 g
Agar	12 g
Defibrinated sheep blood	50 mL
Gentamicin sulphate	4 mg
Nalidixic acid	30 mg
Amphotericin B	2 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

G. vaginalis is a facultative anaerobic, non-motile, pleomorphic, gram-negative or gram-variable bacillus, oxidase and catalase negative.
A normal vaginal flora is characterized by the presence of only *Lactobacillus* species or with the presence of small numbers of *G. vaginalis* morphotypes; the shift in vaginal flora associated with bacterial vaginosis is characterised by a decrease in numbers of lactobacilli which are replaced by a mixed flora of aerobic, anaerobic and microaerophilic species, including *G. vaginalis*.[1]
Diagnosis of bacterial vaginosis is based on the Amsel criteria, which is considered 90% accurate with three or four of the following findings: 1-thin, white, yellow homogeneous vaginal discharge, 2-amine (fishy) odour when potassium hydroxide solution is added to vaginal secretions (commonly called the "whiff test"), 3-presence of clue cells (greater than 20%) on microscopy, 4-vaginal pH greater than 4.5.[2]
Although not recommended for routine laboratory procedures, the isolation of *G. vaginalis* can support the diagnosis of bacterial vaginosis.[3]
Gardnerella Selective Agar is based on the use of selective compounds proposed by Ison[4] and the observation that the colony sizes on Columbia agar base with 5% sheep blood are optimal[5].
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, maize starch is included to absorb toxic by-products contained in the specimen, sheep blood enhances the growth of *G. vaginalis,* though it is not useful for the differentiation of *G. vaginalis* colonies that are β-haemolytic only with human and rabbit blood. Gentamicin is inhibitory for Gram-negative and Gram-positive organisms other than *G vaginalis*[4], nalidixic acid is inhibitory for Gram-negative bacteria, amphotericin B is an antifungal agent.

PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25°C	7.3 ± 0.2

SPECIMENS

Gardnerella Selective Agar can be directly inoculated with vaginal swab. It is best to take one swab for direct examination and to take another for culture.[3] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[6]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate by rolling the swab over a small area of the surface at the edge and streak with a sterile loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Incubate at 35–37°C in 5–10% carbon dioxide for 44-48 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
G. vaginalis colonies are white, small (<0,5 mm in diameter), non β-haemolytic.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
G. vaginalis	ATCC	14018	35-37°C / 44-48H / CO_2	good growth, non β- haemolytic colonies
C. albicans	ATCC	10231	35-37°C / 44-48H / CO_2	inhibited
E. faecalis	ATCC	29212	35-37°C / 44-48H / CO_2	inhibited

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since the medium doesn't contain human or rabbit blood, the presumptive identification of *G. vaginalis* colonies by β-haemolysis evidence is not possible.
- The presence of *G. vaginalis* in a vaginal specimen does not necessarily indicate that the isolated organism is the cause of an infection.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Public Health England. Investigation of Genital Tract and Associated Specimens. UK Standards for Microbiology Investigations. 2017, B 28 Issue 4.6.
2. Colonna C, Steelman M. Amsel Criteria. *In*Statpearls. StatPearls Publishing; Last update, July 1, 2019
3. Funke G, Bernard KA. Coryneform Gram-Positive Cocci. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.474
4. Ison CA. Dawson SG, Hilton J, Csonka GW, Easmon CSF. Comparison of culture and microscopy in the diagnosis of Gardnerella vaginalis infection. J. Clin. Pathol 1982; 35:550
5. Catlin BV. Gardnerella vaginalis: characteristics, clinical considerations, and controversies. ClinMicrobiol Rev 1992; 5:213
6. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.

PACKAGING
Product	Type	REF	Pack
Gardnerella Selective Agar	Ready-to-use plates	549993	2 x 10 plates ø 90 mm

IFU rev1, 2020/11

GASSNER MEDIUM
Dehydrated culture medium

INTENDED USE
For the detection and isolation of pathogenic *Enterobacteriaceae* in foodstuffs and other materials.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Peptone	14.00 g
Sodium chloride	5.00 g
Lactose	43.000 g
Aniline Blue	0.62 g
Metachrome yellow	1.25 g
Agar	12.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Gassner Medium is based on the formulation proposed by Gassner[1] for the detection and isolation of pathogenic *Enterobacteriaceae* in foodstuffs and other materials. This medium is also known as Water-blue Metachrome-Yellow Lactose Agar.
Peptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Methachrome yellow inhibits the accompanying Gram-positive microbial flora. Lactose, at high concentration, is the fermentable carbohydrate and a source of carbon and energy. The colour of the medium is green and when lactose is fermented by coliforms, an acidic environment is created that induces a colour change of the pH indicator aniline blue from green to deep blue; at alkaline pH levels the yellow colour of methachrome yellow becomes increasingly evident. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 76 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes.
Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	blue-green, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS
Foodstuffs and other materials of sanitary interest. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
The method followed will depend upon the sample under test.
Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate in aerobic conditions at 35-37°C for 18-24 hours.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies
Coliforms grow on the medium with blue colonies surrounded by a deep-blue halo.
Lactose non-fermenting *Enterobacteriaceae* grow with yellow or pale green colonies surrounded by a halo of the same colour.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37°/ 18-24 H-A	good growth, blue colonies with blue halo
S. Typhimurium ATCC 14028	35-37°/ 18-24 H-A	good growth, yellow colonies with yellow halo
E. faecalis ATCC 19433	35-37°/ 18-24 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Gassner G. Ein neuer Dreifarbennährboden zur Tyhus-Ruhr-Diagnose. Centralbl f Bakt I Orig 1918; 80:219-222.

PACKAGING

Product	Type	REF	Pack
Gassner Medium	Dehydrated medium	4015012	500 g (6.57 L)

IFU rev 1, 2022/08

GC MEDIUM BASE
BIOVITEX, VCN, VCNT
Dehydrated culture medium and supplements

N. gonorrhoeae: on Modified Thayer Martin Medium

VCN ANTIMICROBIC SUPPLEMENT
VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	1.50 mg
Colistin	3.75 mg
Nystatin	6250 IU

VCNT ANTIMICROBIC SUPPLEMENT
VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	1.50 mg
Colistin	3.75 mg
Nystatin	6250 IU
Trimethoprim	2.50 mg

INTENDED USE

In vitro diagnostics. General purpose medium, used with various enrichments and selective supplements, for the isolation and cultivation of *Neisseria gonorrhoeae*, *Haemophilus* spp. and other fastidious microorganisms, from clinical specimens.

COMPOSITION*

GC MEDIUM BASE
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Peptocomplex	15 g
Starch	1 g
Dipotassium hydrogen phosphate	4 g
Potassium dihydrogen phosphate	1 g
Sodium chloride	5 g
Agar	12 g

BIOVITEX/RESTORING FLUID
VIAL CONTENTS FOR 500 mL OF MEDIUM

Diphosphopyridine nucleotide	1.250 mg
Cocarboxylase	0.500 mg
p-Aminobenzoic acid	0.065 mg
Thiamine HCl	0.015 mg
Vitamin B_{12}	0.050 mg
L-glutamine	50.000 mg
L-cystine	5.500 mg
L-cysteine HCl	129.500 mg
Adenine	5.000 mg
Guanine HCl	0.150 mg
Ferric nitrate	0.100 mg
Glucose	500.000 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In 1945, Johnston[1] described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 hours. This medium was later modified by Carpenter and Morton[2] using GC Medium Base with the addition of haemoglobin and a yeast concentrate (chocolate agar). The medium was further improved by replacing yeast concentrate with a chemically defined supplement, formulated specifically to facilitate the growth of gonococci.[3] In 1964 Thayer and Martin[4] proposed a selective medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*, incorporating haemoglobin, yeast supplement B, polymyxin B and ristocetin into GC Agar. Thayer and Martin improved in 1966[5] the formulation substituting the original antibiotics with vancomycin, colistin and nystatin (VCN). In 1970 Martin and Lester[6] modified the new Thayer Martin Medium by increasing agar and glucose content and by incorporating an additional antibiotic, trimethoprim lactate; this improved medium is called Modified Thayer-Martin (MTM) medium. Martin and Lewis[7] in 1977 further improved the selectivity of MTM by increasing the vancomycin concentration from 3.0 µg/mL to 4.0 µg/mL to achieve greater inhibition of gram-positive bacteria and replacing nystatin with anisomycin (VCA/ VCAT) to achieve greater inhibition of yeasts; this medium is known as Martin-Lewis Agar.
In 1969 Hovig and Aandahl[8] formulated a selective medium for the isolation of *Haemophilus* spp. from respiratory tract, incorporating bacitracin into chocolate agar. In 1973 Chapin and Doern[9] described a chocolatized medium with bacitracin, vancomycin and clindamycin for the selective recovery of *H. influenzae* from specimens contaminated with upper respiratory tract microbial flora.
GC Medium Base is therefore the basal medium of choice to be supplemented with enrichments and selective compounds for the isolation and cultivation of *Neisseria* spp., *Haemophilus* spp. and other fastidious pathogenic microorganisms from clinical specimens.

Peptocomplex provides carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, dibasic and monobasic potassium phosphates buffer prevent pH changes due to amine production, corn starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth.[10]
With GC Medium Base it is possible to prepare a variety of enriched and selective media including: chocolate agar enriched, Thayer Martin medium and Modified Thayer Martin medium; the formulas of the mentioned media are summarized in the table below.
Chocolate agar enriched (CAE) is intended for cultivation and isolation of fastidious microorganisms such as *Haemophilus* spp. and *Neisseria spp.* from a variety of sterile and non-sterile clinical specimens. For normally non-sterile human sites it is advised to use chocolate agar enriched together with a selective medium. Heated horse blood provides hemin (X factor) required for growth of *Haemophilus* and enhancing growth of *Neisseria*. The medium is supplemented with Biovitex that supplies V factor (NAD) for *Haemophilus* growth and vitamins, amino acids, coenzymes, dextrose, ferric ions and other factors which improve the growth of pathogenic *Neisseria*.
Thayer Martin Medium (TM) and Modified Thayer Martin Medium (MTM) are selective and enriched media intended for the isolation of *Neisseria gonorrhoeae* from non-sterile human sites contaminated by mixed flora of bacteria and/or fungi. Vancomycin inhibits Gram positive bacterial contamination, nystatin is an anti-fungal agent, colistin inhibits Gram negative microbial flora and almost all saprophytic *Neisseria* spp., trimethoprim suppresses *Proteus* swarming.

DIRECTIONS FOR MEDIA PREPARATION

Chocolate agar enriched (haemoglobin)
Prepare a double strength GC Medium Base by suspending 19 g of in 250 mL of purified water. Mix thoroughly, heat with frequent agitation and boil for about 1 min.
Prepare a 2% haemoglobin solution by dissolving 5 g of haemoglobin powder in 250 mL of warm purified water.
Autoclave separately the GC Medium Base and haemoglobin solution at 121 °C for 15 min.
Cool the autoclaved solutions to approximately 47-50 °C.
To 250 mL of cooled double strength GC Medium Base, aseptically add 250 mL of haemoglobin solution and the contents of one vial of Biovitex reconstituted with 5 mL of Restoring Fluid (REF 424009). Mix gently but thoroughly and distribute into sterile Petri dishes or tubes, or other sterile containers.
Chocolate agar enriched (cooked blood)
Suspend 19 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and aseptically add 5-10% of the defibrinated horse blood and heat to 80°C for 15 minutes with agitation. Cool to 47-50°C and add 5 mL of Biovitex reconstituted as described above. Mix gently but thoroughly and distribute into sterile Petri dishes or tubes, or other sterile containers.
Selective media for Neisseria (TM and MTM)
Suspend 19 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and aseptically add 5-10% of the defibrinated sheep blood and heat to 80°C for 15 minutes with agitation. Cool to 47-50°C and add 5 mL of reconstituted Biovitex as described above and the contents of one vial of VCN Antimicrobic Supplement (cat. No. 4240007), reconstituted with 5 mL of sterile purified water (Thayer-Martin medium) or the contents of one vial of VCNT Antimicrobic Supplement (cat. No. 4240008) reconstituted with 5 mL of sterile purified water (modified Thayer-Martin's medium). Instead of heated sheep blood, GC Medium Base can be supplemented with a sterile solution of bovine haemoglobin: 5 g of haemoglobin in 250 mL of water, sterilized in an autoclave + 250 mL of double strength GC Medium Base, autoclaved.
Mix gently but thoroughly and distribute into sterile Petri dishes or tubes, or other sterile containers.

PHYSICAL CHARACTERISTICS

GC Medium Base, dehydrated medium	
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	beige, limpid
Prepared plates appearance	brown, opaque
Final pH at 20-25 °C	7.2 ± 0.2
VCN Antimicrobic Supplement	
Appearance of lyophilised product	short, dense, yellow pastille
Appearance of reconstituted product	yellow, turbid solution
VCN Antimicrobic Supplement	
Appearance of lyophilised product	short, dense, yellow pastille
Appearance of reconstituted product	yellow, turbid solution
Biovitex / Restoring Fluid	
Appearance of lyophilised Biovitex	short, close, pink pastille
Appearance of Restoring Fluid	colourless, limpid solution
Appearance of reconstituted Biovitex	pale pink, limpid solution

SPECIMENS

Chocolate agar enriched: plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens' types, related to specific infections.[11-13] Chocolate agar enriched is not suitable for direct inoculation of blood samples.
Thayer Martin (TM) and Modified Thayer Martin (MTM) Media: plates can be directly inoculated with specimens from non-sterile human sites contaminated by mixed flora of bacteria and/or fungi (e.g. urogenital tract, upper respiratory tract, pus and exudates).[11,13-14] This medium is not useful for the isolation of *Neisseria* spp. from supposedly sterile sites.[10]

TEST PROCEDURE

Allow plates to come to room temperature. The agar surface should be smooth and moist, but without excessive water. Process the specimen as soon as possible after it is received in the laboratory to avoid loss of gonococci viability and overgrowth of contaminants.
Roll the swab over one quadrant of the surface then streak the specimen over the other quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Alternatively, since swabs for gonococcal culture may contain only small numbers of organisms, roll swab directly on the medium in a large "Z" pattern to sufficiently transfer the specimen; cross-streak the "Z" pattern with a sterile loop.
Chocolate agar enriched: incubate at 35-37°C in aerobic conditions with 5 -10% CO_2, and record the results after 18-24 and 48 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols. Consult the procedures outlined in the references for further information.[12,15]
Thayer Martin and Modified Thayer Martin media: incubate at 35-36.5°C in a moist atmosphere supplemented with 3-7% CO_2; cultures should be examined daily for growth and held for a maximum of 72 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic characteristics of the colonies.

Chocolate agar enriched

Colonies of *Haemophilus influenzae* have a diameter of about 1-2 mm, are colourless, transparent, moist and tend to be translucent, with a characteristic "mousy" odour.

Colonies of *N. gonorrhoeae* are of variable diameter (0,5 - 2 mm), moderately convex, raised, finely granular, glistening, with entire or lobate margins.

For other fastidious microorganisms, refer to appropriate references and procedures for results reading and interpretation.[12,15]

Thayer Martin and Modified Thayer Martin media

N. gonorrhoeae colonies are variable in size, usually small (0,5-2 mm), moderately convex, raised, granular, glistening, moist, with entire to lobate margins, usually greyish-white to translucent; almost all strains become mucoid after 48 hours.

A Gram staining must be performed on suspected *Neisseria* colonies to confirm the presence of uniform Gram-negative diplococci. Performance of oxidase test is mandatory for colonies suspected to belong to *Neisseria* that shall be positive for *N. gonorrhoeae*.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

TEST STRAINS			INCUBATION (T°/ t / ATM)	EXPECTED RESULTS
Chocolate agar enriched				
H. influenzae	ATCC	10221	35-37°C / 18-24H / A or CO_2	good growth
N. gonorrhoeae	ATCC	43069	35-37°C / 18-24H / CO_2	good growth
Thayer Martin and Modified Thayer Martin Media				
N. gonorrhoeae	ATCC	43069	35-37°C / 24-48H / CO_2	good growth
P. mirabilis	ATCC	43071	35-37°C / 24-48H / CO_2	inhibited
E. coli	ATCC	25922	35-37°C / 24-48H / CO_2	inhibited
N. sicca	ATCC	9913	35-37°C / 24-48H / CO_2	growth partially inhibited
S. epidermidis	ATCC	12228	35-37°C / 24-48H / CO_2	inhibited
C. albicans	ATCC	60193	35-37°C / 24-48H / CO_2	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

GC Medium Base used for the preparation of chocolate agar enriched

- The growth on chocolate agar enriched depends on the metabolic requirements of each microorganism; it is possible that some strains are unable to grow on the medium.
- Depending on the specimens analysed and the microorganisms being tested for, it is recommended to use also additional selective media such as Thayer Martin for the isolation of gonococcus and Haemophilus selective agar for the isolation of *H. influenzae*.
- For the growth of *N. gonorrhoeae,* it is necessary that the surface of the plates is moist; if it appears dry, humidify with a few drops of sterile purified water. Place damp gauze or paper towels in the CO_2 container before incubation or use an incubator with humidifier.[10]
- The gonococci are one of the most fragile Gram-negative bacteria. It is recommended that any suspected *Neisseria* containing specimen should be inoculated onto primary isolation medium immediately on collection to avoid any loss in viability and/or overgrowth of contaminants; if this is not possible *N. gonorrhoeae* swabs are better held at 4-6° C for not more than 3 hours.[10]
- If *N. gonorrhoeae* is suspected the incubator temperature should be set at 35-36,5°C with 5% CO_2, because many strains of *N. gonorrhoeae* will not grow well at 37°C and grow poorly with 10% CO_2.[10,17]
- The number and type of fastidious species present in the specimens as infectious agents is very high. Therefore, before the chocolate agar enriched is routinely used for rarely isolated or recently described microorganisms, its suitability must be verified by the user.
- The presence of colonies on chocolate agar enriched is not an indication, by itself, of the presence of pathogenic microorganisms: user must differentiate potential pathogens requiring biochemical, immunological, molecular, or mass spectrometry testing for identification and antimicrobial testing from contaminants that represent member of normal microbiota.

GC Medium Base used for the preparation of Thayer Martin and Modified Thayer Martin media

- Vancomycin sensitive strains of some auxotypes of *N. gonorrhoeae* which fail to grow on MTM, have been reported from 3% to 10% of the total isolates.[18,19] Some gonococci are susceptible to trimethoprim too.[20]
- It is recommended that both a selective and a non-selective medium be used when isolating pathogenic *Neisseria* in order to avoid the loss of vancomycin and/or trimethoprim sensitive strains.[10]
- TM and MTM are not useful for the isolation of *Neisseria* spp. from supposedly sterile sites as cerebrospinal fluid, conjunctival swab, skin biopsy, joint fluid for which non-selective media are recommended.[10]
- For the growth of *N. gonorrhoeae,* it is necessary that the surface of the plates is moist; if it appears dry, humidify with a few drops of sterile purified water. Place damp gauze or paper towels in the CO_2 container before incubation or use an incubator with humidifier.[10]
- On TM and MTM *N. gonorrhoeae* grows with smaller and more granular colonies than with non-selective chocolate agar.
- Some saprophytic non-target microorganisms, resistant to antimicrobials present in the media may grow. *N. lactamica* may grow on TM and MTM with colonies smaller and less moist than gonococci, occasionally with a yellowish tint.[10]
- The gonococci are one of the most fragile Gram-negative bacteria. It is recommended that any suspected *Neisseria* containing specimen should be inoculated onto primary isolation medium immediately on collection to avoid any loss in viability and/or overgrowth of contaminants; if this is not possible *N. gonorrhoeae* swabs are better held at 4-6° C for not more than 3 hours.[10]
- The incubator temperature should be set at 35-36,5°C[17] because many strains of *N. gonorrhoeae* will not grow well at 37°C. [10,17]
- Examine plates after 24 hours incubation. At 48 hours the Gram morphology may exhibit atypical forms.
- Many standard protocols[10,13,14,16] describe the use of Thayer Martin and Modified Thayer Martin media for the detection of meningococcal carriage in oropharyngeal and nasopharyngeal swabs. This application is out of Biolife GC Medium Base intended use. The end user should validate this application before routinely using those selective media for *N. meningitidis* detection in clinical specimens.

All media prepared with GC Medium Base

- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N. gonorrhoeae*.[10]
- Incorrect specimen collection, incubation temperature, CO_2 level, humidity and pH can adversely affect growth and viability of the microorganisms.
- Inactivation or deterioration of antibiotics into selective media can allow the growth of contaminants.
- It is recommended to measure the pH of complete media. GC Medium Base has sufficient buffering capability however sometimes it could be necessary to adjust the final pH.

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Selective and enrichment supplements
Store the products in the original package at +2°C/ +8°C away from direct light.

REFERENCES

1. Johnston. J. J Vener Dis Infor 1945; *26*:239.
2. 2 Carpenter, C.M., and H.E. Morton. 1947. An improved medium for isolation of the gonococcus in 24 hours. Proc. N.Y. State Assoc. Public Health Labs. *27*:58-60.
3. *3 Martin, J.E., Jr., T.E. Billings, J.F. Hackney, and J.D. Thayer. 1967. Public Health Rep. 82:361.*
4. Thayer JD, E. Martin Jr E. A selective medium for cultivation of N. gonorrhoeae and N. meningitidis. Pub. Health Rep. 1964; 79:49.
5. Thayer JD, E. Martin Jr E. Improved medium selective for cultivation of N. gonorrhoeae and N. meningitidis. Pub. Health Rep. 1966; 81:559-562.
6. Martin JE Jr, Lester A. Transgrow, a maedium for transport and growth of N. gonorrhoeae and N. Meningitides. HSMHA Helth Service Rep. 1971: 86:30
7. Martin and Lewis. 1977, Public Health Rep. 35:53.
8. Hovig B, Aandahl EH. A selective method for the isolation of Haemophilus in material from the respiratory tract. Acta Pathol Microb Scand 1969; 77:676-84
9. Chapin KC, Doern GV. Selective Media for Recovery of Haemophilus influenzae From Specimens Contaminated With Upper Respiratory Tract Microbial Flora.J Clin Microbiol 1983;17(6):1163-5.
10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
11. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
12. Vandepitte J, Verhaegen J, P. Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd edition Geneve: World Health Organization Geneva; 2003.
13. Public Health England: Standards for microbiology investigations (UK SMI)- Bacteriology : UK SMI B2:2017, UK SMI B9:2015, UK SMI B14:2016; UK SMI B28:2017; B51:2014
14. Elias J, Frosh M, Vogel U. Neisseria. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.635.
15. Ledeboer NA, Doern GV. Haemophilus. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.667.
16. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019.
17. CDC: Morbidity and Mortality Weekly Report (MMWR).Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae. Infections Recommendations and Reports. October 18, 2002 / Vol. 51 / No. RR-15
18. Talbot V. et al. Vancomycin sensitive penicillinase producing Neisseria gonorrhoea. Br.J Ven Dis. 1983; 59:277
19. Mirret S, Reller B, Knapp JS. Neisseria gonorrhoeae Strains inhibited by vancomycin in selective media and correlation with auxotype.J Clin Microbiol 1981; 14: 94
20. Lai-King Ng, Martin IE.The laboratory diagnosis of Neisseria gonorrhoeae Can J Infect Dis Med Microbiol. 2005; 16(1): 15–25.

PACKAGING

Product	Type	REF	Pack
GC Medium Base	Dehydrated medium	4015202	500 g (13,1 L)
Biovitex-Restoring Fluid	Enrichment supplement	4240009	5 + 5 vials, each for 500 mL of medium
Biovitex-Restoring Fluid	Enrichment supplement	42185011	1 vial of Biovitex+1 vial of 50 mL Restoring Fluid for 5000 mL of medium.
VCN Antimicrobic Supplement	Selective supplement	4240007	10 vials, each for 500 mL of medium
VCNT Antimicrobic Supplement	Selective supplement	4240008	10 vials, each for 500 mL of medium

IFU rev 2, 2022/04

GELATIN PEPTONE BIOS AGAR

Dehydrated culture medium

INTENDED USE

For the enumeration of microorganisms in ice-cream, ice-cream related products, fresh pasta.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Gelatin peptone	5 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Gelatin Peptone Bios Agar is free of fermentable carbohydrates, has low nutritive characteristics and is particularly useful for growing non-fastidious organisms. Gelatin Peptone Bios Agar may be used for the total microbial count of ice-cream and ice-cream related products[1] and fresh pasta.[2] The medium contains a gelatin peptone which provide the essential growth factors for microbial growth, substantially reducing the growth of microorganisms related to production process, in particular lactobacilli, thus favouring the growth of spoiling microorganisms. Agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 20 g in 1000 ml of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes.
Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	nearly colourless, limpid
Final pH at 20-25 °C	7.0 ± 0.1

SPECIMENS
Ice cream, ice cream related products, fresh pasta. Refer to applicable regulations for the collection of food samples.[1,2] Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE
1. Prepare the initial suspension of the sample and the decimal dilutions with the suitable diluent.
2. Transfer by means of sterile pipettes 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of Gelatin Peptone Bios Agar, cooled to approximately 45°C, into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.
5. Incubate at 32°C ± 1°C for 48h ± 3h (ice cream)[1] or at 30°C for 72 hours (fresh pasta)[2].

READING AND INTERPRETATION
Enumerate the number of colonies per plate and calculate the microbial count. Do not count pin-point colonies.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 25922	32°C/ 48 H-A	good growth
L. delbrueckii subsp. *bulgaricus* DSM 20081	32°C/ 48 H-A	very poor growth, pin-point colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- The isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. O.M. 11/10178: Limiti di cariche microbiche tollerabili in determinate sostanze alimentari e bevande. G.U. Supp. ord. n. 346 del 13/12178.
2. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti. Raccolta a cura di D.De Medici, L.Fenicia, L.Orefice e A.Stacchini.

PACKAGING
Product	Type	REF	Pack
Gelatin Peptone Bios Agar	Dehydrated medium	4015102	500 g (25 L)

IFU rev 1, 2022/08

GIOLITTI CANTONI STAPHYLOCOCCI BROTH
Dehydrated culture medium

INTENDED USE
Selective enrichment broth for detection and enumeration of coagulase-positive staphylococci in foodstuffs

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Tryptone	10.0 g
Beef extract	5.0 g
Yeast extract	5.0 g
Lithium chloride	5.0 g
D-Mannitol	20.0 g
Sodium chloride	5.0 g
Glycine	1.2 g
Sodium pyruvate	3.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Staphylococcus aureus has been identified as the causative agent in many foods poisoning outbreaks. Processed foods may contain relatively small numbers of debilitated viable cells, whose presence must be demonstrated by appropriate methods.
Giolitti Cantoni Staphylococci Broth is based on the formulation devised by the Italian microbiologists Giovanni Giolitti and Carlo Cantoni for the recovery of low numbers of stressed *S. aureus* in foods[1,2] and applied by Mossel[3] for the detection of *S. aureus* in dried milk and infant foods.
It is recommended by ISO 6888-3[4] for the detection or enumeration with MPN technique of coagulase positive staphylococci in foodstuffs, where staphylococci are expected to be stressed and in low numbers. Compared to the classic formula of Giolitti and Cantoni, the medium proposed by ISO 6888-3 additionally contains sorbitan mono-oleate.
Essential growth factors are provided by tryptone, beef extract and yeast extract. D-mannitol is the carbohydrate source. Sodium pyruvate and sorbitan mono-oleate aid in resuscitation of stressed cells. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Gram-negative bacteria are inhibited by lithium chloride while Gram positive contaminants are inhibited by the combination of glycine and potassium tellurite added to the medium base. The anaerobic conditions created by paraffin oil layered on the tubes restrict growth of micrococci. Tellurite is reduced by *S. aureus* and related species to tellurium giving the medium a black colour.[5]

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 54.2 g (or 108.4 g in the case of double strength medium) in 1000 mL of cold purified water. If required, add 1 mL (2 mL in the case of double strength medium) of sorbitan mono-oleate (Tween 80 REF 42120502). Warm slightly to completely dissolve the powder. Dispense the

single strength medium in quantities of 9 mL into tubes of suitable dimensions (e.g. 16 mm × 160 mm) and 10 mL of double-strength medium in 20 mm x 200 mm tubes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 44 °C to 47 °C and aseptically add to each tube of single strength medium 0.1 mL of a filter sterilised Potassium Tellurite 1% solution (REF42211501) and 0.2 mL to a double-strength medium.
After inoculation, cover the surface of the tubed broth with a 30 mm layer of sterile plain agar or paraffin oil.
If the medium base is stored before the addition of potassium tellurite solution, shortly before use heat it for 15 min at 100 °C to expel air.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	6.9 ± 0.2

SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[4]

TEST PROCEDURE

1. Following the procedure for enumeration and detection by MPN given by ISO 6888-3[4], inoculate 9 mL of single-strength Giolitti Cantoni Staphylococci Broth with 1 g or 1 mL of the initial suspension or 10 mL of double-strength broth with 10 mL or 10 g sample. For larger volumes of test portions, add the test portion of x g or x mL to 9x g or 9x mL of single-strength broth, previously de-aerated and with potassium tellurite added, and have a minimum air volume in the flask or container.
2. Proceed as specified above for each of the subsequent dilutions.
3. Carefully mix the inoculum and medium, in each case avoiding the introduction of air.
4. Carefully pour a plug of agar, cooled to between 44 °C to 47 ºC, onto the top of the medium in each inoculated tube and allow it to solidify to form a seal. 30 mm layer of sterile paraffin oil may be used instead of agar solution.
5. Incubate the inoculated tubes of at 37 °C ± 1 ºC for 24 h ± 2 h. Subculture any tubes showing any blackening or black precipitate.
6. Incubate the remainder of the inoculated tubes for a further 24 h ± 2 h and subculture all tubes (i.e. those that do or do not develop a black precipitate after 48 h ± 2 h).
7. For subculturing, aseptically remove the plug of agar or paraffin by using a sterile spatula.
8. With a sterile loop, spread a loop full of each selected broth onto the surface of separate plates of Baird Parker Agar (REF 541116) or Baird Parker RPF Agar (REF 543101) to obtain isolated colonies. Invert the prepared dishes and incubate at 37 °C ± 1 ºC for 24 h ± 2 h and 48 h ± 2 h.

READING AND INTERPRETATION

Formation of a black precipitate or the blackening of the broth suggests the presence of coagulase positive staphylococci.
For the reading and interpretation of plating-out media and for confirmation tests consult the instructions for use of Baird Parker Agar and Baird Parker RPF Agar and follow the procedure given by ISO 6888-3.[4]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538 P	37°C/48 H/A	growth, blackening of the broth
E. coli ATCC 25922	37°C/48 H/A	no growth after subculture in Tryptic Soy Agar

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- A lot of literature has shown that exist false negative and also false positive results of tellurite reduction test (blackening of the medium) and therefore all tubes have to be confirmed by streaking on selective agar media.[4]
- Coagulase enzyme is primarily produced by *S. aureus* but also *Staphylococcus intermedius* and some strains of *Staphylococcus hyicus* are positive to coagulase test.[4]

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to Curtis GDW *et al*. the prepared medium base may be stored 2 weeks at +4°C, while the complete medium should be used the same day of preparation.[5]

REFERENCES

1. Giolitti G, Cantoni C. Att Soc Sci Vet 1965;19: 509
2. Giolitti G, Cantoni C. A medium for the isolation of staphylococci from foodstuffs. J Appl Bacteriol 1966 Aug; 29(2):395-8
3. Mossel DAA, Harrewijn GA, Elzebroek JM. 1973 UNICEF
4. ISO 6888-3:2003. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coagulase positive staphylococci (Staphylococcus aureus and other species) – part 3: MPN technique for low number.
5. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II) Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:247-8.

PACKAGING

Product	Type	REF	Pack
Giolitti Cantoni Staphylococci Broth	Dehydrated medium	4015162	500 g (9.2 L)

IFU rev 1, 2022/08

GLUCOSE OF MEDIUM

Dehydrated and ready-to-use culture medium

Glucose OF Medium - on the left, a test tube inoculated with *E. coli*, on the right, a non-inoculated tube.

INTENDED USE

Semi-solid medium for the confirmation test of *Enterobacteriaceae*.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Enzymatic digest of casein	2.00 g
Dipotassium hydrogen phosphate	0.30 g
Sodium chloride	5.00 g
Glucose	10.00 g
Bromothymol blue	0.08 g
Agar	3.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Glucose OF Medium is based on the formulation described by Hugh and Leifson[1] and meets the requirements of ISO 21528[2,3]. The fermentation of glucose, together with oxidase test, is recommended by ISO 21528 for the confirmation of *Enterobacteriaceae* colonies isolated on Violet Red Bile Glucose Agar.

The medium contains glucose as fermentable carbohydrate and bromothymol blue as a pH indicator: the high concentration of acid produced during fermentation will turn the bromothymol blue indicator from green to yellow in the presence or absence of oxygen. The persistence, after incubation, of a green colour or the appearance of a blue colour, due to an alkaline transformation of the medium, indicates that there was no degradation of glucose. Glucose OF Medium is a semi-solid medium: the presence of agar at a concentration of 0.3% enables the determination of motility in addition to fermentation test and also aids in preventing the distribution of any acid produced towards the surface of the medium, with a consequent dilution. Dipotassium phosphate promotes carbohydrate fermentation and acts as a pH control buffer; enzymatic digest of casein provides carbon, nitrogen and trace elements for microbial growth; sodium chloride maintains the osmotic balance.

DIRECTIONS FOR MEDIUM PREPARATION

Dissolve 20.4 g in 1000 mL of cold purified water. Heat to boiling to completely dissolve the medium, dispense 10 mL into tubes and sterilize by autoclaving at 121° C for 15 minutes. Allow the tubes to solidify vertically.

Before use, if necessary, heat the medium in boiling water or steam for 15 minutes to remove the oxygen present and then cool rapidly to the incubation temperature.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	green, clear
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Pure culture of bacterial strains under examination.

TEST PROCEDURE

Colonies of presumptive *Enterobacteriaceae* isolated on Violet Red Bile Glucose are sub cultured on a non-selective medium (Nutrient Agar) and then confirmed by means of oxidase test and the fermentation of glucose.

Using a wire, stab the colonies that gave a negative oxidase test into tubes containing Glucose OF medium.

Overlay the surface of the medium with minimal 1 cm of sterile mineral oil. Incubate the tubes at 37 ± 1°C for 24 h ± 2 h.

READING AND INTERPRETATION

Positive reaction (acid formation) is indicated by a yellow colour throughout the content of the tube.

Negative reaction: the medium remains green or changes to blue.

If the colonies are oxidase-negative and glucose-positive, the sample shall be regarded as being positive for *Enterobacteriaceae*.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below some test strains useful for the quality control of the medium overlayed with paraffin oil.

CONTROL STRAINS	INCUBATION T°/ T –ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C x 24 h-A	growth with colour change to yellow
P. aeruginosa ATCC 27852	37°C x 24 h-A	growth with absence of colour change or yellow only at the top of the tube.

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Carbohydrate fermentation is one of the tests used to identify pure bacterial cultures. For complete identification, other suitable tests must be carried out.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to ISO 21528 the prepared tubes may be stored for up to 4 weeks at 2-8 °C.[2,3]

Ready-to-use medium in tubes

Store tubes in their original pack at +2°C / +8°C away from direct light.

REFERENCES

1. Hugh R, Leifson E. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J Bacteriol 1953; 66:24-26
2. ISO 21528-1:2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae —Part 1: Detection of Enterobacteriaceae
3. ISO 21528-2:2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count technique

PACKAGING

Product	Type	REF	Pack
Glucose OF Medium	Dehydrated medium	4015252	500 g (24.5 L)
Glucose OF Medium	Ready-to-use tubes	551525	20 x 10 mL

IFU rev 1, 2022/08

GN BROTH HAJNA

Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. Selective enrichment medium for the isolation and cultivation of Gram-negative enteric pathogenic bacteria (*Salmonella* and *Shigella*) from clinical samples and other materials.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Tryptose	20.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	4.0 g
Potassium dihydrogen phosphate	1.5 g
Sodium citrate	5.0 g
Sodium deoxycholate	0.5 g
Mannitol	2.0 g
Dextrose	1.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

GN (Gram Negative) Broth is prepared according to the formulation devised by Hajna in 1955.[1] The medium is used for the enrichment of Gram-negative enteric pathogenic bacteria (*Salmonella* and *Shigella*), in samples of clinical, industrial and environmental origin.[2]

Carbohydrates are balanced with an excess of mannitol over glucose to favour growth of mannitol-fermenting *Salmonella* and *Shigella* over *Proteus* and *Pseudomonas* during the first 6 hours of incubation.[2] The phosphate buffers prevent over-acidification of medium by acidic metabolic production. The selective compounds are sodium citrate and sodium deoxycholate: the medium inhibits all Gram-positive bacteria, particularly enterococci, normal intestinal flora, the coliforms during the first 6 hours of incubation, aerobic and anaerobic spore-forming bacilli.

Croft and Miller[3] and Taylor and Schelhart[4] reported that the enrichment of stool cultures, compared to direct inoculation of plates, increases the sensitivity of isolation of *Salmonella* and *Shigella*, as these infections may be caused by low numbers of bacteria. In another study, Taylor and Schelhart[5] showed that GN Broth was superior to selenite enrichment media for the isolation of *Shigella*. GN Broth is also recommended for use in the microbiological examination of foods[6] and water[7].

For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised, followed by subculture on two different selective media: XLD Agar and a second less selective medium (e.g., Mac Conkey Agar).[8]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 39 g in 1000 mL of cold purified water. Heat to dissolve, distribute 10 mL into tubes and sterilise by autoclaving at 121 °C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, limpid
Final pH at 20-25°C	7.0 ± 0.2

SPECIMENS

GN Broth Hajna may be inoculated with human clinical specimens such as faeces or rectal swab. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied. For food and environmental samples refer to the quoted references.[6,7]

TEST PROCEDURE

For stool testing, inoculate the tubes with 1 g of faeces or 1 mL of faecal suspension obtained by suspending 1 g of faeces in 1 mL of saline. Rectal swabs can be inserted directly into the broth.

Incubate the tube with loosened caps at 35 ± 2°C for 6-8 hours, but if microbial growth is observed already at the 6th hour, subculture to selective and differential plating media such as Mac Conkey Agar, XLD Agar, Hektoen Enteric Agar. Subculture again after 18-24 hours of incubation.

Consult appropriate references for information about processing and inoculation of other clinical specimens[8,9] or food samples[6,7].

READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by turbidity of the broth. Subculture by streaking a loopful of broth on selective enteric plating media. The plating media should be chosen as a combination of greater and lesser inhibitory selective agars.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[10]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028	33-37°/ 18-24H / A	good growth after subculture on Mac Conkey Agar
S. flexneri ATCC 12022	33-37°/ 18-24H / A	good growth after subculture on Mac Conkey Agar
E. coli ATCC 25922	33-37°/ 6-8 H / A	partial or complete inhibition after subculture on Mac Conkey Agar

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since heavy growth of some saprophytes (non-pathogens) may exhibit growth on extended incubation, 6-8 hours is the recommended time period for initial subculturing.[2]
- GN Broth Hajna is not the optimal growth medium for *Shigella dysenteriae*.
- Enteric pathogens isolation techniques should include a variety of enrichment broths and isolation media.
- After the enrichment in GN Broth Hajna, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Ready-to-use medium in tubes

Store tubes in their original pack at +2°C / +8°C away from direct light.

REFERENCES

1. Hajna AA. A new enrichment broth medium for gram-negative organisms of the intestinal group. Public Health Lab 1955; 13:83-89.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Croft CC, Miller MJ. Isolation of *Shigella* from rectal swabs with Hajna "GN" broth. Am J Clin Pathol 1956; 26:411-417.
4. Taylor WI, Schelhart D. Isolation of shigellae. V. Comparison of enrichment broths with stools. App Microbiol 1968; 16:1383-1386
5. Taylor WI, Schelhart D. Isolation of shigellae. IV. Comparison of plating media with stools. Amer J Clin Pathol 1967; 48:356-362.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Bonadonna L, Ottaviani M. Rapporti ISTISAN 07/5 Metodi analitici di riferimento per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. Metodi microbiologici. ISS, 2007.
8. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. *Escherichia, Shigella* and *Salmonella*. *In* Jorgensen JH, Pfaller MA et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015
9. Buchan BW et al. *Escherichia*, *Shigella* and *Salmonella*. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

PACKAGING

Product	Type	REF	Pack
GN Broth Hajna	Dehydrated culture medium	4015242	500 g (12.8)
GN Broth Hajna	Ready-to-use tubes	551524	20 x 10 mL glass tubes

IFU rev 1, 2022/03

GN Broth Hajna; from left: un-inoculated tube, growth of *Shigella flexneri*

HAEMOPHILUS TEST MEDIUM

Ready-to-use plates

Haemophilus Test Medium:
AST of *H. influenzae*

INTENDED USE

In vitro diagnostic device. Culture medium for Antimicrobial Susceptibility Testing (AST) by disk diffusion method of clinical isolates of *Haemophilus* species, according to the Clinical and Laboratory Standards Institute (CLSI).

COMPOSITION - TYPICAL FORMULA *

Beef extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Yeast Extract	5.0 g
NAD	15.0 mg
Bovine haematin	15.0 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The development of bacterial resistance to antimicrobials in the first half of the twentieth century resulted in the need for physicians to request the microbiology lab to test a patient's pathogen against various concentrations of a given antimicrobial to determine susceptibility or resistance to that drug.[1] The culture medium proposed for Kirby-Bauer method was Mueller Hinton Agar, originally developed by Howard Mueller and Jane Hilton in 1941 for the isolation of gonococcus and meningoccus.[2]
Currently, the Clinical Laboratory Standards Institute (CLSI) for USA and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Europe are responsible for updating and modifying the original procedure through a global consensus process.[3,4] Interpretative guidelines for inhibition zone sizes are included in their publications.[3,5]
Mueller Hinton Agar supplemented with yeast extract, NAD and haematin (Haemophilus Test Medium-HTM), as growth-promoting additives, has been proposed by Jorgensen *et al*.[6] in 1987 and is currently recommended by CLSI[3] for testing *Haemophilus* spp. CLSI document M100[4] reports the zone diameter breakpoints and interpretative categories for *H. influenzae* and *H. parainfluenzae*; testing and reporting recommendations for other species of *Haemophilus* are included in the CLSI document M45.[7] Disk diffusion tests performed with HTM allows accurate categorization of susceptible and resistant strains and is easier to interpret than tests performed with Mueller-Hinton chocolate agar.[6]

PHYSICAL CHARACTERISTICS

Medium appearance	beige-amber, limpid
Final pH at 20-25 °C	7.3 ± 0.1

SPECIMENS

AST by disk diffusion method is designed to be used with pure cultures of strains isolated from clinical specimens. Haemophilus Test Medium is not intended for microbial isolation directly from clinical specimens.

TEST PROCEDURE

- The surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. Do not over-dry plates.
- Prepare the inoculum using colonies from an overnight (20 to 24 hours) culture on a chocolate agar plate. Suspend the colonies in saline and mix to an even turbidity. Adjust the density of the organism suspension to 0.5 McFarland by adding saline or more bacteria. Colonies grown on sheep blood agar may be used for inoculum preparation. The 0.5 McFarland suspensions contain approximately 1 to 4 x 10^8 CFU/mL. Use care in preparing this suspension because higher inoculum concentrations may lead in false-resistant results with some β-lactam antimicrobial agents, particularly when β-lactamase producing strains of *H. influenzae* are tested.
- Dip a sterile cotton swab into the suspension. Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
- Allow disks to reach room temperature before opening cartridges or containers used for disk storage.
- Apply disks firmly to the surface of the inoculated agar plate within 15 minutes of inoculation. Disks must be in close and even contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from disks is very rapid.
- The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. Test a maximum of 9 disks on a 140 mm plates and 4 disks on a 90 mm plate.
- Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition.
- Incubate at 35 ± 2°C, 5% CO_2 for 16-18 hours.

READING AND INTERPRETATION

Measure the diameter of zones of complete inhibition as judged by the unaided eye, including the diameter of the disk. Hold the plates a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.
With trimethoprim and sulphonamides, antagonists in the medium allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.
Interpret zone diameters into susceptibility categories according to the current breakpoint tables reported by CLSI M100[3] for *H. influenzae* and *H. parainfluenzae* and by CLSI M45 for other species of *Haemopilus*.[7]

USER QUALITY CONTROL

All manufactured lots of Haemophilus Test Medium plates are released for sale after the Quality Control has been performed to check the compliance with the specifications, according to CLSI rules[3]. However, the end user can perform its own Quality Control in accordance with the

local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Select the quality control strain specified by CLSI (*H. influenzae* ATCC 49247) to monitor the performance of the test.
For the details about the suggested QC frequency, the choice of antibiotics and the acceptability ranges, consult the CLSI document.[3]

LIMITATIONS OF THE METHOD

- With trimethoprim and sulphonamides, antagonists in the medium my allow some slight growth; therefore, read the end point at the concentration in which there is ≥80% reduction in growth compared with the control.[3]
- For isolates of *H. influenzae* from CSF, only results of testing with ampicillin, any of 3rd-generation cephalosporins listed below, chloramphenicol and meropenem are appropriate to report.[3]
- Amoxicillin-clavulanate, azithromycin, cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime and clarithromycin are used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. The results of susceptibility tests with these antimicrobial agents are often not necessary for management of individual patients.[3]
- EUCAST has evaluated the disk potency of 16 strategically important antibiotic disks from nine manufacturers of disks for antimicrobial susceptibility testing. The study disclosed some good and some poor quality among disks and manufacturers. It is the responsibility of laboratories to perform quality control to guarantee that the material used performs to the standards of the laboratory and the health care system.[8]
- Incorrect inoculum concentration, improper storage of antimicrobial discs, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.[9] Therefore, strict adherence to protocol is required to ensure reliable results.
- Consult the CLSI papers for the details of disc diffusion methodology, reading and interpretations of inhibition zones, warnings, guidance documents in susceptibility testing, guidelines for detection of resistance mechanisms, clinical breakpoints.
- Informational supplements to CLSI document M100, or revised versions, are periodically published, containing revised tables of antimicrobial discs and interpretative standards. The latest tables should be consulted for current recommendations.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology (ASM), December 8, 2009.
2. Bauer AW, Perry DM, Kirby WM. Single disk antibiotic sensitivity testing of staphylococci. Analysis of technique and results. Arch Intern Med 1959; 104:208
3. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.
4. The European Committee on Antimicrobial Susceptibility Testing. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 8.0 (January 2020). http://www.eucast.org.
5. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. http://www.eucast.org.
6. Jorgensen JH, Redding JS, Maher LA, Howell AW. Improved medium for antimicrobial susceptibility testing of Haemophilus influenza. J Clin Microbiol 1987; 25:2105- 2113.
7. Clinical and Laboratory Standards Institute (CLSI). Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2016.
8. Åhman J, Matuschek E, Kahlmeter G. The quality of antimicrobial discs from nine manufacturers, EUCAST evaluations in 2014 and 2017. Clinical Microbiology and Infection 2019; 25:346-352
9. Matushek E. EUCAST Educational Workshop. Technical problems and controversies in antimicrobial susceptibility testing. ECCMID 2017, Vienna, Austria.

PACKAGING

Product	Type	REF	Pack
Haemophilus Test Medium	Ready-to-use plates	549901	2 x 10 plates ø 90 mm

IFU rev 1, 2020/10

HEART INFUSION BROTH

Dehydrated culture medium

INTENDED USE

General purpose liquid medium for the cultivation of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria and fungi from a variety of specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef heart infusion solids	10.0 g
Tryptose	10.0 g
Sodium chloride	5.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Heart Infusion Broth is a general-purpose nutrient medium for the cultivation of a large variety of microorganisms, including nutritional fastidious, such as streptococci, meningococci and pneumococci.
Several modifications of heart infusion media have been described.[1] Supplemented with 6.5% sodium chloride, the medium may be used for differentiation of enterococci from streptococci.[2] By adding 0.1-0.2% of agar to the Heart Infusion Broth, its viscosity is increased, thus promoting the growth of anaerobes in the lower layers of the medium. The addition of carbohydrates or other ingredients results in media used for a variety of purposes.
Heart Infusion Broth can be used for the mass cultivation of microorganisms, and is thus suitable for the preparation of vaccines.
Beef heart infusion and tryptose provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 25 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS
Heart Infusion Broth can be used for the sub-culture of colonies grown on primary isolation media. It can also be inoculated with a variety of samples following the procedures described in the literature. Good laboratory practices for collection, transport and storage of specimens should be applied.

TEST PROCEDURE
With a bacteriological needle or loop inoculate the liquid medium in a test tube or bottle with a colony grown on a plating medium or with one or two drops of the specimen, if liquid, using a sterile pipette. Swab specimens may be inserted into broth after inoculation of plated media. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION
The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of growth is closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24H / A	good growth
E. coli ATCC 25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

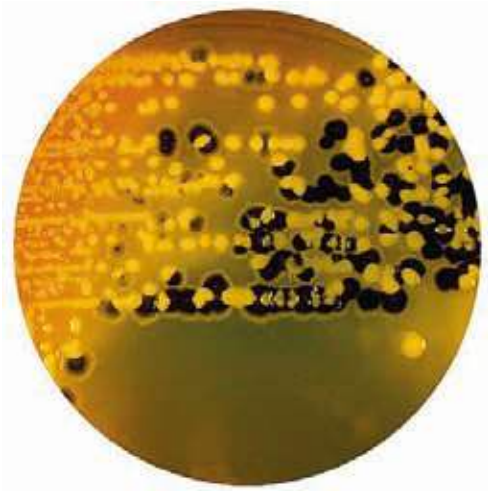
REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985

PACKAGING
Product	Type	REF	Pack
Heart Infusion Broth	Dehydrated medium	4015402 4015404	500 g (20) 5 kg (200 L)

IFU rev 1, 2022/08

HEKTOEN ENTERIC AGAR
Dehydrated and ready-to-use culture medium



HEA: *Salmonella* colonies with large black centre and yellow-orange *K. pneumoniae* colonies.

INTENDED USE
In vitro diagnostics. Selective and differential medium for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella,* from clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE PLATES AND FLASKS

Tryptose	12.000 g
Yeast Extract	3.000 g
Bile salts n° 3	9.000 g
Lactose	12.000 g
Sucrose	12.000 g
Salicin	2.000 g
Sodium chloride	5.000 g
Sodium thiosulphate	5.000 g
Fe-ammonium citrate	1.500 g
Bromothymol blue	0.065 g
Acid fuchsin	0.100 g
Agar	15.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In the first half of the twentieth century, several culture media were developed and proposed for the isolation of enteric pathogens from faeces and other materials. Some of them were moderately selective and allowed the growth of faecal contaminants, others showed excessive toxicity for the growth of pathogens, especially of *Shigella*.[1]

Sylvia King and William I. Metzger, working at the Hektoen Institute in Chicago, formulated HE agar in 1968[2] with the goal to increase the recovery of *Shigella* species from mixed cultures. They enriched SS Agar formulation, evaluated in 1941 by Catherine Mayfield and Maud Gober[3], with extra amounts of carbohydrates and peptones to offset the inhibitory effects of the bile salts. The two dyes added to the medium, bromothymol blue and acid fuchsin, have lower toxicity than other dyes, thus pathogens recovery was improved.[4]

Hektoen Enteric Agar is a selective and differential medium intended for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella* from clinical and non-clinical specimens.[5] Hektoen Enteric Agar is recommended by ISO 21567[6] as plating medium for the detection of *Shigella* and by FDA-BAM[7] for detection of *Salmonella,* in food.

Animal peptone and yeast extract provide carbon, nitrogen, vitamins and trace elements for bacterial growth; the high concentration of bile salts n°3 and dyes inhibits Gram-positive organisms and most of the non-pathogenic coliform flora of the intestinal tract. Since the enteric pathogens *Salmonella* and *Shigella* can tolerate these inhibitory substances, they generally grow faster and larger than the coliforms.[1] Lactose, sucrose and salicin are fermented by coliforms, that are able to grow in the presence of the bile salts, and by some *Proteus* species with production of acids. The acid condition causes the bromothymol blue indicator to change from its neutral green colour to an orange-yellow colour and to bile salts to precipitate appearing as a hazy zone around the colonies. Ferric ammonium citrate is an indicator of the formation of hydrogen sulphide. *Salmonella* spp. produce thiosulphate reductase that causes the release of a sulphide molecule from the sodium thiosulfate present in the medium. This sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric ammonium citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 76.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to 47-50°C mix well and pour into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	dark green, limpid or slightly opalescent
Final pH at 20-25 °C	7.5 ± 0.2

SPECIMENS

Hektoen Enteric Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swab[8,9] and non-clinical specimens such as food and animal feeding stuffs [6,7]. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[8] Collect specimens before antimicrobial therapy where possible. Consult appropriate standard methods for details on food sample collection and preparation.[6,7]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment step in Selenite Broth followed by subculture on Hektoen Enteric Agar and on a second plating medium.[9]

For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised, followed by subculture in two different selective media: Hektoen Enteric Agar and a second less selective medium (Mac Conkey Agar).[9]

Incubate inoculated Hektoen Enteric Agar plates with the specimen or with a specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.

Consult appropriate references for the detection of *Shigella* and *Salmonella* in non-clinical specimens.[6,7]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Do not examine areas of confluent growth as false negative fermentation reactions may occur.

The different colour characteristics of isolates may be interpreted as follows:[1]

Greenish-blue, light green, or transparent colonies with black centres: no fermentation present, H_2S production present: suspect *Salmonella*.

Greenish-blue, light green, or transparent colonies: no fermentation present, H_2S production absent: suspect Shigella or H_2S negative *Salmonella.*

Yellow colonies with an orange-yellow precipitate: fermentation of lactose, sucrose, or salicin: not likely to be *Salmonella* or *Shigella*.

Salmon to orange colonies: fermentation of salicin, H_2S production absent: not likely to be *Salmonella* or *Shigella*.

Yellow, salmon to orange colonies with black centre: fermentation of lactose or sucrose, or salicin, H_2S production present: not likely to be *Shigella* or *Salmonella* (other than rare lactose positive *Salmonella*).

Since some *Proteus* spp. may grow with greenish blue colonies with black centre and if *Proteus* colonies are mixed with H_2S positive *Salmonella* colonies, it could be difficult to choose the colonies for further biochemical and serological identification.

It is advised to screen the colonies by flooding the plate with one drop of MUCAP reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C_8 esterase enzyme, typical of *Salmonella* spp.[10]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[11]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, light green colonies with black centre
S. flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, light green colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, yellow to salmon colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Be aware that carbohydrates non-fermenting strains of *Proteus* spp. may or may not be inhibited and colonies may resemble *Salmonella*.[5] Rapid differentiation between very similar colonies may be performed with MUCAP test.[10]
- Some lactose fermenting *Shigella* and *Salmonella* strains may resemble coliforms and are not recognized on Hektoen Enteric Agar.
- Do not incubate longer than 24 hours since a loss of yellow/salmon colour may occur due to the utilisation of peptones for growth with the productions of alkaline end-products.[1]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella* and/or *Shigella*, with lower selectivity such as Mac Conkey Agar and with higher selectivity such as SS Agar, should be used; other media for the isolation of other enteric pathogens must be inoculated with the specimen.[8,9]
- Over time and during the shelf-life, bile salts in Hektoen Enteric Agar plates may crystallize and form a precipitate in the medium. This does not affect the performance of the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the self-prepared plates can be stored at +2°C +8°C for up to 6-8 weeks.[5]

Ready-to-use plates

Store plates in their original pack at +2°C/+8°C away from direct light.

Ready-to- flasks

Store flasks in their original pack at +2°C/+8°C away from direct light.

References

1. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010.
2. King S, WI Metzger WI. A new plating medium for the isolation of enteric pathogens: I. Hektoen enteric agar. Appl Microbiol 1968; 16:577–578.
3. Mayfield CR, M Gober M. Comparative efficiency of plating media for the isolation of Shigella dysenteriae. Am J Public Health 1941; 31:363–368.
4. King S, WI Metzger WI. A new plating medium for the isolation of enteric pathogens: II. Comparison of Hektoen Enteric Agar with S S and E M B Agar. Appl Microbiol 1968;16: 579–581.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. ISO 21567 :2005. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.
7. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019.
8. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
9. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.685.
10. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526.
11. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
12. King S. Department of Microbiology, Cook County Hospital, Chicago. Personal communication.1968.

Packaging

Product	Type	REF	Pack
Hektoen Enteric Agar	Dehydrated medium	4015412 4015414	500 g (6.5 L) 5 kg (65 L)
Hektoen Enteric Agar	Ready-to-use plates	541541	2 x 10 plates ø 90 mm
Hektoen Enteric Agar	Ready-to use flasks	5115412 5115413	6 x 100 mL 6 x 200 mL

IFU rev 2, 2021/12

HERELLEA AGAR

Dehydrated culture medium

Herellea Agar*: A. calcoaceticus*

Intended use

In vitro diagnostic. For isolation, cultivation and differentiation of Gram-negative fermentative and non-fermentative bacteria. It is especially recommended for the differentiation of *Acinetobacter* (formerly *Herellea*) species in urethral and vaginal specimens.

Composition - typical formula * (after reconstitution with 1 L of water)

Tryptone	15.00 g
Soy peptone	5.00 g
Sodium chloride	5.00 g
Lactose	10.00 g
Maltose	10.00 g
Bile salts N.3	1.25 g
Bromocresol purple	0.02 g
Agar	16.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Herellea Agar has been formulated by Mandel, Wright and McKinnon[1] in 1964 as a selective medium for enhancing the isolation of *Mima* and *Herellea* organisms in gonorrhoeal specimens in the presence of large numbers of Gram-positive cocci and Gram-negative rods (usually members of the family *Enterobacteriaceae*) frequently encountered in urethral and vaginal discharges.
Herellea Agar is used for isolation, cultivation and differentiation of Gram-negative fermentative and non-fermentative bacteria and it is especially recommended for the differentiation of *Mima polymorpha* and *Herellea vaginicola* (included together in the species *Acinetobacter)* from *Neisseria gonorrhoeae* in urethral and vaginal specimens.[2]
Casein and soy peptones provide nitrogen, carbon and other essential nutrients for bacterial growth. Inhibition of Gram-positive bacteria and *N. gonorrhoeae* is achieved by the incorporation of bile salts n°3. Sodium chloride maintains the osmotic balance of the medium. Lactose and maltose are fermentable carbohydrates: fermenting bacteria produce acid end-products that make the pH indicator (bromocresol purple) turn yellow. *Acinetobacter* organisms do not ferment the carbohydrates and grows with pale lavender colonies, the same colour of the medium. All acid-producing colonies are yellow, surrounded by a yellow zone.[1]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 62 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes, cool to approximately 47-50ºC and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale violet, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Herellea Agar is used for the bacteriological processing of clinical specimens such as urethral and vaginal specimens.[1,2,] Collect clinical specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate inoculated plates, in aerobic conditions at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Interpretation of colonies' colours:
Acinetobacter spp. do not ferment lactose and maltose and grow with colonies of the same colour of the medium, sometimes with a slight colour change to a more intense violet.
Lactose and maltose fermenting *Enterobacteriaceae* grow with yellow colonies surrounded by yellow halos.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[8]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
A. calcoaceticus ATCC 19606	35-37°C / 18-24H / A	good growth, pale lavender colonies
E. coli ATCC 25922	35-37°C / 18-24H / A	good growth, yellow colonies and medium
S. aureus ATCC 25923	35-37°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Cultures

LIMITATIONS OF THE METHOD

- *Pseudomonas* and *Proteus* spp. are not inhibited; however, they do not produce acids. *Proteus* colonies are colourless, *Pseudomonas* colonies are grey-green with a diffusible pigment.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the self-prepared plates can be stored at +2°C +8°C for up to 6-8 weeks.[3]

REFERENCES

1. Mandel AD, Wright K, McKinnon JM. Selective medium for isolation of Mima and Herellea organisms. J Bacteriol 1964; 88:1524
2. Ronald M. Atlas, James W. Snyder. Handbook of Media for Clinical and Public Health Microbiology. CRC Press, 2014
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Herellea Agar	Dehydrated medium	4015432	500 g (8,1)

IFU rev 2, 2022/03

HHD BROTH

Dehydrated culture medium

INTENDED USE

For the detection and differentiation of heterofermentative and homofermentative lactic acid bacteria.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Fructose	2.500 g
Potassium dihydrogen phosphate	2.500 g
Tryptic digest of casein	10.000 g
Soy peptone	1.500 g
Acid digest of casein	3.000 g
Yeast extract	1.000 g
Bromocresol green	0.066 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactic Acid Bacteria are widely distributed throughout nature and are involved not only in the production, but also in the spoilage of acid food products. The Lactic Acid Bacteria (LAB), although consisting of a number of diverse genera, are grouped as either homofermenters or heterofermenters on the basis of their metabolic properties: the heterofermentative LAB, which produce CO_2, lactic acid, acetic acid, ethanol, and mannitol from hexoses, and the homofermentative LAB, which produce primarily lactic acid from hexose.
HHD Broth, prepared according to the formula of McDonald et al.[1], allows differentiation of the two groups of bacteria on the basis of a different acidification of the substrate that contains a low amount of fructose (14mM). The addition of agar to HHD Broth allows differentiation by colony colour in a solid medium. HHD Agar is recommended by APHA[2] for enumeration of acid-producing microorganisms in fermented and acidified vegetables.
Bromocresol green is used as a pH indicator that differentiates the degree of acidification induced by both groups of bacteria. The indicator is yellow at pH values below 8.8 and blue at values above 5.6. The homofermentative bacteria produce 2 moles of lactic acid from fructose and grow on HHD Broth with a colour change of the indicator to green and blue-green sedimented cells at the bottom of the tube.
Heterofermentative bacteria induce less acidification of the substrate and grow on HHD Broth without significantly changing the colour of the medium, which remains blue with white sedimented cells.
If agar is added to HHD Broth, the homofermentative bacteria grow with blue-green colonies, the heterofermentative ones with colourless colonies. McDonald *et al.*[1] recommend recording the colour of the cell sediment and colonies for the differentiation of the two bacterial groups rather than the colour change of the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 20.6 g in 1000 ml of cold purified water and add 1g of Tween 80 (REF 42120502). Mix thoroughly and warm if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.
For the preparation of HHD Agar, add 13 g/L of Agar Bios LL (REF 411030) and add 1g of Tween 80 (REF 42120502) to HHD Broth before sterilisation.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	blue, limpid
Final pH at 20-25 °C	7.0 ± 0.1

SPECIMENS

Food samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

TEST PROCEDURE

HHD Broth

1. Inoculate the tubes with the sample suspension and/or its decimal dilutions or with pure cultures.
2. Incubate at 30°C for 3 days.

HHD Agar[2]

1. Samples are pour-plated with melted HHD Agar and after solidification are overlayed with additional HHD Agar.
2. Plates are incubated at 30 °C for 72 ± 3 hours.

READING AND INTERPRETATION

HHD Broth: the homofermentative bacteria grow with a green/blue-green cell sediment at the bottom of the tube whereas the heterofermentative bacteria grow without significantly changing the colour of the medium, which remains blue with a white cell sediment.
HHD Agar: homofermentative organisms grow with blue to green colonies, whereas heterofermentative colonies remains white.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T	EXPECTED RESULTS
L. mesenteroides ATCC 14935	30°/ 72 hours	growth, the medium remains blue, white sedimented cells
L. plantarum ATCC 14917	30°/ 72 hours	growth, the medium turn to green, green sedimented cells

ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place

REFERENCES

1. Mc Donald LC, Mc Feeters RF, Daeschel MA, Fleming HP. Differential medium for the enumeration of homofermentative and heterofermentative lactic acid bacteria. App Environ Microbiol 1987; 53:1382-1384.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

PACKAGING

Product	Type	REF	Pack
HHD Broth	Dehydrated medium	4015292	500 g (24.2 L)

IFU rev 1, 2022/08

IRON SULFITE AGAR

Dehydrated culture medium

INTENDED USE

For the detection and enumeration of sulphite-reducing *Clostridium spp.* in samples of the food chain.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	15.0 g
Soy Peptone	5.0 g
Yeast extract	5.0 g
Sodium metabisulphite	0.5 g
Iron (III) ammonium citrate	1.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Sulphite-reducing clostridia are large, rod-shaped, Gram-positive, spore-forming, anaerobic bacteria, present widely in dust, soil, and vegetation and as normal flora in mammalian gastrointestinal tracts. The most important species which belong to this group are *C. perfringens, C. bifermentans, C. sporogenes* and *C. botulinum*. Sulphite-reducing clostridia may be sought as an index organism for clostridial contamination, as general hygiene indicators, or as a means of detecting faults in food processing.
Iron sulphite agar was first developed by Wilson and Blair[1] to enumerate *C. perfringens* (*B. welchii*) in water.
The current formulation, with a reduced concentration of sodium metabisulphite, complies with ISO 15213-1.[2]
The medium utilizes the ability of *Clostridium* spp. to reduce sulphite which reacts with the iron ammonium citrate to form ferrous sulphide which stains the colonies black.[3] Peptones and yeast extract provide nitrogen, carbon, minerals, vitamins and amino acids for the microbial growth. Agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47 °C mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates/flasks/tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.6 ± 0.2

SPECIMENS

Foods and animal feeding stuffs, environmental samples in the area of food and feed production and handling; samples from the primary production stage. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2]

TEST PROCEDURE

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.
2. If it is the intention to count only spores, within 15 min after preparation of the initial suspension, heat the decimal dilution series to 80° C in a water bath for 10 min ± 1 min.
3. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1mL of each decimal dilution, in duplicate, to the centres of empty Petri dishes. When, for certain products, it is necessary to estimate low numbers of sulphite-reducing *Clostridium* spp. the limit of enumeration may be lowered by examining 10 mL of the initial suspension in three large (140 mm) Petri dishes.
4. Pour about 12 -15 mL for 90 mm Petri dishes or 45 - 50 mL for 140 mm Petri dishes of the Iron Sulfite Agar molten and tempered at 44 C to 47 °C, into each Petri dish and mix well with the inoculum.
5. When the medium has solidified pour about 5 mL of the ISA medium for 90 mm Petri dishes or 10 mL for 140 mm Petri dishes as overlay, to prevent the development of spreading colonies on the surface of the medium.
6. Allow to solidify and incubate in anaerobic jars or other suitable containers and incubate at 37 °C for 48 ± 2 hours. Longer incubation may result in excess blackening of the plates

Note: a special protocol must be applied for the enumeration of sulphite-reducing *Clostridium* spp. in feed. For the detailed procedure consult the ISO 15213-1.[2]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Count the black colonies on the plates containing less than 150 characteristic colonies (for 90 mm Petri dishes) or less than 360 colonies (for 140 mm Petri dishes. Choose at random five such colonies for subculturing for the confirmation tests.
For a complete explanation of the identification criteria and methods, refer to the quoted reference.[2]

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[2]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C/ 48 H / AN	growth, black colonies
E. coli ATCC 25922	37°C/ 48 H / AN	Growth, no blackening of colonies

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Diffuse, unspecific blackening of the medium can occur. The growth of anaerobic bacteria, which produce hydrogen (not H_2S), can also reduce the sulphite present and lead to a general blackening of the medium, which makes enumeration of typical colonies difficult.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 15213-1 the medium may be stored at +2°C /+8°C for up to 4 weeks in closed containers or tubes.[2]

REFERENCES

1. Wilson WJ, Blair EMMV. The application of a sulphite-glucose-iron agar medium to the quantitative estimation of B. welchii and other reducing bacteria in water supplies. J Pathol Bacteriol 1924; 119:121.
2. ISO 15213:2023. Microbiology of the food chain - Horizontal method for the detection and enumeration of Clostridium spp. Part 1: Enumeration of sulfite-reducing Clostridium spp. by colony-count technique.
3. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:204-5.

PACKAGING

Product	Type	REF	Pack
Iron Sulfite Agar	Dehydrated medium	4015652	500 g (12 L)

IFU rev 0, 2023/02

IUT MEDIUM

Ready-to-use tubes

IUT Medium: from the left: *M. kansasii* and uninoculated tube

INTENDED USE

In vitro diagnostic device. For the cultivation and isolation of *Mycobacterium* species, especially *M. tuberculosis*.

COMPOSITION -TYPICAL FORMULA *

Magnesium sulphate	0.24 g
Magnesium citrate	0.60 g
Monopotassium phosphate	2.50 g
L-asparagine	3.60 g
Malachite green	0.40 g
Glycerol	12.00 mL
Purified water	600 mL
Homogenized whole eggs	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The medium originally described by Löwenstein in 1931[1] contained congo red and malachite green to limit the growth of unwanted bacteria. In 1932 Jensen[2], modified the medium by suppressing the congo red, modifying the concentration of magnesium citrate and potassium phosphate and increasing malachite green concentration. Today it is generally accepted that the use of an egg-based medium in combination with a liquid medium is essential for good laboratory practice in the isolation of mycobacteria;[3]
IUT Medium differs from Lowenstein-Jensen Medium since it does not contain potato flour/starch. This medium has been recommended by the International Union against Tuberculosis for the Diagnosis of Mycobacterial Infections.[4] IUT Medium has been reported by LaPlaca et al.[5] to provide a higher rate of positive isolates.
In IUT Medium, during the cooking process, the egg albumin coagulates thus providing a solid surface for bacterial growth. The concentration of malachite green is selected to maximize the growth of mycobacteria while inhibiting other microorganisms. L-asparagine is a source of nitrogen and vitamins. Monopotassium phosphate and magnesium sulphate enhance organism growth and act as buffers. Egg suspension provides fatty acids and proteins required for the metabolism of mycobacteria. Glycerol is a carbon source and is favourable to the growth of the human type tubercle bacillus while being unfavourable to the bovine type.

PHYSICAL CHARACTERISTICS

Medium appearance	green, opaque slanted medium
Final pH at 20-25°C	not applicable

SPECIMENS

Specimens submitted for mycobacterial culture fall into two categories:
1- specimens normally contaminated with resident flora: the majority originates from respiratory tract, including sputum, tracheal and bronchial aspirates, and bronchoalveolar lavage specimens; other commonly submitted specimens types include urine, gastric aspirates, tissues, biopsy specimens.

2- specimens from normally sterile sites such as pleural and pericardial aspirates.
Contaminated specimens require a decontamination step before culture to reduce the likelihood of overgrowth by organisms other than mycobacteria. Specimens from normally sterile sites should be concentrated by centrifugation. Consult appropriate references for the applicable techniques[3,6] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.[3,6]

TEST PROCEDURE

Remove any condensation water present at the bottom of the slope and inoculate the surface of the slope with 0.2 mL (3-5 drops) of decontaminated and/or concentrated specimen.
Briefly angle slopes to allow the specimen to inoculate the entire surface. Ensure that the caps are tightly closed.
Incubate at 35 to 37°C for 6-8 weeks, extending to 12 weeks if necessary. 5-10% CO_2 in air stimulates the growth of mycobacteria in primary isolation cultures. It is necessary to incubate under CO_2, with loosening the caps to promote the circulation of carbon dioxide, for only the first 7 to 10 days after inoculation, subsequently cultures can be removed to ambient air incubators if space is limited and incubated with the caps tightly screwed to prevent dehydration of the medium.[3]
Specimens with positive smears that are culture negative should be held for an additional 4 weeks. The same should be done for culture negative specimens that were positive for mycobacteria by nucleic acid-based amplification assays.[3]
The cultures should be examined within 2 to 5 days after inoculation to permit early detection of rapidly growing mycobacteria. Young cultures (up to 4 weeks of age) should be examined twice a week, whereas older cultures could be examined at weekly intervals.[3]
For samples obtained from surface sites, such as skin, or when the clinician suspects the presence of particular mycobacterial species (*M. marinum, M. ulcerans, M. chelonae,* or *M. haemophilum*), it is recommended to inoculate two sets of media, one of which incubated at 35-37°C and one at a lower temperature (30-32°C).
Consult appropriate references for the detailed procedures about the treatment, inoculation and incubation of clinical specimens.[3,6]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
M. tuberculosis appears as granular, rough, dry colonies; *M. kansasii* appears as smooth to rough photochromogenic colonies; *M. gordonae* appears as smooth yellow-orange colonies; *M. avium* appears as smooth, colourless colonies; *M. smegmatis* appears as wrinkled, creamy white colonies.[7]
Confirm the presence of Acid-Fast Bacilli in positive cultures with the Ziehl-Nielsen or auramine-phenol stain.[6]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[8]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
M. tuberculosis H37Ra ATCC 25177	35-37°C / <21 days / CO_2	growth
M. kansasii Group I ATCC 12478	35-37°C / <21 days / CO_2	growth
M. intracellulare Group III ATCC 13950	35-37°C / <21 days / CO_2	growth
M. fortuitum Group IV ATCC 6841	35-37°C / <21 days / CO_2	growth

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- To shorten the isolation time as much as possible and to obtain a faster identification, the combination of a solid medium and a liquid medium is strongly recommended. The latter allows to reduce the incubation time and egg-based media allow the growth of some strains of *M. tuberculosis* complex and some non-tuberculous species that are unable to develop in liquid media.[6]
- It should be noted that if there is not enough specimen volume for PCR and culture, then only culture should be done. All samples, even if PCR positive, should be submitted for culture.[6]
- *M. bovis* grows poorly, or not at all on IUT Medium.[10] For isolation and cultivation of *M. bovis*, the medium without glycerol and supplemented with sodium pyruvate should be used.
- *M. leprae* and *M. genavense* fail to grow on IUT Medium.[3,10]
- A negative culture does not exclude an ongoing mycobacterial infection. There are several factors that can be responsible for negative cultures even in the presence of an infection: un-representative sample, mycobacteria destroyed during digestion and decontamination of the sample, presence of contaminants that mask or inhibit the growth of mycobacteria, inadequate incubation conditions.
- False positive cultures may result from mislabelling, specimen switching during handling, specimen carryover, contaminated reagents, or cross-contamination between cultures tubes.[3]
- IUT Medium contains malachite green and is photosensitive and should not be exposed to light during storage.[10]
- IUT Medium may display some variation in the light-green colour throughout the tube. This doesn't interfere with the growth of mycobacteria; however, colour changes showing bright yellow or dark blue zones may indicate contamination.[10]
- The presence of yellow granules due to the lipid part of the egg, does not interfere with the performance of the medium.
- It is recommended that suitable identification and susceptibility tests be performed on isolates. For the detailed procedures consult appropriate references.[3,6,11]

STORAGE CONDITIONS

Store tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Lowenstein E. Die Zachtung der Tuberkelba zillen aus dem stramenden Blute. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg Abt I Orig 1931; 120:127.
2. Jensen KA. Rinzuchtung und Typenbestim mung von Tuberkelbazillentammen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg Abt I Orig 1932; 125:222-239.
3. Martin I, Pfyffer GE, Parrish N. Mycobacterium: general characteristics, laboratory detection and staining procedures. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. International Tuberculosis Year Book, 1955, Bulletin of the International Union against Tuberculosis, pg. 89.
5. La Placa, Bubani and Raspi., 1956, Riv. Patol. Clin. Tuberc., 29:133.
6. Public Health England. Investigation of specimens for Mycobacterium species. UK Standards for Microbiology Investigations. B 40, Issue 7.3, 2020.
7. Atlas R, Snyder J. Media Reagents and Stains. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
8. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.

9. Manuale tecnico per la diagnosi microbiologica della tubercolosi: http://www.salute.gov.it/imgs/C_17_pubblicazioni_614_allegato.pdf.
10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
11. Warshauder DM, Salfinger M, Desmond E, Grace Lin SY. *Mycobaterium tuberculosis* complex. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
IUT Medium	Ready-to-use tubes	551634	20 glass tubes with slanted medium, 18 x145 mm,

IFU rev 1, 2021/02

KANAMYCIN AESCULIN AZIDE AGAR BASE
KANAMYCIN SELECTIVE SUPPLEMENT
Dehydrated culture medium and selective supplement

INTENDED USE
Selective and differential basal medium and selective supplement for the isolation, enumeration and differentiation of enterococci in foodstuffs.

COMPOSITIONS*
KANAMYCIN AESCULIN AZIDE AGAR BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	20.00 g
Yeast extract	5.00 g
Sodium chloride	5.00 g
Sodium citrate	1.00 g
Aesculin	1.00 g
Ferric ammonium citrate	0.50 g
Sodium azide	0.15 g
Agar	10.00 g

KANAMYCIN SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Kanamycin sulphate	10 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Kanamycin Aesculin Azide Agar is prepared according to the formulation described by Mossel *et al.*[1,2] It is a bile-free selective medium for the isolation, enumeration and differentiation of enterococci in foodstuffs. The medium utilises the selective inhibitory components sodium azide and kanamycin: growth of the majority of unwanted organisms is suppressed. The medium contains the indicator system aesculin and ferrous iron: enterococci hydrolyse aesculin producing black zones around the colonies due to the formation of black iron phenolic compounds derived from the aglucon.[3] Essential growth factors are provided by tryptone which is a source of nitrogen, carbon and minerals whereas yeast extract provides vitamins, particularly of the B-group. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium.

DIRECTIONS FOR MEDIA PREPARATION
Suspend 21.3 g of dehydrated medium in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of Kanamycin Selective Supplement (REF 4240055) reconstituted with 5 mL of a sterile purified water, under aseptic conditions. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Kanamycin Aesculin Azide Agar Base	
Dehydrated medium appearance	beige, fine, free-flowing powder
Solution and prepared plates appearance	tan with trace blue cast, limpid
Final pH at 20-25 °C	7.0 ± 0.2
Kanamycin Selective Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless, limpid solution

SPECIMENS
Food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

TEST PROCEDURE
1. Prepare tenfold dilutions of sample with a suitable diluent.
2. Within 3 hours from the sample preparation, spread 0.1mL of the inoculum onto the plates.
3. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium).

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical enterococci colonies are round, grey, about 2 mm in diameter surrounded by a brown-black zone.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecium ATCC 19434	37°C / 24 H / A	grey colonies with black halo
B. subtilis ATCC 6633	37°C / 24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The identification of isolated strains must be confirmed by suitable tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Selective supplement
Store the product in the original package at **+**2°C / +8°C away from direct light.
According to Baird RM *et al* the self-prepared plates can be stored at +2°C / +8°C for 7 days.[3]

REFERENCES

1. Mossel DAA, Bijker PGH, Eeldering J.: Streptokokken der Lancefield-Gruppe D in Lebensmitteln und Trinkwasser - Ihre Bedeutung, Erfassung und Bekämpfung. - Arch. f. Lebensmittelhyg., 1978; 29: 121-127
2. Mossel DAA, Bijker PGH, Eeldering J. VanSpreekens KA. In: Streptococci, edited by Skinner LH and Quesnel LB. SAB Symposium Sereies n° 7. Academic Press, London.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:195-196

PACKAGING

Product	Type	REF	Pack
Kanamycin Aesculin Azide Agar Base	Dehydrated medium	4015522	500 g (11.7 L)
Kanamycin Selective Supplement	Freeze-dried supplement	4240055	10 vials, each for 500 mL of medium

IFU rev 1, 2023/02

KLIGLER IRON AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. For the differentiation of *Enterobacteriaceae*, based on sugar fermentation and hydrogen sulphide production.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Beef extract	3.000 g
Yeast extract	3.000 g
Peptocomplex	20.000 g
Lactose	10.000 g
Glucose	1.000 g
Ferric ammonium citrate	0.500 g
Sodium thiosulphate	0.300 g
Sodium chloride	5.000 g
Phenol red	0.025 g
Agar	11.700 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The formulation of Kligler iron agar medium is based on several microbiologists' attempts to develop a medium to aid in the identification of intestinal gram-negative bacilli. In 1911, Russell[1] developed a double sugar tube medium for the differentiation of typhoid bacilli from urine and faeces. In 1917 Kligler[2] reported the use of lead acetate to detect hydrogen sulphide production. In 1918 Kligler[3] combined the use of lead acetate with Russel's double sugar agar for simultaneous differentiation of typhoid, dysentery and allied bacilli. Bailey and Lacy[4] simplified the formula by using phenol red as the pH indicator instead of Andrade indicator. The current formulation of Kligler Iron Agar combines features of all the differential media described above.
Kligler Iron Agar (KIA) is intended for the differentiation of *Enterobacteriaceae*, grown on primary isolation media, based on the fermentation of glucose and lactose with production of acids and gas and the production of hydrogen sulphide.[5]
The fermentation of the two sugars can take place both on the surface of the slant and in the butt with or without the presence of gas (CO_2 + H_2).
Regarding the fermentation of sugars on KIA, 3 reaction models can be registered:
1-fermentation of glucose; 2-fermentation of glucose and lactose; 3-no fermentation.
In the first case, after 18-24 hours of incubation, an alkaline reaction on the slant and an acid reaction on the butt is observed. The complete consumption of glucose, present at a concentration of 0.1%, on the surface, where aerobic conditions exist, after 18-24 hours induces the oxidative degradation of peptones, with production of ammonia, alkalinity and a red colour change of phenol red (reversal of the acid-alkaline reaction). However, in the anaerobic butt the bacteria metabolize the glucose producing ATP and pyruvate, which is converted into stable acid end-products with a colour change of the indicator to yellow (acid pH).
In the second case, the microorganisms ferment glucose and lactose: after 18-24 hours of incubation an acid reaction is recorded on the slant and in the butt. This is due to the high concentration of lactose: after 18-24 hours their degradation is not exhausted on the surface and therefore there is no utilisation of peptones and no reversal of the reaction.
In the third model an alkaline reaction is recorded both on the slant and in the butt. This behaviour is not typical of *Enterobacteriaceae* but of some non-enteric non-fermenting Gram-negative bacteria that can utilise the peptones for growing (*Alcaligenes faecalis, Acinetobacter, Pseudomonas*).
If the degradation of the peptones is anaerobic the indicator will turn to red (alkaline pH) both on the surface and in the butt, if the degradation is aerobic, there is no colour change of phenol red in the butt.

Ferrous sulphate is an indicator of the formation of hydrogen sulphide. Thiosulphate reductase producing organisms causes the release of a sulphide molecule from the sodium thiosulfate. The hydrogen sulphide will react with ferric ions in the medium to produce iron sulphide, a black insoluble precipitate.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 54.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute in screw capped tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool in a slanted position to give short slopes and deep butts.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	red, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Kligler Iron Agar Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

TEST PROCEDURE

With an inoculating needle, pick the centre of a single pure colony, inoculate the slant by first stabbing the butt to the bottom; withdraw the needle, and then streak the surface of the slant. Loosen the closure of the tube before incubating.
Incubate aerobically at 35-37°C for 18 to 24 hours.

READING AND INTERPRETATION

Three kinds of data may be obtained from the reactions.[6]

Sugar fermentation

Acid (yellow) butt, alkaline (red) slant: glucose fermented, lactose not fermented.
Acid (yellow) butt, acid (yellow) slant: glucose and lactose fermented.
Alkaline (red) butt, alkaline (red) slant: neither glucose nor lactose fermented.

Gas production

Presence of bubbles in the butt. With large amounts of gas, the agar may be cracked and displaced

Hydrogen sulphide production

Hydrogen sulphide production from thiosulfate is indicated by the blackening of the butt as a result of the reaction of H_2S with the ferric ions to form black ferrous sulphide. Formation of H_2S requires an acidic environment; sometimes the butt will be entirely black; in such a case, it is assumed that the butt portion of the tube is acid (the yellow colour is masked by H_2S production)
All combinations of the reactions described above can be observed on Triple Sugar Iron Agar, therefore it is important to record the results of all the reactions (sugar fermentations, gas production, H_2S production). The following table, taken from MacFaddin[7] shows the reaction patterns of some *Enterobacteriaceae*.

Microorganism	Lac	Glu	Gas	H_2S
Edwarsiella	-	A	+	+
Escherichia coli	A [1]	A	V^+	-
Shigella	V^- [3]	A	V^- [2]	-
Klebsiella	A	A	+	-
Enterobacter	V	A	V^- [6]	-
Hafnia	V^-	A	V^+	-
Serratia	V^-	A	V^-	-
Morganella	-	A	V^+	-
Proteus mirabilis	-	A	+	+
Proteus vulgaris	-	A	V[7]	+
Salmonella	-[4]	A	V^+	+[5]
Salmonella arizonae	V^{+1}	A	+	+
Citrobacter amalonaticus	V	A	+	-
Citrobacter diversus	V	A	+	-
Citrobacter freundii	A[1]	A	+	+
Yersinia	-	A	V	-

Notes
Lac: lactose fermentation; Glu: glucose fermentation; A: acid reaction; V: variable; V^+: variable, usually positive; V^-: variable, usually negative. 1: the reaction may by delayed; 2:*S.flexneri* ser.6 gas production positive (slight amount); 3: usually negative except *S. sonnei* (acid reaction may be delayed); 4: although rare, lactose positive variants of S. Typhi exist; 5: S. Typhi may have a ring of H_2S but its presence is not diagnostic. S. Paratyphi A if positive may be weak.; 6: *E. agglomerans* gas production variable; 7: if gas produced, a slight amount.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

E. coli ATCC 25922	growth, yellow slant, yellow butt, gas +, H_2S -
S. Typhimurium ATCC 14028	growth, red slant, yellow butt, gas +, H_2S +

Aerobic incubation at 35-37°C for 18-24 h.

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It is necessary to inoculate the medium with a microbiological needle without breaking the agar (do not use loops).
- Perform the reading between 18 and 24 hours of incubation; early readings can induce false acidity results of the A/A type or there is not enough time for the sugar fermentation with consequent colour change of the indicator; delayed readings can give false K/K results due to the use of peptones and alkaline change of the medium.[7]
- H_2S production can mask the acid reaction in the butt, however the production of H_2S requires acidic conditions therefore the butt must be considered acid when there is blackening.
- The medium does not contain inhibitors therefore a large variety of microorganisms can grow on it; for this reason, before inoculation, make sure that the organisms are catalase positive, Gram-negative bacilli.[7]
- A pure culture is essential when inoculating the medium. If the culture is not pure, irregular results may be obtained.[7]

- Some organisms such as the *Klebsiella-Enterobacter* group produce such an abundance of gas that the medium may be completely displaced by gas resulting in the medium being blown up into the cap. If this occurs, handle the culture with caution when sub-culturing to avoid contaminations.
- Make sure that the caps are loosened during incubation since for a correct medium performance a free exchange of air is necessary. If the caps are too closed, an acid reaction occurs on the slant only even in the presence of glucose fermentation.[7]
- If no reaction is observed on the slant and in the butt, check the tube carefully to see if there is growth. If there is no growth, possibly the tube has not been correctly inoculated. If growth is present, proceed with other identification systems for Gram-negative bacilli.[7]
- Occasionally a KIA tube exhibits a yellow slant and no colour change in the butt. This could be the result of the inoculation of a Gram-positive strain or of the failure to stab the butt.[7]
- It is recommended that biochemical, immunological, molecular or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks.[7]

Ready-to-use medium in tubes

Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Russell FF. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J Med Res 1911; 25:21
2. Kligler IJ. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am J Public Health 1917; 7:1042-1044
3. Kligler IJ. Modification of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J Med Res1917; 37:225.
4. Bailey Sadie F, Lacey GR. J. Bact. 1927; 13:82-189.
5. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition CRC Press,1997
6. Lehman D. Triple sugar iron agar protocols. American Society for Microbiology 2015.
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Kligler Iron Agar	Dehydrated culture medium	4015602	500 g (9.2 L)

IFU rev 3, 2022/10

Kligler Iron Agar
from left: uninoculated tube, *E. coli,* S. Typhimurium.

LACTOSE BROTH

Dehydrated and ready-to-use culture medium

INTENDED USE

Non-selective medium for detection and enumeration of coliform bacteria in materials of sanitary importance.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Beef extract	3 g
Gelatin peptone	5 g
Lactose	5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactose broth was formulated by American Public Health Association (APHA) to test dairy products and water[1,2] and it is no longer included in recent editions. Lactose Broth may be used for study of lactose fermentation of bacteria in general and it is reported by FDA-BAM and as an alternative to Lauryl Sulfate Broth in the presumptive MPN test for total coliforms and faecal coliforms in foodstuffs[3] and as a pre-enrichment medium for detection of *Salmonella* in some foods[4]. The medium is indicated by ISTISAN Report 96/35 for the enumeration of *E. coli* in frozen pasta using the MPN technique.[5]

Essential growth factors are provided by beef extract and gelatin peptone which are sources of nitrogen, carbon, amino acids and minerals. Lactose is the fermentable carbohydrate for coliforms.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 13 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into test tubes containing inverted Durham tube or 225 mL into flasks. Sterilise by autoclaving at 121°C for 15 minutes. In the case of double strength, suspend 26 g in 1000 ml of cold purified water and dispense 10 mL into 20x200 mm tubes. The Durham tubes shall not contain air bubbles after sterilization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	very pale yellow, limpid
Final pH at 20-25 °C	6.9 ± 0.2

SPECIMENS

Foods and materials of sanitary importance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[3,4]

TEST PROCEDURE

Coliforms and faecal coliforms (MPN technique).[3]

- Prepare decimal dilution of the sample with the suitable diluent.
- Using at least 3 consecutive dilutions, inoculate 1 mL aliquots from each dilution into 3 Lactose Broth tubes for a 3 tube MPN analysis (some analysis may require the use of 5 tubes for each dilution).
- Incubate Lactose Broth tubes at 35°C ± 0.5°C. Examine tubes and record reactions at 24 ± 2 h for gas.
- Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 3 h.
- Perform confirmed test on all presumptive positive (gas) tubes.
- Completed test for coliforms: from each gassing lactose broth tube, transfer a loopful of suspension to a tube of Brilliant Green Bile Broth (REF 401265) and incubate at 35°C ± 0.5°C and examine for gas production at 48 ± 3 h.
- Completed test for faecal coliforms: from each gassing Lactose Broth tube transfer a loopful to a tube of EC broth (REF 401425) and incubate 24 ± 2 h at 44.5°C and examine for gas production. If negative, re incubate and examine again at 48 ± 2 h.

READING AND INTERPRETATION

The medium becomes turbid when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.

Consider as coliforms the growth in Lactose Broth that show gas formation in the Durham tube of Brilliant Green Bile Broth test tubes incubated at 35°C for 24-48 hours.

Consider as faecal coliforms the growth in Lactose Broth that show gas formation in the Durham tube of EC Broth test tubes incubated at 44.5°C for 24-48 hours.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35° C/24 H/A	growth, with gas production
C. freundii ATCC 43864	35 °C/24 H/A	growth, with gas production

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin the self-prepared screwcap tubes and flasks may be stored at +2°C /+8°C for 6 months.[6]

Ready-to-use medium in tubes

Store tubes in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. American Public Health Association. Standard methods for the examination of dairy products, 1948. 9th ed. APHA, New York, N.Y.
2. American Public Health Association. Standard methods for the examination of water and sewage, 9th ed. 1946. APHA, New York, N.Y.
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Rev October 2020.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev March 2022.
5. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti. Raccolta a cura di D.De Medici, L.Fenicia, L.Orefice e A. Stacchini
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Lactose Broth	Dehydrated medium	4015752	500 g (38.5 L)
Lactose Broth	Ready-to-use tubes	551575	20 x 10 mL

IFU rev 1, 2022/08

LACTOSE GELATIN MEDIUM

Dehydrated culture medium

INTENDED USE

For the confirmation of *Clostridium perfringens* colonies isolated from foods and other materials.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	15.00 g
Yeast extract	10.00 g
Lactose	10.00 g
Gelatin	120.00 g
Phenol red	0.05 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactose Gelatin Medium is prepared according to the formulation recommended by ISO 7937[1] and by FDA-BAM[2,3] for the confirmation of *C. perfringens* colonies isolated from foods and other materials based on lactose fermentation and gelatin liquefaction. Lactose Gelatin Medium should be used in combination with Motility Nitrate Medium for confirmatory purposes.[1,3]
Essential growth factors are provided by enzymatic digest of casein which is a source of nitrogen, carbon and minerals. The yeast extract is a source of vitamins, particularly of the B-group. Lactose is the fermentable carbohydrate: most of the end products of its fermentation are organic acids, which produce a colour change of phenol red from red to yellow; moreover, lactose fermentation is indicated by the presence of gas bubbles in the medium. Gelatin is a protein derived from collagen, a component of vertebrate connective tissue. Organism producing proteolytic enzymes, and gelatinases, hydrolyse gelatin into polypeptides and individual amino acids. In the process, gelatin loses its structure and becomes liquid.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 155 g in 1000 mL of cold purified water. Mix thoroughly and heat gently to completely dissolve the powder. Dispense 10 mL portions into test tubes and sterilise by autoclaving at 121°C for 15 minutes. If not used the same day, store in a refrigerator at 3 ± 2°C. If not used within 8 h, just before to use, heat in boiling water or flowing steam for 15 minutes, then cool rapidly to the incubation temperature.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	red, limpid
Final pH at 20-25 °C	7.5 ± 0.2

SPECIMENS

Colonies isolated on plating media such as TSC Agar.

TEST PROCEDURE

For the confirmation of *C. perfringens* one of the following two techniques may be followed[1,3]:

- confirmation technique using Nitrate Motility Medium (REF 401726) and Lactose Gelatin Medium (REF 4015762)
- confirmation technique using Lactose Sulfite Medium (REF 401579).

Confirmation using Lactose Gelatin Medium

- Inoculate each well-isolated characteristic colony selected from TSC Agar into freshly deaerated Lactose Gelatin Medium.
- Incubate under anaerobic conditions at 37°C for 24 hours.

READING AND INTERPRETATION

Examine the tubes for the presence of gas and yellow colour (due to acid formation) indicating lactose fermentation.
Chill the tubes for 1 hour at 5°C and check for gelatin liquefaction. If the medium has solidified, re-incubate for additional 24 hours and again check for gelatin liquefaction
Bacteria that produce black colonies in TSC Agar, are non-motile, reduce nitrate to nitrite, produce acid and gas from lactose, and liquefy gelatin in 48 h are considered to be *C. perfringens*. Cultures that show a faint reaction for nitrite (i.e. a pink colour) shall be eliminated, since *C. perfringens* consistently gives an intense and immediate reaction.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C/ 24-48 H / AN	growth with gas production, positive to gelatinase test

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 7937, the complete medium Lactose Gelatin Medium in tubes can be stored at 3 °C ± 2 °C for 3 weeks but, just prior to use, heat in boiling water or flowing steam for 15 min, then cool rapidly to the incubation temperature.[1]

REFERENCES

1. ISO 7937:2004. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of Clostridium perfringens -- Colony-count technique
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Media M75: Lactose-Gelatin Medium (for Clostridium perfringens).
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens

PACKAGING
Product	Type	REF	Pack
Lactose Gelatin Medium	Dehydrated medium	4015762	500 g (3.2 L)

IFU rev 1, 2022/08

LACTOSE SULFITE MEDIUM
Dehydrated culture medium

INTENDED USE
For the confirmation of *Clostridium perfringens* colonies isolated from foods and other materials.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Enzymatic digest of casein	5.000 g
Yeast extract	2.500 g
Sodium chloride	2.500 g
Lactose	10.000 g
L-cysteine hydrochloride	0.300 g
Disodium disulfite anhydrous	0.750 g
Ammonium iron (III) citrate	0.625 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Lactose Sulfite Medium is prepared according to the formulation recommended by ISO 7937[1] for the confirmation test of *C. perfringens* based on lactose fermentation and sulfite reduction. The reaction obtained in Lactose Sulfite Medium when incubated at 46 °C is very specific for *C. perfringens* and some colonies of *Clostridium paraperfringens* and *Clostridium absonum*.[1]
Essential growth factors are provided by enzymatic digest of casein which is a source of nitrogen, carbon and minerals. The yeast extract is a source of vitamins, particularly of the B-group. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Lactose is the fermentable carbohydrate. Ferric ammonium citrate and disodium disulfite are indicators of sulphite reduction by *C. perfringens* which produces blackening of the medium.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 21.7 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Dispense 9 mL portions into test tubes with inverted Durham tubes and sterilise by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS
Colonies isolated on plating media such as TSC Agar.

TEST PROCEDURE
For the confirmation of *C. perfringens* in foods, ISO 7937[1] recommends to choose one of the following two techniques:
- confirmation technique using Lactose Sulfite Medium.
- confirmation technique using Nitrate Motility Medium (REF 401726) and Lactose Gelatin Medium (REF 4015762)

Confirmation using Lactose Sulfite Medium
After incubation of the enumeration plates (TSC Agar REF 402158), inoculate each selected typical colony into fluid Thioglycolate Medium (REF 402137) and incubate under anaerobic conditions at 37 °C for 18 h to 24 h.
After incubation, transfer with no delay 5 drops of the thioglycolate culture to the Lactose Sulfite Medium by means of a sterile pipette. Incubate aerobically at 46 °C for 18 h to 24 h in the water bath.

READING AND INTERPRETATION
Examine the tubes of Lactose Sulfite Medium for the production of gas and the presence of a black colour (iron sulphide precipitate). Durham tubes more than one-quarter full of gas and tubes having a black precipitate are considered positive.
In case of doubt, when the Durham tube in a blackened medium is less than one-quarter full of gas, transfer with no delay, using a sterile pipette, 5 drops of the previous growth in Lactose Sulfite Medium to another tube of Lactose Sulfite Medium. Incubate in the water bath at 46 °C for 18 h to 24 h. Examine this tube as described above.
Bacteria which form characteristic colonies in the TSC Medium and which give a positive confirmation with the Lactose Sulfite Medium are considered as being *C. perfringens*. In all other cases, the tubes should be considered as negative.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	46°C/ 18-24 H / A	growth with gas production into Durham tubes, presence of a black precipitate

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 7937 the complete medium Lactose Sulfite Medium in tubes should be used the same day of the preparation.[1]

REFERENCES

1. ISO 7937:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique

PACKAGING

Product	Type	REF	Pack
Lactose Sulfite Medium	Dehydrated medium	4015792	500 g (23 L)

IFU rev 3, 2022/08

LAURYL PEPTO BIOS BROTH

(Lauryl Sulphate Broth-Lauryl Tryptose Broth)

Dehydrated culture medium

INTENDED USE

Selective medium for detection and enumeration of *Escherichia coli,* coliforms and faecal coliforms in materials of sanitary importance.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of plant and animal tissues	20.00 g
Lactose	5.00 g
Sodium chloride	5.00 g
Sodium lauryl sulphate	0.10 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lauryl Pepto Bios Broth, also known as Lauryl Sulfate Broth or Lauryl Tryptose Broth or Lauryl Sulfate Tryptose Broth, is prepared according to the formulation devised by Mollan and Ourby.[1]
This medium is recommended by ISO 7251[2] for detection and enumeration of *E. coli* in foodstuffs, by ISO 4831[3] for the detection of coliforms in foodstuffs, by FDA-BAM[4] for the MPN test for coliforms, faecal coliforms and *E. coli* in foods, bottled water, seawater and shellfish, by APHA[5-7] for the detection of coliforms in water, dairy products and other foods. The broth is specifically designed to allow rapid multiplication and copious gas production from a small inoculum of target organisms.[8] Essential growth factors are provided by enzymatic digest of plant and animal tissues which is a source of nitrogen, carbon, amino acids and minerals; lactose is a fermentable carbohydrate. Phosphates are used as buffering agents to control the pH in the medium and sodium chloride maintains the osmotic balance. The surface-active agent sodium lauryl sulphate acts as the selective agent in restricting the growth of bacteria other than coliforms.[8]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.6 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into 16 x160 mm test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. In the case of double strength suspend 71.2 g in 1000 mL of cold purified water and dispense 10 mL into 20x200 mm tubes. The Durham tubes shall not contain air bubbles after sterilization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Prepared tubes appearance	colourless, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.[2,3] Foods, bottled water, seawater and shellfish.[4] Water, dairy products and other foods.[5-7] For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Test for detection and enumeration of presumptive *E. coli*.[2]

1. Inoculate tubes of Lauryl Pepto Bios Broth at single and double strength.
2. For enumeration, follow the MPN scheme with 3 single-strength and 3 double-strength tubes inoculated with the sample suspension and its decimal dilutions. For detection method inoculate one tube with single strength medium or one tube with the double strength medium with the initial suspension, depending of the limit required.
3. Incubate at 37 °C ± 1 for 24 h ± 2 h. If, at this stage, neither gas production nor opacity preventing the observation of gas production is observed, incubate for up to 48 h ± 2 h
4. From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth showing opacity, cloudiness or any visible gas, inoculate with a sampling loop a tube of EC Broth (REF 401425).

5. Incubate the EC Broth tubes at 44 °C ± 1°C for 24 h ± 2 h. If, at this stage, there is no visible gas in the EC Broth, extend the incubation up to a total of 48 h ± 2 h.
6. For the confirmatory test of E. coli proceed as following:
7. After incubation, if visible gas is observed, inoculate a tube of Peptone (Tryptone) Water (REF 401891), preheated to 44 °C, using a sampling loop.
8. Incubate for 48 h ± 2 h at 44 °C.
9. Add 0.5 mL of Kovacs' Reagent (REF 19171000) to the incubated tubes, mix well and examine after 1 min. A red colour in the alcoholic phase indicates the presence of indole.

Test for detection and enumeration of presumptive coliforms.[3]

1. Inoculate tubes of Lauryl Pepto Bios Broth at single and double strength.
2. For enumeration, follow the MPN scheme with 3 single-strength and 3 double-strength tubes inoculated with the sample suspension and its decimal dilutions. For detection method inoculate one tube with single strength medium or one tube with the double strength medium with the initial suspension, depending of the limit required.
3. Incubate the tube of double-strength medium at 30 °C or 37 °C (as agreed) for 24 h ± 2 h.
4. Incubate the tube of single-strength medium at 30 °C or 37 °C (as agreed) for 24 h ± 2 h or, if neither gas formation nor opacity prevent this, gas formation is observed at this stage, continue incubation for another 24 h ± 2 h.
5. From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth inoculate with a loop a tube of Brilliant Green Bile Broth 2% (REF 401265) and incubate at 30 °C ± 1 °C or 37 °C ± 1 °C for 24 h ± 2 h or, if gas formation is not observed, extend the incubation up to a total of 48 h ± 2 h.

READING AND INTERPRETATION

Lauryl Pepto Bios Broth becomes turbid when bacteria are growing; gas formation can be observed as bubbles production accumulated into Durham tubes.
Consider as positive for *E. coli* bacteria the growth in Lauryl Pepto Bios Broth that has given rise to visible gas in tube of EC Broth and to indole production.[2]
Consider as coliforms the growth in Lauryl Pepto Bios Broth that show gas formation in Brilliant Green Bile Broth tube.[3]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C/24-48 H/A	growth, with gas production
C. freundii ATCC 43864	37°C/24-48 H/A	growth, with gas production
E. faecalis ATCC 19433	37°C/24-48 H/A	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to Baird RM *et al*., the prepared Lauryl Pepto Bios Broth can be stored in the refrigerator for up to 1 month in screw-capped containers.[8]

REFERENCES

1. Mallmann WL, Darby CW. Uses of a Lauryl Sulfate Tryptose Broth for the Detection of Coliform Organisms. Am J Public Health Nations Health.1941 Feb;31(2):127-34.
2. ISO 7251:2005 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique,
3. ISO 4831:2006 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique
4. FDA-BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Content current as of:10/09/2020
5. APHA Standard Methods for the Examination of Water and Wastewater, 23rd ed. 2017.
6. APHA Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., 2015.
7. APHA Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., 2004.
8. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 195-196.

PACKAGING

Product	Type	REF	Pack
Lauryl Pepto Bios Broth	Dehydrated medium	4015802	500 g (14 L)

IFU rev 1, 2022/07

LAURYL SULPHATE BROTH MUG IDF FORMULATION

Dehydrated culture medium

INTENDED USE

Selective, fluorogenic medium for the enumeration of *Escherichia coli* and coliforms in milk, dairy products and other materials of sanitary importance.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	20.00 g
Lactose	5.00 g
Sodium chloride	5.00 g
Sodium lauryl sulphate	0.10 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 g
L-tryptophan	1.00 g
4-methylumbelliferone beta-D-glucuronide (MUG)	0.10 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lauryl Sulfate Broth (LSB) was first introduced by Mollan and Ourby.[1] Rapid assay for *E. coli* was developed by Feng and Harman[2] by using LSB supplemented with the compound 4-methylumbelliferone glucuronide (MUG), which is hydrolysed by glucuronidase to yield a fluorogenic product. Lauryl Sulphate Broth with MUG (LSB MUG) is recommended by ISO 11866-1:2005 [IDF 170-1:2005][3] for simultaneous detection of *E. coli* and coliforms in milk and dairy products and by FDA-BAM[4] and AOAC[5] for detecting *E. coli* in chilled or frozen foods, exclusive of shellfish.
The broth is specifically designed to allow rapid multiplication and copious gas production from a small inoculum of target organisms.[6]
Essential growth factors are provided by tryptose which is a source of nitrogen, carbon, amino acids and minerals; lactose is a fermentable carbohydrate. Phosphates act as buffer system and sodium chloride maintains the osmotic balance. The surface-active agent sodium lauryl sulphate acts as the selective agent in restricting the growth of bacteria other than coliforms.[6] Coliforms grown in LSB MUG, ferment lactose and produce gas while other bacteria are either inhibited or grow without producing gas. MUG is cleaved by β-D-glucuronidase produced by *E. coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be determined directly by using a long-wave ultraviolet light (Wood's lamp). The presence of 1 g/L tryptophan enhances the detection of tryptophanase by the indole reaction.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 36.7 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into 16 x160 mm test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. In the case of double strength suspend 71.2 g in 1000 ml of cold purified water and dispense 10 mL into 20x200 mm tubes. The Durham tubes shall not contain air bubbles after sterilization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	light yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Milk, dairy products, chilled or frozen foods, exclusive of shellfish. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Take 3 tubes of double strength and 3 tubes of single-strength medium. Inoculate to each tube 10 mL and 1 mL respectively of the test sample if liquid or of the initial suspension. Proceed in the same way for each dilution.
Carefully mix the inoculum with the medium.
Incubate the tubes at 30 °C ± 1 °C for 24 ± 2 hours. If, at this stage, neither gas formation nor opacity preventing of gas formation is observed, incubate for up to 48 ± 2 hours.

READING AND INTERPRETATION

Presence of growth and gas production are considered a positive test for the presence of coliforms.
Perform the confirmatory test for *E coli* on all tubes:

- add to each tube 0.5 ml of NaOH 0.1M and observe for fluorescence under a Woods lamp
- add to each tube showing fluorescence 0.5 ml of Kovacs Reagent (cat. n° 19171000). Mix well and examine after 1 minute for the formation of a distinct red-purple colour in upper layer.

Identify those tubes which develop fluorescence and are positive to indole test as *E. coli.*

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	30°C/24H/A	growth, with fluorescence under Wood's lamp and gas
C. freundii ATCC 43864	30°C/24H/A	growth with gas, without fluorescence under Wood's lamp
E. faecalis ATCC 19433	30°C/48 H/A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive and fluorescent under Wood's lamp; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[7-9]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[8]
- Up to 10% of *E. coli* have been reported to be slow or non-lactose fermenting but should be MUG-positive.[4,10]

STORAGE CONDITIONS

Store at +2°C / +8°C away from direct light in a dry place.

REFERENCES

1. Mallmann WL, Darby CW. Uses of a Lauryl Sulfate Tryptose Broth for the Detection of Coliform Organisms. Am J Public Health Nations Health.1941 Feb;31(2):127-34.
2. Feng, P. C. S., and P. A. Hartman. 1982. Fluorogenic assay for immediate confirmation of Escherichia coli. Appl. Environ. Microbiol. 43:1320–1329.
3. ISO 11866-1:2005 [IDF 170-1:2005] Milk and milk products — Enumeration of presumptive Escherichia coli — Part 1: Most probable number technique using 4-methylumbelliferyl-beta-D-glucuronide (MUG).
4. FDA-BAM Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria. Content current as of:10/09/2020
5. Moberg, L.J., M.K. Wagner, and L.A. Kellen. Fluorogenic assay for rapid detection of Escherichia coli in chilled and frozen foods: collaborative study. J. Assoc.Off. Anal. Chem.1988; 71:589-602.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 195-196.
7. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.'
8. Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 48:285-288
9. Kaluzewski SD, Tomczuk D. Evaluation of the Usefulness of Tests for Productionof Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
10. Gokul Yaratha, MD, Sarah Perloff, DO, Kinesh Changala, MBBS. Lactose vs non-lactose fermenting E. coli: Epidemiology, Clinical Outcomes, and Resistance. Open Forum Infect Dis 2017; V4 (Suppl 1)

PACKAGING

Product	Type	REF	Pack
Lauryl Sulfate Broth MUG IDF Formulation	Dehydrated medium	401580F2	500 g (13.6 L)

IFU rev 1, 2023/02

LEGIONELLA BCYE AGAR BASE
SELECTIVE SUPPLEMENTS
READY-TO-USE MEDIA

Dehydrated culture medium, supplements and ready-to-use plates and flasks

INTENDED USE

Medium base, growth and selective supplements and ready-to-use media for the isolation and enumeration of *Legionella* spp. from clinical specimens and water samples.

COMPOSITIONS *

LEGIONELLA BCYE AGAR BASE
DEHYDRATED AND READY-TO-USE BOTTLED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Activated charcoal	2.0 g
Yeast extract	10.0 g
Agar	13.0 g

LEGIONELLA BCYE α-GROWTH SUPPLEMENT W/O CYSTEINE
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

ACES Buffer/Potassium hydroxide	6.4 g
α–ketoglutarate	0.5 g
Ferric pyrophosphate	125.0 mg

LEGIONELLA GVPC SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Glycine	1.5 g
Vancomycin HCl	0.5 mg
Polymyxin B	40.000 IU
Cycloheximide	40.0 mg

LEGIONELLA AGAR (BCYE)
READY-TO-USE PLATES, TYPICAL FORMULA

Legionella BCYE Agar Base	25.0 g
Potassium hydroxide /ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
L-cysteine HCl	400.0 mg
α–ketoglutarate	1.0 g
Purified water	1000 mL

LEGIONELLA AGAR W/O CYSTEINE
READY-TO-USE PLATES, TYPICAL FORMULA

Legionella BCYE Agar Base	25.0 g
Potassium hydroxide /ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
α-ketoglutarate	1.0 g
Purified water	1000 mL

LEGIONELLA SELECTIVE AGAR MWY ISO
READY-TO-USE PLATES, TYPICAL FORMULA

Legionella BCYE Agar Base	25.0 g
ACES Buffer/Potassium hydroxide	12.8 g
α–ketoglutarate	1.0 g
Ferric pyrophosphate	250.0 mg
L-cysteine HCl	400.0 mg
Glycine	3.0 g
Vancomycin HCl	1.0 mg
Polymyxin B	50,000 UI
Anisomycin	80.0 mg
Bromothymol blue	10.0 mg
Bromocresol purple	10.0 mg
Purified water	1000 mL

LEGIONELLA BCYE α-GROWTH SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

ACES Buffer/Potassium hydroxide	6.4 g
α–ketoglutarate	0.5 g
Ferric pyrophosphate	125.0 mg
L-Cysteine HCl	200.0 mg

LEGIONELLA AB SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefazolin	4.5 mg
Polymyxin B	40,000 UI
Pimaricin (natamycin)	35 mg

LEGIONELLA MWY SELECTIVE SUPPLEMENT (ISO) (with anisomycin)
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Glycine	1.5 g
Vancomycin HCl	0.5 mg
Polymyxin B	25.000 UI
Anisomycin	40 mg
Bromothymol blue	5.0 mg
Bromocresol purple	5.0 mg

LEGIONELLA SELECTIVE AGAR (GVPC)
READY-TO-USE PLATES, TYPICAL FORMULA

Legionella BCYE Agar Base	25.0 g
Potassium hydroxide /ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
L-cysteine HCl	400.0 mg
α –ketoglutarate	1.0 g
Glycine	3.0 g
Vancomycin HCl	1.0 mg
Polymyxin B	80,000 IU
Cycloheximide	80.0 mg
Purified water	1000 mL

LEGIONELLA AB SELECTIVE AGAR
READY-TO-USE PLATES, TYPICAL FORMULA

Legionella BCYE Agar Base	25.0 g
Potassium hydroxide /ACES Buffer	12.8 g
Ferric pyrophosphate	250.0 mg
L-cysteine HCl	400.0 mg
α –ketoglutarate	1.0 g
Glycine	3.0 g
Cefazolin	9.0 mg
Polymyxin B	80,000 UI
Pimaricin (natamycin)	70.0 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Legionellae are mesophilic, motile, a-saccharolytic, obligately aerobic, nutritionally fastidious, Gram-negative, non-spore-forming gammaproteobacteria.[1] *Legionella pneumophila*, the most widely studied species, displays pleomorphism, demonstrating coccoid, bacillary and/or long filamentous forms that are influenced by temperature, available nutrients or metabolites, growth environment and medium type.[2] *Legionella* species share growth dependence for L-cysteine and growth enhancement by iron.[1] Legionellae grow on several types of complex artificial media,

however, the most successful medium is buffered charcoal yeast extract (BCYE) agar containing ferric pyrophosphate, α-ketoglutarate and L-cysteine.[2]
The culture is the reference technique for laboratory diagnosis: it has 100% specificity and a variable sensitivity depending on the characteristics of the sample, on the experience and technical proficiency of laboratory personnel, as well as on the delays in respiratory sample processing, the prior use of antimicrobial therapies and culture overgrowth by other oropharyngeal bacteria.[2,3]
Optimal yield of *Legionella* spp. from clinical specimens usually requires that a variety of media be used: one plate with non-selective medium (BCYE) and two with selective media.[1]
The choice of the method used for the enumeration of *Legionella* spp. in water depends on the origin and characteristics of the sample, the reason of sampling or investigation, the expected concentration of interfering microorganisms and the desired lower limit of detection level; a decision matrix for choosing an appropriate method is described in ISO 11731.[4]
Buffered Charcoal Yeast Extract Agar (BCYE) was developed by Feeley et al.[5] and then further modified by Edelstein[6] by introducing α-ketoglutarate, and by Pasculle et al.[7] by the addition of ACES buffer.
Wadowsky and Yee[8] proposed a selective version of BCYE, by introducing in the formulation glycine, vancomycin and polymyxin B, resulting in the formation of GVP medium. Another modification in 1984 by Dennis et al.[9] made the medium even more selective for *Legionella* by the addition of cycloheximide, resulting in GVPC medium.
Vickers et al.[10] introduced 0.001% of bromocresol purple and bromothymol blue into BCYE agar for the differentiation between members of the family *Legionellaceae*. Edelstein in 1982[11] proposed MWY medium as a modification of the GVP medium of Wadowsky and Yee, including bromothymol blue and bromocresol purple and an antifungal agent.
Legionella BCYE Agar Base (401582), GVPC Supplement (423215), AB Selective Supplement (423225), MWY Selective Supplement ISO (423220), BCYE α-Growth Supplement (423210) and BCYE α-Growth Supplement w/o Cysteine (423212) are prepared according to the formulations recommended by ISO 11773.[4]
Yeast extract is a source of nitrogen, carbon, and vitamins for microbial growth. Activated charcoal removes hydrogen peroxide and other toxic products. ACES Buffer is used for pH stabilisation, α-ketoglutarate and ferric pyrophosphate stimulate *Legionella* growth. L-cysteine, is an essential amino acid and an important energy source for *Legionella* spp. Glycine and polymyxin B are inhibitors of Gram-negative bacteria, cefazolin is active against Gram-positive bacteria and some Gram-negative bacteria, vancomycin suppress the growth of Gram-positive bacteria while cycloheximide, natamycin and anisomycin are used as antifungal agents.

DIRECTIONS FOR MEDIA PREPARATION

Suspend12.5 g of Legionella BCYE Agar Base in 450 mL of cold purified water. Heat to boiling with agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C. Add the suitable growth and selective supplements. After supplements addition, keeping the medium under stirring, distribute into sterile Petri dishes.

SELECTIVE MEDIUM BCYE-GVPC

To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella GVPC Selective Supplement (REF 423215) reconstituted with 10 mL of sterile purified water.

SELECTIVE MEDIUM BCYE-AB

To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella AB Selective Supplement (REF 423225), reconstituted with 5 mL of sterile purified water.

SELECTIVE MEDIUM BCYE-MWY (WITH ANISOMYCIN)

To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella MWY Selective Supplement (ISO) (REF 423220), reconstituted with 10 mL of sterile purified water.

NON-SELECTIVE MEDIUM WITH CYSTEINE: BCYE W/ L-CYSTEINE

To the medium base cooled to 47-50°C add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210), reconstituted with 50 mL of sterile purified water.

NON-SELECTIVE MEDIUM WITHOUT CYSTEINE: BCYE W/O L-CYSTEINE

To the medium base cooled to 47-50°C add the contents of one vial of Legionella BCYE α-Growth Supplement w/o Cysteine (REF 423212), reconstituted with 50 mL of sterile purified water.

PHYSICAL CHARACTERISTICS

Legionella BCYE Agar Base (REF 4015822-4015824)	
Dehydrated medium appearance:	fine grain size, blackish
Prepared plates and flasks appearance	black, homogeneously opaque
Final pH at 20-25°C	6.9 ± 0.1
Legionella BCYE α-Growth Supplement (REF 423210)	
Freeze-dried supplement appearance	medium size, pink pastille
Aspect of the solution	light yellow, opalescent
Legionella BCYE α-Growth Supplement w/o Cysteine (REF 423212)	
Freeze-dried supplement appearance	medium size, dark-pink pastille
Aspect of the solution	light yellow, opalescent
Legionella GVPC Selective Supplement (REF 423215)	
Freeze-dried supplement appearance	high size, white pastille
Aspect of the solution	colourless, clear
Legionella AB Selective Supplement (REF 423225),	
Freeze-dried supplement appearance	high size, white pastille
Aspect of the solution	whitish, cloudy
Legionella MWY Selective Supplement (ISO) (REF 423220),	
Freeze-dried supplement appearance	high size, bluish pastille
Aspect of the solution	blue, cloudy
Ready-to-use plates (REF 499995, 549943, 549945, 549947, 54994854995)	
Prepared plates appearance	black, homogeneously opaque
Final pH at 20-25°C	6.9 ± 0.1

SPECIMENS

Legionella BCYE Agar base, Legionella BCYE α-Growth Supplement and the selective supplements GVPC, are intended for the bacteriological processing of several human clinical specimens including those from the lower respiratory tract, such as sputum, pleural fluid, bronchial aspirates, and bronchial alveolar lavage (BAL) fluid; lung tissue and biopsy specimens are also appropriate for attempting culture.[1,12] Collect specimens

before antimicrobial therapy where possible. Transfer the sample as soon as possible to the laboratory; use a transport medium if the sample cannot be processed immediately. Legionella BCYE Agar Base, Legionella BCYE α-Growth Supplement and the selective supplements GVPC, MWY-ISO and AB are intended for the bacteriological processing of non-clinical specimens: all kinds of water samples such as potable, industrial, waste, natural waters and water related samples (e.g. biofilms, sediments, etc.).[4] Consult the ISO Standard 11731 for sampling methods and for sample treatment procedures.[4] Good laboratory practices for collection, transport and storage of the specimens should be applied.
Legionella BCYE Agar Base supplemented with Legionella BCYE α-Growth Supplement w/o Cysteine, must be inoculated with colonies cultivated on selective or non-selective isolation media for the presumptive confirmation of *Legionella* colonies.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Isolation from clinical specimens[1,12]

Optimal yield of *Legionella* spp. from clinical specimens usually requires[1]:

- That specimen be diluted 1:10 in Tryptic Soy Broth or purified water to reduce inhibition by tissue and serum factors, as well as antibiotics. If the sputum is very dense, it must be re-suspended with 0.2-1 mL of dithiothreitol-based fluidifying.
- That the specimen be pre-treated to reduce contaminating flora. This is done by diluting 1:10 the specimen with a low pH KCl-HCl buffer (pH 2.2) and incubating at room temperature for 4 minutes. An alternative to specimen acidification is heating at 50°C for 30 min.
- That a variety of media be used: one plate with non-selective medium (BCYE) and two with selective media.

Inoculate approximately 0.1 mL onto each plate, with the bulk of inoculum applied to the first quadrant and streak with a loop over the other quadrants of the plate to obtain well isolated colonies.
Incubate at 35-37°C in humidified air. A small amount of CO_2 (2.5%) supplementation may enhance the growth of some of the more fastidious *Legionella* spp. such as *L. sainthelensi* and *L oakridgensis*. This low level of CO_2 supplementation will not harm the growth of *L. pneumophila*, but CO_2 levels higher than 2.5% may inhibit growth.
Colonies are normally microscopically visible after 2 days and, macroscopically, after 3-5 days.

Enumeration in environmental samples[4]

The work procedures described in the ISO 11731 Standard differ in relation to the origin of the sample, its characteristics, the purposes of the research and in relation to the expected concentrations of the target microorganism and the contaminating flora.
Schematically, the different possibilities of treatment and inoculation of the samples are summarized below.

1. For samples with a high number of *legionellae* and a low number of contaminants: direct inoculation of the sample on a non-selective BCYE w/L-cysteine medium and on a selective BCYE-AB medium plate.
2. For samples with a low number of legionellae and a low number of contaminants: membrane filtration and positioning of the untreated filter on a BCYE w/ L-cysteine non-selective medium plate, positioning of the filter(s) treated with acids on one or more selective or highly selective medium plates (BCYE-AB or BCYE-GVPC or BCYE-MWY); wash the untreated and acid or heat treated membrane and inoculate 0.1-0.5 mL on a non-selective medium plate and on plates of one or more selective and highly selective media (BCYE-AB or BCYE-GVPC or BCYE-MWY).
3. For samples with a high number of contaminants: inoculate the non-concentrated, concentrated and diluted 1:10 sample; divide each sub-sample into three aliquots: one untreated, one treated with heat and one treated with acids; inoculate 0.1-0.5 mL of each aliquot on a selective medium plate (BCYE-GVPC or BCYE-MWY).
4. For samples with a very high number of contaminants: inoculate the un-concentrated and diluted sample 1:10 and 1: 100 after a pre-treatment with a combination of heat followed by the acid solution. Prepare dilutions with the appropriate diluent after acid treatment. After vortexing, inoculate 0.1-0.5 mL of each aliquot on a selective medium (BCYE-GVPC or BCYE-MWY) plate.

Allow the inoculum to absorb well then incubate the inverted plates in a humid atmosphere at 36 ± 2°C for 7-10 days, observing the plates at days 2, 3, 4, 5 and then at the end of the incubation period.
The procedural elements reported above are entirely schematic. For details of *Legionella* counting techniques in water, refer to the ISO 11731 Standard[4] or other applicable guidelines.

Confirmation of the colonies

A first criterion to differentiate *Legionell*a colonies is their inability to grow, with rare exceptions (*L. oakridgensis*, *L. jordanis*, and *L. nagasakiensis, L. spiritensis*)[2,4,12], on medium lacking L-cysteine.
When there is only one colony type, pick three presumptive colonies; if more morphological different types of presumptive colonies of *Legionella* are growing on the plate, take at least one colony from each type.[4]
Subculture onto a plate of BCYE w/cysteine and a plate of BCYE w/o cysteine.
Be careful not to carry over any culture media with the colony and first inoculate a plate of Legionella Agar w/o Cysteine.
Incubate at 36 ± 2°C for 2 to 5 days.[4]

READING AND INTERPRETATION

Isolation and enumeration - After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristic of the colonies. *Legionella* spp. colonies begin to appear on culture plates on day 2 of incubation. It is very unusual for the bacterial colonies to appear on plates after 5 days of incubation. Some very rarely isolated *Legionella* spp. may require up to 14 days of incubation before growth appears; this is an extremely rare event. Regardless, it is reasonable to inspect culture plates on days 2 to 5 ad than again at day 14.[1]
In the first 24-36 hours of incubation the observation of the plate under a low power binocular microscope with incident light illuminating the agar surface at an acute angle may help in the *Legionella* and contaminants colonies recognition.
Legionella colonies, in principle, appear white-grey, with entire, shiny edges, rounded with a diameter of 1 to 4 mm. Generally, and especially in the first 2 days of incubation, the edge shows a pink or blue-green iridescence while the centre is opalescent gray with an appearance similar to ground glass. Observed under UV lamp (366 nm), some species (*L. anisa, L. bozemanii, L cherrii, L. dumoffii, L. gormanii, L. gratiana, L. parisiensis, L. steigerwaltii* and *L. tucsonensis*) show a blue-white auto-fluorescence, others (*L.erythra and L.rubrilucens*) a bright red auto-fluorescence. *L. pneumophila* and common legionellae, normally do not show auto-fluorescence. With the prolongation of the incubation time, the colonies become wider, the centre assumes a creamy white colour and lose much of their iridescence. A common feature of *Legionella* colonies is the difficulty in taking them with the loop from the surface of the agar.
For the details of *Legionella* spp. enumeration in water samples consult the ISO Standard.[4]
Confirmation of the colonies - After incubation, observe the bacterial growth on both inoculated plates. Regard as *Legionella* those colonies which grow on the plate of BCYE w/cysteine but fail to grow on the plate of BCYE w/o cysteine.
Presumptive identification should be completed by Gram staining prepared from cysteine containing agar only: *Legionella* cells are Gram-negative poorly/faintly staining thin rods, which may be filamentous in older cultures.[4]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of *Legionella* strains and non-target microorganisms must be made depending on of the prepared, selective or non-selective, media and the field of application (clinical or water analysis). Consult the quoted literature for the details of the quality control procedures.[13,14,15]

LIMITATIONS OF THE METHOD

- Some legionellae cannot be grown on routine Legionella culture media and have been termed Legionella-like amoebal pathogens (LLAPs), because they grow in certain host species of amoeba.[16]
- Colonies of *Legionella* grown on white membrane filters may have a different appearance to those that develop against a black or dark background filter.
- Feeley et al.[5] recommend not to incubate the medium in CO_2 higher than 2.5% due to the possibility that *L. pneumophila* growth may be inhibited.
- The glycine contained in the medium may inhibit some of non-*pneumophila* strains.[17]
- Selective BCYE media that contain vancomycin may not support the growth of all *Legionella* spp.[18]
- Culture media performance is a critical factor in the isolation of legionellae from respiratory samples. It has been reported[3] that WY media yielded significantly higher isolation rates than GVPC and BCYE media in regard to performance with samples that harboured low *Legionell*a inocula and high contamination levels.
- Not all *Legionella*-positive samples may be identified by a single culture method. A combination of non-selective and selective media is strongly recommended.[1,12,19]
- The plates with characteristic growth and with colonies presumptively identified as *Legionella*, must undergo confirmation tests with biochemical, immunological, molecular or mass spectrometry techniques. If relevant, perform antimicrobial susceptibility testing.
- In clinical microbiology, the diagnosis of legionellosis must be based on an interdisciplinary approach that includes radiological results, cultural results, determination of urinary antigen. Legionella BCYE Agar Base and the supplements are intended as an aid to the diagnosis of the infection: the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Supplements, ready-to-use plates and flasks
Store the products in the original package at +2°C /+8°C away from direct light.

REFERENCES

1. Edelstein PH, Luck C. *Legionella. In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
2. Mercante JW, Winchell JM. Current and Emerging Legionella Diagnostics for Laboratory and Outbreak Investigations. Clin Microbiol Rev. 2015; 28:95-147
3. Descours G, Cassier P, Forey F, Ginevra C, Etienne J, G. Jarraud LS. Evaluation of BMPA, MWY, GVPC and BCYE media for the isolation of Legionella species from respiratory samples. J Microbiol Meth 2014; 98:119–121
4. ISO 11731:2017 Water quality — Enumeration of Legionella
5. Feeley JC, Gibson RJ, Gorman GW, Langford NC, Rasheed JK, Mackel DC, Baine WB, Charcoal-yeast extract agar: primary isolation medium for Legionella pneumophila, J Clin Microbiol 1979; 10:437-441.
6. Edelstein P.H., Improved semiselective medium for isolation of Legionella pneumophila from contaminated clinical and environmental specimens. J Clin Microbiol 1981; 14:298-303
7. Pasculle AW, Feeley JC, Gibson RJ et al. Pittsburgh Pneumonia Agent: Direct Isolation from Human Lung Tissue. J Infect Dis 1980; 141:727.
8. Wadowsky RM, Yee RB.. Glycine-Containing Selective Medium for Isolation of Legionellaceae from Environmental Specimens. Appl Environ Micro 1981; 42:768-772
9. Dennis PJL, Bartlett CLR, Wright AE. 1984. Comparison of Isolation Methods for Legionella spp. In Thronsbury, C. et al. (ed.) Legionella: Proceedings of the 2nd International Symposium. Washington, D.C. ASM.; 294- 296.
10. Vickers RM, Brown A, Garrity GM. Dye-containing BCYE medium for differentiation of members of the family Legionellaceae. J Clin Microbiol 1981; 13:380.
11. Edelstein PH Comparative Study of Selective Media for Isolation of Legionella pneumophila from Potable Water.J Clin Microbiol 1982; 16:697.
12. Public Health England. UK Standards for Microbiology Investigations. Identification of Legionella species. ID18, Issue no: 3, Issue date: 14.04.15
13. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media
14. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
15. The Australian Society for Microbiology. Guidelines for Assuring Quality of Medical Mycological Culture Media. 2012
16. Legionella and the prevention of legionellosis- Edited by: Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S. World Health Organization 2007.
17. Lück PC, Igel L, Helbig JH, Kuhlisch E, Jatzwauk L. Comparison of commercially available media for the recovery of Legionella species. Int J Hyg Environ Healt 2004; 207(6):589-93.
18. Lee TC, Vickers RM, Yu VL, Wagener MM. Growth of 28 Legionella spp on selective culture media: a comparative study. J Clin Microbiol 1993;31(10):2764-8.
19. Kusnetsov JM, Jousimies-Somer HR, Nevalainen AI, Martikainen PJ. Isolation of Legionella from water samples using various culture methods. J Appl Bacteriol. 1994 76(2):155-62.

PACKAGING

Product	Type	REF	Pack
Legionella BCYE Agar Base	Dehydrated medium	4015822 4015824	500 g (20 L) 5 kg (200 L)
Legionella BCYE α-Growth Supplement	Lyophilized supplement	423210	4 vials, each for 500 mL of medium
Legionella BCYE α-Growth Supplement w/o Cysteine	Lyophilized supplement	423212	4 vials, each for 500 mL of medium
Legionella GVPC Selective Supplement	Lyophilized supplement	423215	4 vials, each for 500 mL of medium
Legionella AB Selective Supplement	Lyophilized supplement	423225	10 vials, each for 500 mL of medium For water microbiological control only
Legionella MWY Selective Supplement (ISO)	Lyophilized supplement	423220	4 vials, each for 500 mL of medium For water microbiological control only
Legionella Selective Agar (GVPC)	Ready-to-use plates	549995 499995	2 x 10 plates ø 90 mm 6 x 5 plates ø 55 mm
Legionella Agar (BCYE)	Ready-to-use plates	549945	2 x 10 plates ø 90 mm
Legionella Agar w/o Cysteine	Ready-to-use plates	549943	2 x 10 plates ø 90 mm
Legionella Selective Agar MWY ISO	Ready-to-use plates	549948	2 x 10 plates ø 90 mm
Legionella BCYE Agar Base	Ready-to-use flasks	5115824	6 x 180 mL

IFU rev 9, 2022/04

LETHEEN BROTH AOAC

Dehydrated culture medium

INTENDED USE

Liquid medium for determining the phenol coefficient of cationic surface-active materials.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	5.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Lecithin	0.7 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Quisno, Gibby and Foter[1] found that the addition of lecithin and polysorbate 80 to a liquid medium resulted in a neutralisation of high concentrations of quaternary ammonium salts. This new medium was called “letheen broth”, an expression of the contracted name of the main components: lecithin and tween. The medium was originally developed as a subculture broth for the neutralization of quaternary ammonium salts. Letheen Broth AOAC is used for determining the phenol coefficient of cationic surface-active materials[2] according to AOAC procedures.[3] It is recommended also for recovering bacteria from the solutions containing residues of sanitizers from food utensils and equipment.
Phenol coefficient is the measure of the disinfecting power of a substance, determined by dividing the figure indicating the degree of dilution of the disinfectant that kills a microorganism within a given time by that indicating the degree of dilution of phenol killing the microorganism under similar conditions.
Letheen Broth contains beef extract and a meat peptone which provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Lecithin and polysorbate 80 neutralize quaternary ammonium compounds, phenols, hexachlorophene, formalin.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 20.7 g in 1000 ml of cold purified water, add 5 g of polysorbate 80 (Tween® 80 REF 42120502), heat to boiling with frequent agitation to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Disinfectants and products containing disinfectants. Refer to applicable International Standards and regulations and operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE, READING AND INTERPRETATION

Consult the current edition of the appropriate references[3,4] for the recommended procedure for sample preparation, inoculation, analysis and interpretation of results.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24H / A	good growth
P. aeruginosa ATCC 27853	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared medium may be stored at 2-8°C for 4 weeks.[2]

REFERENCES

1. Quisno R, Gibby IW, Foter MJ. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am J Pharm Sci Support Public Health 1946; 118:320-323
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
3. AOAC International. Official Methods of Analysis 21st Edition; 2019.
4. US Environmental Protection Agency Office of Pesticide Programs. Standard Operating Procedure for AOAC Use Dilution Method for Testing Disinfectants. Online Revision 01-17-20.

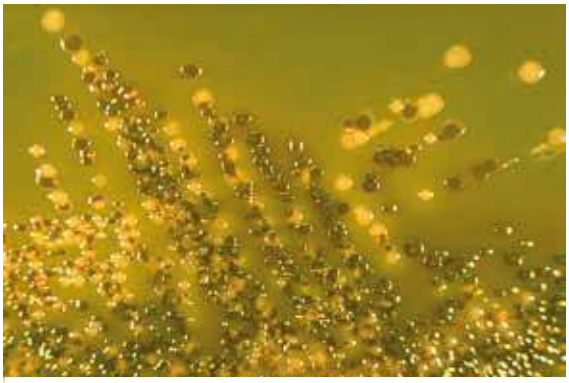
PACKAGING

Product	Type	REF	Pack
Letheen Broth AOAC	Dehydrated medium	4015912	500 g (24.2 L)

IFU rev 1, 2022/08

LEVINE EMB BLUE AGAR

Dehydrated and ready-to-use culture medium



Levine EMB Blue Agar: colonies of *E. coli* with greenish metallic sheen and S. Typhimurium (cream colonies)

INTENDED USE

In vitro diagnostics. Medium for the isolation and differentiation of *Enterobacteriaceae* from clinical and non-clinical specimens.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Peptone	10.000 g
Lactose	10.000 g
Dipotassium hydrogen phosphate	3.000 g
Eosin yellow	0.400 g
Methylene blue	0.065 g
Agar	14.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Levine EMB Blue Agar is prepared on the basis of the formulation described by Levine in 1918[1], as a modification of the Holt-Harris & Teague EMB (HHT) medium of 1916[2]. Compared to the latter, Levine EMB Agar contains a single sugar, lactose, at higher concentration; according to Levine, this modification better differentiates between the species that are now called *Escherichia coli* and *Enterobacter aerogenes*.[1]

Levine EMB Blue Agar is a versatile, moderately selective medium for the isolation and differentiation of *Enterobacteriaceae* based on the fermentation of lactose, from clinical specimens and other materials. Its use for cosmetics[3], food[4], dairy products[5], water[6] and pharmaceutical products[7] has been described. Levine EMB Blue Agar can also be used for the isolation and differentiation of *Candida* spp. from specimens of clinical origin, adding to the medium chlortetracycline HCl 0.1 g/L.[8]

Peptone provides nitrogen, carbon, minerals for microbial growth; lactose is incorporated as a fermentable carbohydrate; eosin yellow and methylene blue have a slight inhibitory activity towards Gram-positive microorganisms; the optimal ratio between the contents of the two dyes is required for the differentiation of lactose-fermenting enteric bacteria from lactose non-fermenters. The differentiation between *E. coli* and *E. aerogenes* is made possible by the presence of phosphate buffer which minimizes the acidifying effects produced by the slow fermentation of lactose by *E. aerogenes*.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37.5 g in 1000 mL of cold purified water. Heat to boiling, stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to about 60°C and before distributing to plates gen-tly shake the flask to oxidise the medium and to disperse the floc-culent precipitate that is formed during sterilisation. Flocculent precipitate should not be removed.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder
Solution appearance	green-violet, flocculent, hazy
Prepared plates appearance	green-violet, limpid or slightly hazy
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

Levine EMB Blue Agar is intended for the bacteriological processing a variety of clinical specimens on which detect *Enterobacteriaceae*. Good laboratory practices for collection, transport and storage of the specimens should be applied. For non-clinical samples, refer to the applicable international standards.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Clinical specimens

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic conditions at 35-37°C for 18-24 hours.

For the isolation of *Candida* spp. with the medium supplemented with chlortetracycline, incubate at 35-37°C for 24-48 hours with 10% CO_2.

Non-clinical specimens

Consult applicable norms and standards for details of operating procedures.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

E. coli colonies are 2-3 mm in diameter, slightly raised, concave, rarely convex; they are violet-cyclamen with a darker centre that extends for about 3/4 of the diameter, with greenish metallic sheen.

E. aerogenes colonies are convex with a diameter of about 4-6 mm, pink to lavender in colour, with a darker centre smaller than that observed with *E. coli*; they are normally free of greenish metallic sheen.

The colonies of lactose non-fermenting organisms (*Salmonella, Shigella, Proteus* etc.) are transparent, amber or pink or colourless.

C. albicans and other yeasts grow with, small, dull, round, smooth colonies, appearing “spidery” or “feathery”.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth, violet-cyclamen colonies with a darker centre with metallic sheen
E. aerogenes ATCC 13048	35-37°C / 18-24 H / A	growth, dark pink colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 H / A	growth, colourless or whitish colonies
E. faecalis ATCC 19433	35-37°C / 18-24 H / A	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Levine EMB Blue Agar is only moderately selective; some staphylococci, streptococci and yeasts grow exhibiting small, pinpoint colonies. As well, other Gram negative non fermenting bacilli exhibit growth appearing as non-lactose fermenters (e.g. *Aeromonas*, *Acinetobacter* and *Pseudomonas*).[9]
- Some strains of *Salmonella* and *Shigella* will not grow on the medium.[9]
- Store prepared medium in the dark at 2-8°C; the photosensitive dyes in the medium may inhibit growth of certain bacteria, mainly *Proteus*, if stored in light.[10]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for 6-8 weeks.

Ready-to-use plates

Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Holt-Harris JE, Teague O. A new culture medium for the isolation of Bacillus typhosus from stools. J Inf Dis 1916; 18:596-600
2. Levine M. Differentiation of B coli and B aerogenes on a simplified eosin-methylene blue agar J Inf Dis 1918; 23:43-47
3. Curry, Graf and McEwen (ed.). 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
4. U.S. Food and Drug Administration Bacteriological Analytical Manual, 8th Edition, Revision A, 1998.
5. Standard Methods for the Examination of Dairy Products, 13th Ed. APHA, 1972
6. Standard Methods for the Examination of Water and Wastewater, 14th Ed APHA, 1975
7. United States Pharmacopoeia XXI (1985) Microbial. Limit Tests. Rockville. Md.
8. Weld JT. Candida albicans: Rapid identification in pure culture with carbon dioxide on Modiofied Eosine Methylene Blue medium. Arch Dermat Syph 1952; 66: 691-694
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. Girolami RL Stamm JM (1976) Inhibitory Effect of Light on Growth-Supporting Properties of Eosin Methylene Blue Agar Appl Environ Microbiol 1976;31: 141–142

PACKAGING

Product	Type	REF	Pack
Levine EMB Blue Agar	Dehydrated medium	4015952 4015954	500 g (13.3L) 5 kg (133 L)
Levine EMB Blue Agar	Ready-to-use plates	541595	2 x 10 plates ø 90 mm

IFU rev 3, 2022/03

LINDEN GRAIN BROTH

Dehydrated culture medium

INTENDED USE

Liquid medium for media fill process simulation of beverage bottling.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Glucose	20.0 g
Yeast extract	3.5 g
Tryptone	2.0 g
Ammonium sulphate	2.0 g
Potassium dihydrogen phosphate	1.0 g
Magnesium sulphate	1.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The bottling plants are validated after installation and subsequently according to quality assurance plans. The media fill process simulation is a part of the validation of an aseptic manufacturing and is performed using a nutrient medium to obtain an accurate overall assessment of the filling process with regard to microbial contamination.

Linden Grain Broth is a standardised nutrient liquid medium designed for media fill process simulation of aseptic beverage bottling.

It is a highly nutritious medium due to the presence of tryptone which provides nitrogen, amino acids, minerals for microbial growth, yeast extract which is a source of vitamins, especially of B-group and a high glucose concentration which is a source of carbon and energy. The medium at pH 4.2 allows the growth of organisms (fungi and acidophilic bacteria) that can spoil low-acid beverages such as soft drinks and juices.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 29.5 g in 1000 ml of cold purified water. Mix thoroughly and heat if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	4.2 ± 0.2

SPECIMENS
Aseptic beverage bottling plants.

TEST PROCEDURE
Perform media fill procedure. Fill bottles or cans with sterile Linden Grain Broth.
Incubate bottles or cans up to 7 days at 25°C.

READING AND INTERPRETATION
The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of growth is closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	25°C / 72H / A	good growth
A. brasiliensis ATCC 9642	25°C / 72H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

PACKAGING

Product	Type	REF	Pack
Linden Grain Broth	Dehydrated medium	4015874	5 kg (169.5 L)

IFU rev 3, 2022/09

LISTERIA BUFFERED ENRICHMENT BROTH

Dehydrated culture medium

INTENDED USE
Buffered enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in food and environmental samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	17.00 g
Soy peptone	3.00 g
Yeast extract	6.00 g
Sodium chloride	5.00 g
Glucose	2.50 g
Dipotassium hydrogen phosphate	9.60 g
Potassium dihydrogen phosphate	3.85 g
Acriflavine HCl	15.00 mg
Nalidixic acid	40.00 mg
Cycloheximide	50.00 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Listeria Buffered Enrichment Broth is a modification of the formulation devised by Lovett et al.[1] with added buffering strength and is used for the detection of *L. monocytogenes* in food and environmental samples. The enrichment and selective properties have been improved by increasing the buffering capacity of the original medium. Casein peptone, yeast extract and soy peptone provide essential nitrogen and carbon-based nutrients, vitamins and trace elements for microbial growth; glucose is a carbohydrate that increases the growth rate of *Listeria*; phosphates act as a buffer system; sodium chloride maintains osmotic balance. Selectivity is provided by cycloheximide, an antifungal compound, nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine, an acridine derivative with bacteriostatic properties towards many Gram-positive bacteria and weak antifungal activity. Because all these antimicrobials are thermostable, they are included in the powdered medium and can be sterilised by autoclaving.[2,3]

DIRECTIONS FOR MEDIA PREPARATION
Suspend 47 g in 1000 mL of cold purified water. Mix thoroughly and warm if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	pale yellow, limpid
Final pH of complete media (at 20-25°C)	7.2 ± 0.2

SPECIMENS
Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Add 25 g of sample to 225 mL of Listeria Buffered Enrichment Broth. Blend until the test portion is thoroughly dispersed.
Incubate the enrichment medium for 24 and 48 hours at 30°C.
After 24- and 48-hours incubation, streak a loopful of the enrichment culture onto ALOA Agar plate (code 401605) and PALCAM Agar plate (code 401604) or Oxford Agar plate (code 401600).
Incubate at 37°C and examine for the presence of typical colonies at both 24 h and 48 h.

READING AND INTERPRETATION
After incubation, *Listeria* spp. produce a turbidity into the enrichment broth. After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 19111	30°C / 48h / A	growth
S. aureus ATCC 25923	30°C / 48h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.
- Techniques for the detection of Listeria in foods vary, depending on material under examination and local laws. Refer to various compendia or to national regulations for the complete procedures.

STORAGE CONDITIONS
Store at +10/+30°C away from direct light in a dry place.

REFERENCES

1. Lovett J, Francis DW, Hunt JM. Listeria monocytogenes in Raw Milk: Detection, Incidence, and Pathogenicity. J Food Prot 1987; 50:188-19
2. Martindale The Extra Pharmacopoeia (1982) Twenty-eighth Edition. The Pharmaceutical Press, London.
3. Haley, L.D., Trandel, J.B., Coyle, M.B. (1980) Practical methods for culture and identification of fungi in the clinical microbiological laboratory. Cumitech n. 11, ASM, Washington, D.C.

PACKAGING
Product	Type	REF	Pack
Listeria Buffered Enrichment Broth	Dehydrated medium	401601B2	500 g (10.5 L)

IFU rev 1, 2022/07

LISTERIA ENRICHMENT BROTH
Dehydrated culture medium

INTENDED USE
Selective enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in food and environmental samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Tryptic digest of casein	17.0 g
Soy peptone	3.0 g
Yeast extract	6.0 g
Sodium chloride	5.0 g
Glucose	2.5 g
Dipotassium hydrogen phosphate	2.5 g
Acriflavine HCl	15.0 mg
Nalidixic acid	40.0 mg
Cycloheximide	50.0 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Listeria Enrichment Broth (LEB) is based on the formula devised by Lovett et al. in 1987 as an alternative to the cold enrichment procedure.[1]
This medium has been shown to recover an inoculum of less than 10 CFU/mL from raw milk.
Casein peptone and soy peptone provide essential nitrogen, carbon-based nutrients, and trace elements for microbial growth; yeast extract is a source of vitamins, particularly of the B-group, for growth stimulation. Glucose is a carbohydrate that increases the growth rate of *Listeria*; dipotassium phosphate is used as buffering agent to control the pH in the medium; sodium chloride maintains osmotic balance. Selectivity is provided by cycloheximide, an antifungal compound, nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine, an acridine derivative with bacteriostatic properties towards many Gram-positive bacteria and weak antifungal activity. Because all these antimicrobials are thermostable, they are included in the powdered medium and can be sterilised by autoclaving.[2,3]

DIRECTIONS FOR MEDIA PREPARATION
Suspend 36.1 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder. Distribute and sterilize by autoclaving at 115°C for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	pale yellow, limpid
Final pH of complete media (at 20-25°C)	7.3 ± 0.2

SPECIMENS
Foods, dairy products, environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards

TEST PROCEDURE
- Add a test portion of 25 g or 25 mL to 225 mL of Listeria Enrichment Broth and homogenize.
- Incubate at 30 °C for 48 h.
- Streak the enrichment broth onto one or more plating-out media (e.g., ALOA, Oxford or PALCAM plates).
- Incubate the agar plates at 37°C for 24-24 ± 2 hours.

READING AND INTERPRETATION
After incubation, *Listeria* spp. produce a turbidity into the enrichment broth.
After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Follow the procedure described by International Standards for the identification of colonies.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[2]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 19111	30°C / 48h / A	growth
S. aureus ATCC 25923	30°C / 48h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *L. monocytogenes* must be confirmed by suitable tests.
- Injured *Listeria* cells may fail to grow on this medium.[4]
- Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or national regulations for the complete procedures.

STORAGE CONDITIONS
Store at +10/+30°C away from direct light in a dry place.

REFERENCES

1. Lovett J, Francis DW, Hunt JM. Listeria monocytogenes in Raw Milk: Detection, Incidence, and Pathogenicity. J Food Prot 1987; 50:188-19
2. Martindale The Extra Pharmacopoeia (1982) Twenty-eighth Edition. The Pharmaceutical Press, London.
3. Haley, L.D., Trandel, J.B., Coyle, M.B. (1980) Practical methods for culture and identification of fungi in the clinical microbiological laboratory. Cumitech n. 11, ASM, Washington, D.C.
4. APHA Compendium of Methods for the Microbiological Examination of foods 4th ed.

PACKAGING
Product	Type	REF	Pack
Listeria Enrichment Broth	Dehydrated medium	4016012 4016014	500 g (13.8 L) 5 kg (138.5)

IFU rev 1, 2022/07

LISTERIA UVM1 ENRICHMENT BROTH
Dehydrated and ready-to-use culture medium

INTENDED USE
Primary enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in foods according to USDA-FSIS.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE FLASKS

Proteose peptone	5.00 g
Tryptone	5.00 g
Beef extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Sodium phosphate bibasic	12.00 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Acriflavine	12.00 mg
Nalidixic acid	20.00 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Although improved control measures since the 1990s have significantly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meat and meat products, it remains a significant cause of foodborne illness.[1]
Identification traditionally involves culture methods based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.[2]
ISO, FDA, USDA-FSIS protocols differ in the recommended culture media but they all involve one or more enrichment steps followed by plating into one or two selective isolation media.

Originally described by Donnelly and Baigent[3], the University of Vermont Modified (UVM) medium was adjusted by McClain and Lee[4] decreasing the nalidixic acid concentration and increasing the acriflavine concentration. Listeria UVM1 Enrichment Broth corresponds to the formulation modified by McClain and Lee and meets the requirements of USDA-FSIS.[5]
Listeria UVM1 Enrichment Broth is recommended as primary enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in food according to USDA-FSIS MLG 8.11,[6,7] followed by a secondary enrichment in MOPS-BLEB.
Essential growth factors for microbial growth are provided by peptones which are sources of nitrogen, carbon and minerals and by yeast extract which is a source of vitamins, particularly of the B-group. Phosphates are used as buffering agents to control the pH in the medium. Selectivity is provided by the presence of nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine, an acridine derivative with bacteriostatic properties towards many Gram-positive bacteria and weak antifungal activity. The high salt (NaCl) tolerance of *Listeria* is used to inhibit growth of enterococci. Esculin is hydrolysed by all *Listeria* species.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 54.4 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder. Distribute a suitable volume of broth in flasks and autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared flasks appearance	yellow, limpid
Final pH of complete media (at 20-25°C)	7.2 ± 0.2

SPECIMENS

Red meat, poultry, ready-to-eat siluriformes (fish) and egg products, and environmental samples. When collecting, storing, transporting and preparing food samples, follow the rules of good laboratory practice and refer to USDA-FSIS document MLG 8.11.[6]

TEST PROCEDURE

1. Perform the primary enrichment by adding 225 ml of Listeria UVM1 Enrichment Broth to 25 g or 25 ml of sample. Homogenise for 2 minutes and incubate at 30° ± 2°C for 20-26 hours.
2. Transfer 0.1 mL from the UVM broth into 10 mL of MOPS-BLEB (REF 401601M) and incubate at 35° ± 2°C for 18-24 hours
3. At the same time, from the primary enrichment broth streak 0.1 mL onto a MOX medium plate (401601 Listeria Oxford Agar Base + 4240039 MOX COL Selective Supplement) and incubate at 35° ± 2°C for 24-28 hours.
4. Use the culture in MOPS-BLEB for inoculating a second MOX medium plate (culture procedure only) or for molecular detection of *Listeria monocytogenes*.

READING AND INTERPRETATION

After incubation, *Listeria* spp. produce a turbidity into the enrichment broth.
After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Follow the procedure described by USDA-FSIS MLG method 8.11[6] for the identification of colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 19117	30°C / 24h / A	good growth
E. coli ATCC 25922	30°C / 24h / A	inhibited
E. faecalis ATCC 29212	30°C / 24h / A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.
- Techniques for the detection of *Listeria* in foods vary, depending on the material under examination and local laws. Refer to various compendia or national regulations for the complete procedures.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C / +30° C away from direct light in a dry place.
Ready-to-use medium in flasks
Store flasks in their original pack at 2-8°C away from direct light.

REFERENCES

1. Buchanana RL *et al*. A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments Food Control Volume 75, May 2017, Pages 1-13
2. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. FEMS Microbiol Rev. 2005 Nov;29(5):851-75
3. Donnelly CW, Baigent GJ. Method for flow cytometric detection of Listeria monocytogenes in milk. Appl Environ Microbiol1986; 52:689.
4. McClain D, Lee WH. Development of USDA-FSIS method for isolation of Listeria monocytogenes from raw meat and poultry J Ass Off Assol Chem. 1988; 71: 660
5. Laboratory Guidebook, Notice of Change: Media and Reagents. USDA-FSIS, Chapter MLG Appendix 1.09, 12/29/2017.
6. Laboratory Guidebook, Notice of Change: Isolation and Identification of Listeria monocytogenes from Meat, Poultry, Ready to Eat Siluriformes (Fish) and Egg Products, and Environmental Sponges. USDA-FSIS, Chapter MLG 8.11, 1/02/2019.
7. Laboratory Guidebook, Notice of Change: Flow Chart Specific for FISI Isolation and Identification of Listeria monocytogenes - Isolation and Identification of Listeria monocytogenes (Culture Method only). USDA-FSIS, Chapter MLG 8 Appendix 1.4, 1/02/2019.

PACKAGING

Product	Type	REF	Pack
Listeria UVM1 Enrichment Broth	Dehydrated medium	4015982 4015984	500 g (9.2 L) 5 kg (92L)
Listeria UVM Broth	Ready-to-use flasks	5115983	6 x 225 mL

IFU rev 1, 2022/07

LISTERIA FRASER BROTH BASE
LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)
LISTERIA FRASER BROTH

Dehydrated culture medium, supplement and ready-to-use tubes and flasks

INTENDED USE

Listeria Fraser Broth Base, with the addition of ferric ammonium citrate, is used for secondary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain (ISO 11290-1).

COMPOSITION *

LISTERIA FRASER BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Enzymatic digest of animal tissue	5.00 g
Enzymatic digest of casein	5.00 g
Meat extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Disodium hydrogen phosphate anhydrous°	9.50 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Lithium chloride	3.00 g
Acriflavine HCl	25.00 mg
Nalidixic Acid	20.00 mg

LISTERIA FRASER BROTH
READY-TO-USE TUBES AND FLASKS, TYPICAL FORMULA

Enzymatic digest of animal tissue	5.00 g
Enzymatic digest of casein	5.00 g
Meat extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Disodium hydrogen phosphate anhydrous°	9.50 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Lithium chloride	3.00 g
Acriflavine HCl	25.00 mg
Nalidixic Acid	20.00 mg
Ferric Ammonium Citrate	0.50 g

LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)

	VIAL CONTENTS FOR 500 mL OF MEDIUM REF 4240056	VIAL CONTENTS FOR 5 L OF MEDIUM REF 42185056 and 42185056A
Ferric Ammonium Citrate	0.25 g	2.5 g

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.
° Equivalent to 12 g of disodium hydrogen phosphate dihydrate

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Although improved control measures since the 1990s have significantly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meat and meat products, it remains a significant cause of foodborne illness.[1]
Identification traditionally involves culture methods based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.[2]
ISO,[3,4] FDA,[5] USDA-FSIS[6] protocols differ in the recommended culture media but they all involve one or more enrichment steps followed by plating into one or two selective isolation media. Fraser Broth was developed by Judy A. Fraser and William H. Sperberby[7] by a modification of the USDA secondary enrichment broth through the addition of lithium chloride and ferric ammonium citrate. The efficacy of Fraser Broth was documented by testing a wide range of food and environmental samples from food processing facilities. Listeria Fraser Broth Base contains all the basic ingredients with the exception of ferric ammonium citrate which is contained in the freeze-dried supplement that enable the complete medium Fraser Broth to be prepared. Acriflavine and nalidixic acid, being thermostable, are included in the medium base.
Fraser Broth is used for secondary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples from the food chain according to ISO 11290-1.[3]
Peptones and yeast extract provide nitrogen, carbon, vitamins particularly of the B-group and trace elements for microbial growth; phosphates are used as buffering agents to control the pH in the medium. Selectivity is provided by the presence of nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine which inhibits many Gram-positive bacteria; lithium chloride and the high salt (NaCl) tolerance of Listeria are used to inhibit growth of enterococci. Fraser Broth contains double the concentrations of acriflavine and nalidixic acid compared to Half-Fraser Broth. Aesculin is hydrolysed. to glucose and aesculetin (6-7-dihydroxycoumarin): aesculetin reacts with the iron salts in the medium, giving it a brown-black colour.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 27.5 g in 500 mL (or 275 g in 5 litres) of cold purified water. Heat to boiling with frequent agitation to completely dissolve the powder. Autoclave at 121°C for 15 minutes and cool to 25-50°C. To 500 mL of medium add the contents of one vial of Listeria Fraser Supplement (Fe Ammonium Citrate) (REF 4240056) reconstituted with 5 mL of sterile purified water. To 5 litres of medium add the contents of one vial of Listeria Fraser Supplement (Fe Ammonium Citrate) (REF 42185056 or 42185056A) reconstituted with 20 mL of sterile purified water. Mix well and pour into sterile flasks under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared flasks appearance	yellow-brown, limpid
Freeze-dried selective supplements	low, fragile brown pellet; brown opalescent solutions after reconstitution
Final pH of complete media (at 20-25°C)	7.2 ± 0.2

SPECIMENS

Foods, animal deeding stuffs, food chain and environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[3,4]

TEST PROCEDURE

Detection of *Listeria monocytogenes* and *Listeria* spp. (ISO 11290-1)[3]

- In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 g or 225 mL of Half-Fraser Broth, to obtain a tenfold dilution, and homogenize.
- Incubate the primary enrichment medium at 30 °C ± 1°C for 25 h ± 1 h.

- Transfer 0.1 mL of the culture to a tube or bottle containing 10 mL of secondary enrichment medium (Fraser Broth) and incubate for 24 h ± 2 h at 37 °C ± 1°C. In the case of *Listeria* spp. other than *Listeria monocytogenes* detection, additional 24 h incubation can allow for recovery of more species.
- From the primary enrichment culture inoculate, by means of a loop, the surface of the first selective plating medium (Agar Listeria acc. to Ottaviani and Agosti-ALOA, REF 401505), to obtain well-separated colonies. Proceed in the same way with the second selective plating-out medium of choice (e.g., PALCAM or Oxford Agar, REF 401604 or 401600).
- From the secondary enrichment medium, repeat the procedure with the two selective plating-out media.
- Incubate ALOA plates at 37°C ± 1°C for 24 ± 2 hours; if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
- Incubate the second plating out medium according to the instructions for use
- Examine the dishes for the presence of presumptive colonies of *L. monocytogenes* or *Listeria* spp.

Notes
It is possible to store at 5 °C the pre-enriched sample after incubation before transfer to Fraser broth for a maximum of 72 h.
Half-Fraser broth and Fraser broth can be refrigerated at 5 °C before isolation on selective agar for a maximum of 72.
After incubation, ALOA plates can be refrigerated at 5 °C for a maximum of 48 h before reading.

READING AND INTERPRETATION

After incubation, typically *Listeria* spp. produce a blackening of the two enrichment broths.
After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies on plating out media.
With ALOA plates, consider as presumptive *L. monocytogenes* the blue-green colonies surrounded by an opaque halo; consider as presumptive *Listeria* spp. the blue-green colonies with or without opaque halo.
Second plating-out medium: examine for the presence of typical colonies according to the characteristics of the chosen medium.
Confirm typical colonies by the methods and tests indicated in ISO 11290-1 or ISO 11290-2, after purification of the colonies in Tryptic Soy Yeast Extract Agar (REF 402166).
The mandatory confirmatory tests for *L. monocytogenes*, according to ISO 11290 and using ALOA medium, are the following: β-haemolysis (+), carbohydrate utilization (L-rhamnose +; D-xylose -). Optional confirmatory tests for *L. monocytogenes* are: catalase (+), mobility at 25°C (+), CAMP Test (+). The mandatory confirmatory tests for *Listeria* spp. are: microscopic examination, catalase (+); optional tests are: VP (+), mobility at 25°C (+).
Miniaturized galleries for the biochemical identification of *L. monocytogenes* may be used (Listeria Mono Confirm Test REF 193000)

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes +	ATCC 13932	37°C / 24h / A	> 10 typical colonies after subculture on ALOA
E. faecalis +	ATCC 29212		
E. coli	ATCC 25922		
L. monocytogenes +	NCTC 7973	37°C / 24h / A	> 10 typical colonies after subculture on ALOA
E. faecalis +	ATCC 29212		
E. coli	ATCC 25922		
E. faecalis	ATCC 29212	37°C / 24h / A	< 100 colonies after subculture on TSA
E. coli	ATCC 25922	37°C / 24h / A	totally inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Cultures

LIMITATIONS OF THE METHOD

- Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.
- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C/+30°C away from direct light in a dry place.
Freeze-dried supplement and ready-to-use tubes and flasks
Store the products in the original package at +2°C/+8°C away from direct light.

REFERENCES

1. Buchanana RL *et al*. A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments Food Control Volume 75, May 2017, Pages 1-13
2. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. FEMS Microbiol Rev. 2005 Nov;29(5):851-75
3. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
4. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.
5. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods, April 2022.
6. USDA-FSIS. Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat, Siluriformes (Fish) and Egg Products, and Environmental Samples. MLG 8.13, 10/01/2021
7. Fraser JA, Sperber WH. Rapid Detection of Listeria spp. in Food and Environmental Samples by Esculin Hydrolysis. J Food Prot 1988 Oct;51(10):762-765.

PACKAGING

Product	Type	REF	Pack
Listeria Fraser Broth Base	Dehydrated medium	4015962 4015964	500 g (9.1 L) 5 kg (91 L)
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	4240056	10 vials, each for 500 mL of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056	1 vial for 5 L of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056A	9 vials, each for 5 L of medium
Listeria Fraser Broth	Ready-to-use tubes	551596	20 x 10 mL
Listeria Fraser Broth	Ready-to-use tubes	551596N	20 x 9 mL
Listeria Fraser Broth	Ready-to-use flasks	5115963	6 x 225 mL
Listeria Fraser Broth	Ready-to-use flasks	5115962	6 x 90 mL

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LISTERIA FRASER BROTH BASE HALF CONCENTRATION
LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)
LISTERIA FRASER BROTH HALF CONCENTRATION

Dehydrated culture medium, supplement and ready-to-use flasks

INTENDED USE

Listeria Fraser Broth Base Half Concentration, with the addition of ferric ammonium citrate, is used for primary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples of the food chain (ISO 11290-1) and as diluent for sample preparation in the enumeration procedure (ISO 11290-2).

COMPOSITION *

LISTERIA FRASER BROTH BASE HALF CONCENTRATION, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Enzymatic digest of animal tissue	5.00 g
Enzymatic digest of casein	5.00 g
Meat extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Disodium hydrogen phosphate anhydrous°	9.50 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Lithium chloride	3.00 g
Acriflavine HCl	12.50 mg
Nalidixic Acid	10.00 mg

LISTERIA FRASER BROTH
READY-TO-USE FLASKS, TYPICAL FORMULA

Enzymatic digest of animal tissue	5.00 g
Enzymatic digest of casein	5.00 g
Meat extract	5.00 g
Yeast extract	5.00 g
Sodium chloride	20.00 g
Disodium hydrogen phosphate anhydrous°	9.50 g
Potassium dihydrogen phosphate	1.35 g
Aesculin	1.00 g
Lithium chloride	3.00 g
Acriflavine HCl	12.50 mg
Nalidixic Acid	10.00 mg
Ferric Ammonium Citrate	0.50 g

LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)

	VIAL CONTENTS FOR 500 mL OF MEDIUM REF 4240056	VIAL CONTENTS FOR 5 L OF MEDIUM REF 42185056 and 42185056A
Ferric Ammonium Citrate	0.25 g	2.5 g

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.
° Equivalent to 12 g of disodium hydrogen phosphate dihydrate

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Although improved control measures since the 1990s have significantly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meat and meat products, it remains a significant cause of foodborne illness.[1]
Identification traditionally involves culture methods based on selective enrichment and plating followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.[2]
ISO,[3,4] FDA,[5] USDA-FSIS[6] protocols differ in the recommended culture media but they all involve one or more enrichment steps followed by plating into one or two selective isolation media. Fraser Broth was developed by Judy A. Fraser and William H. Sperberby[7] by a modification of the USDA secondary enrichment broth through the addition of lithium chloride and ferric ammonium citrate. The efficacy of Fraser Broth was documented by testing a wide range of food and environmental samples from food processing facilities.
Listeria Fraser Broth Base Half Concentration contains all the basic ingredients with the exception of ferric ammonium citrate which is contained in the freeze-dried supplement that enables the preparation of the complete Half-Fraser Broth medium. Acriflavine and nalidixic acid, being thermostable, are included in the medium base.
Half-Fraser Broth is used for primary enrichment in the procedure for the detection of *Listeria monocytogenes* and *Listeria* spp. in samples from the food chain, according to ISO 11290-1.[3] Half-Fraser Broth can be used for sample preparation in the enumeration procedure according to ISO 11290-2.[4]
Peptones and yeast extract provide nitrogen, carbon, vitamins particularly of the B-group and trace elements for microbial growth; phosphates are used as buffering agents to control the pH in the medium. Selectivity is provided by the presence of nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine which inhibits many Gram-positive bacteria; lithium chloride and the high salt (NaCl) tolerance of *Listeria* are used to inhibit growth of enterococci. Half-Fraser Broth contains half the concentrations of acriflavine and nalidixic acid compared to Fraser Broth. Aesculin is hydrolysed to glucose and aesculetin (6-7-dihydroxycoumarin): aesculetin reacts with the iron salts in the medium, giving it a brown-black colour.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 27.5 g in 500 mL (or 275 g in 5 litres) of cold purified water. Heat to boiling with frequent agitation to completely dissolve the powder. Autoclave at 121°C for 15 minutes and cool to 25-50°C. To 500 mL of medium add the contents of one vial of Listeria Fraser Supplement (Fe Ammonium Citrate) (REF 4240056) reconstituted with 5 mL of sterile purified water. To 5 litres of medium add the contents of one vial of Listeria Fraser Supplement (Fe Ammonium Citrate) (REF 42185056 or 42185056A) reconstituted with 20 mL of sterile purified water. Mix well and pour into sterile flasks under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared flasks appearance	yellow-brown, limpid
Freeze-dried selective supplements	low, fragile brown tablets; brown opalescent solutions after reconstitution
Final pH of complete media (at 20-25°C)	7.2 ± 0.2

SPECIMENS

Foods, animal deeding stuffs, food chain and environmental samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[3,4]

TEST PROCEDURE

Detection of *Listeria monocytogenes* and *Listeria* spp. (ISO 11290-1)[3]

1. In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 g or 225 mL of Half-Fraser Broth, to obtain a tenfold dilution, and homogenize.
2. Incubate the primary enrichment medium at 30 °C ± 1°C for 25 h ± 1 h.
3. Transfer 0.1 mL of the culture to a tube or bottle containing 10 mL of secondary enrichment medium (Fraser Broth) and incubate for 24 h ± 2 h at 37 °C ± 1°C. In the case of *Listeria* spp. other than *Listeria monocytogenes* detection, additional 24 h incubation can allow for recovery of more species.
4. From the primary enrichment culture inoculate, by means of a loop, the surface of the first selective plating medium (Agar Listeria according to Ottaviani and Agosti-ALOA, REF 401505), to obtain well-separated colonies. Proceed in the same way with the second selective plating-out medium of choice (e.g., PALCAM or Oxford Agar, REF 401604 or 401600).
5. From the secondary enrichment medium, repeat the procedure with the two selective plating-out media.
6. Incubate ALOA plates at 37°C ± 1°C for 24 ± 2 hours; if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
7. Incubate the second plating out medium according to the instructions for use
8. Examine the dishes for the presence of presumptive colonies of *L. monocytogenes* or *Listeria* spp.

Notes
It is possible to store at 5 °C the pre-enriched sample after incubation before transfer to Fraser broth for a maximum of 72 h.
Half-Fraser broth and Fraser broth can be refrigerated at 5 °C before isolation on selective agar for a maximum of 72.
After incubation, ALOA plates can be refrigerated at 5 °C for a maximum of 48 h before reading.

Enumeration of *Listeria monocytogenes* and of *Listeria* spp. (ISO 11290-2)[4]

1. Prepare a sample suspension in Buffered Peptone Water or other suitable enrichment broth according to ISO 6887 (all parts); in case both detection and enumeration are performed according to parts 1 and 2 of ISO 11290, the sample suspension may be made in Half-Fraser Broth.
2. Inoculate 0.1 mL of the sample suspension and 0.1 mL of further decimal dilutions onto 90 mm plates of ALOA medium.
3. For samples with suspected low number of target-strains, inoculate 1 mL of the sample suspension and 1 mL of further decimal dilutions onto 140 mm plates of ALOA medium.
4. Examine after incubation at 37°C for 24 ± 2 hours and, if there is no growth or no typical colonies, re-incubate for a further 24 ± 2 hours.
5. Count *L. monocytogenes* colonies and *Listeria* spp. colonies in plates with less than 150 colonies (90 mm diameter plates) or 360 colonies (140 mm plates) according to the section "reading and interpretation".

READING AND INTERPRETATION

After incubation, typically *Listeria* spp. produce a blackening of the two enrichment broths.
After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies on plating out media.
With ALOA plates, consider as presumptive *L. monocytogenes* the blue-green colonies surrounded by an opaque halo; consider as presumptive *Listeria* spp. the blue-green colonies with or without opaque halo.
Second plating-out medium: examine for the presence of typical colonies according to the characteristics of the chosen medium.
Confirm typical colonies by the methods and tests indicated in ISO 11290-1 or ISO 11290-2, after purification of the colonies in Tryptic Soy Yeast Extract Agar (REF 402166).
The mandatory confirmatory tests for *L. monocytogenes*, according to ISO 11290 and using ALOA medium, are the following: β-haemolysis (+), carbohydrate utilization (L-rhamnose +; D-xylose -). Optional confirmatory tests for *L. monocytogenes* are: catalase (+), mobility at 25°C (+), CAMP Test (+). The mandatory confirmatory tests for *Listeria* spp. are: microscopic examination, catalase (+); optional tests are: VP (+), mobility at 25°C (+).
Miniaturized galleries for the biochemical identification of *L. monocytogenes* may be used (Mono Confirm Test REF 193000).

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM		EXPECTED RESULTS
L. monocytogenes +	ATCC 13932	30°C / 24h / A	> 10 typical colonies after subculture on ALOA
E. faecalis +	ATCC 29212		
E. coli	ATCC 25922		
L. monocytogenes +	NCTC 7973	30°C / 24h / A	> 10 typical colonies after subculture on ALOA
E. faecalis +	ATCC 29212		
E. coli	ATCC 25922		
E. faecalis	ATCC 29212	30°C / 24h / A	< 100 colonies after subculture on TSA
E. coli	ATCC 25922	30°C / 24h / A	totally inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Cultures

LIMITATIONS OF THE METHOD

- Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.
- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C/+30°C away from direct light in a dry place.

Freeze-dried supplement and ready-to-use flasks
Store the products in the original package at +2°C/+8°C away from direct light.

REFERENCES

1. Buchanana RL *et al*. A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments Food Control Volume 75, May 2017, Pages 1-13
2. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. FEMS Microbiol Rev. 2005 Nov;29(5):851-75
3. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
4. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.
5. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods, April 2022.
6. USDA-FSIS. Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat, Siluriformes (Fish) and Egg Products, and Environmental Samples. MLG 8.13, 10/01/2021
7. Fraser JA, Sperber WH. Rapid Detection of Listeria spp. in Food and Environmental Samples by Esculin Hydrolysis. J Food Prot 1988 Oct;51(10):762-765.

PACKAGING

Product	Type	REF	Pack
Listeria Fraser Broth Base Half Concentration	Dehydrated medium	4015942 4015944	500 g (9.1 L) 5 kg (91 L)
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	4240056	10 vials, each for 500 mL of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056	1 vial for 5 L of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056A	9 vials, each for 5 L of medium
Listeria Fraser Broth Base Half Concentration	Ready-to-use flasks	5115942	6 x 90 mL
Listeria Fraser Broth Base Half Concentration	Ready-to-use flasks	5115943	6 x 225 mL

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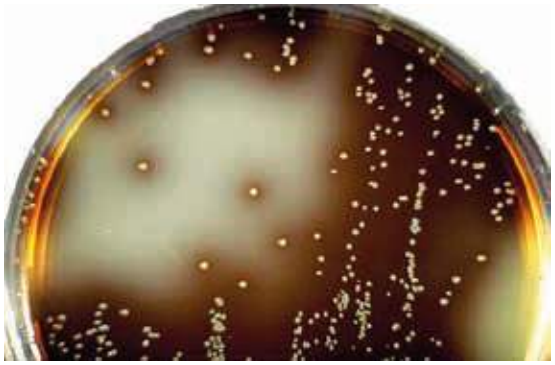
LISTERIA OXFORD AGAR BASE
LISTERIA OXFORD ANTIMICROBIC SUPPLEMENT
LISTERIA MOX-COL ANTIMICROBIC SUPPLEMENT
LISTERIA SELECTIVE AGAR (OXFORD)

Dehydrated culture medium, selective supplements, ready-to use plates



Oxford Medium: colonies of *Listeria monocytogenes*

LISTERIA SELECTIVE AGAR (OXFORD) (READY -TO-USE PLATES)

Listeria Oxford Agar Base	57.4 g
Cycloheximide	400.0 mg
Colistin sulphate	20.0 mg
Cefotetan	2.0 mg
Fosfomycin	10.0 mg
Acriflavine	5.0 mg
Purified water	1000 mL

INTENDED USE

Selective and differential basal medium, selective supplements and ready-to-use plates for the isolation and enumeration of *Listeria* spp. from foodstuffs.

COMPOSITIONS *

LISTERIA OXFORD AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptocomplex	10.00 g
Tryptose	10.00 g
Peptone	3.00 g
Maize starch	1.00 g
Sodium chloride	5.00 g
Aesculin	1.00 g
Ferric ammonium citrate	0.50 g
Lithium chloride	15.00 g
Agar	12.00 g

LISTERIA OXFORD ANTIMICROBIC SUPPLEMENT (VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cycloheximide	200.0 mg
Colistin sulphate	10.0 mg
Cefotetan	1.0 mg
Fosfomycin	5.0 mg
Acriflavine	2.5 mg

LISTERIA MOX-COL ANTIMICROBIC SUPPLEMENT (VIAL CONTENTS FOR 500 mL OF MEDIUM)

Moxalactam	10.0 mg
Colistin sulphate	5.0 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Although improved control measures since the 1990s have significantly reduced the prevalence of *L. monocytogenes* in many food categories, particularly in meat and meat products, it remains a significant cause of foodborne illness.[1]
Identification traditionally involves culture methods based on selective enrichment and plating on chromogenic and aesculin containing media followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.[2]
Listeria Oxford Agar Base is an aesculin based medium prepared without antibiotics and acriflavine; it can be used with Listeria Oxford Antimicrobic Supplement or with Listeria MOX-COL Antimicrobic Supplement for the isolation and enumeration of *Listeria* spp. in foodstuffs.
The complete medium known as "Oxford Medium" is prepared according to the formula developed by Curtis et al.[3] and is recommended by FDA-BAM[4] as one of the aesculin based Listeria selective agars and may be used as second isolation medium as recommended by ISO 11290-1.[5]

The complete Oxford medium contains peptones which provide nitrogen, carbon and minerals for microbial growth. Selectivity is provided by the presence of lithium chloride, active against streptococci, cycloheximide active against yeasts and moulds, cefotetan and fosfomycin active on Gram-positive and Gram-negative bacteria. Aesculin and ferric iron act as indicator system: *Listeria* spp. hydrolyse aesculin, producing black zones around the colonies because of the formation of black iron phenolic compounds derived from the aglucon.
The "MOX" medium is a modification of the formulation described by McClain and Lee[6], with a reduced concentration of moxalactam in order to obtain a better growth of *Listeria* spp. It is recommended by USDA-FSIS[7,8] and FDA-BAM[2] for the detection of *L. monocytogenes*. MOX formulation with moxalactam, colistin and lithium chloride is considered superior for the inhibition of methicillin resistant staphylococci and *Proteus* spp.

Directions for media preparation

Suspend 28.7 g of dehydrated medium in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C.

Oxford medium

Add the content of one vial of Listeria Oxford Antimicrobic Supplement (REF 4240038) reconstituted with 5 mL of a solution of 1:1 ethanol/sterile purified water, under aseptic conditions. Mix well and pour into sterile Petri dishes.

MOX-COL medium

Add the content of one vial of Listeria MOX-COL Antimicrobic Supplement (REF 4240039) reconstituted with 5 mL of sterile purified water, under aseptic conditions. Mix well and pour into sterile Petri dishes.

Physical characteristics

Listeria OXFORD Medium	
Dehydrated medium appearance	beige, fine, free-flowing powder
Solution and prepared plates appearance	amber, slightly opalescent with a blue ring at the surface of the liquid
Final pH at 20-25 °C	7.0 ± 0.2
Listeria OXFORD Antimicrobic Supplement	
Freeze-dried supplement appearance	short, fragile, yellow-orange pellet
Reconstituted supplement appearance	yellow-orange, limpid
Listeria MOX-COL Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless, limpid

Specimens

Food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[4,5,7]

Test procedure

Perform selective enrichment of the sample with the broths recommended by the chosen method of analysis.
Generally, the primary enrichment broth is incubated at 30°C and the secondary enrichment broth is incubated at 37°C for 24 hours.
The enrichment broths recommended by ISO 11290-1 are Half Fraser Broth and Fraser Broth; the selective broths indicated by USDA-FSIS are UVM1 and MOPS-BLEB, while FDA-BAM includes only one medium, Buffered Listeria Enrichment Broth without and with selective agents, with incubation at 30°C for 48 hours.
Streak a loopful of the incubated enriched broth onto the surface of an Oxford Medium plate or MOX-COL Medium plate and of ALOA plate to obtain well isolated colonies.
Examine the plates after incubation at 37°C for 24 ± 2 hours and after 48 ± 4 hours.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
After 24 h incubation at 37° C typical *Listeria* species colonies are approximately 1 mm diameter, grey-brown with brown or black halo.
Following 48 h incubation typical *Listeria* species colonies are approximately 2-3 mm diameter, black with a black halo and sunken centre.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains		Incubation T°/ t / ATM	Expected results
L. monocytogenes	ATCC 19111	37°C / 48 H / A	grey colonies with black-brown halo
E. faecalis	ATCC 19433	37°C / 48 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- The Oxford medium does not allow the differentiation of *L. monocytogenes* from other species of the genus *Listeria*.
- The identification of *L. monocytogenes* must be confirmed by suitable tests.

Storage conditions

Dehydrated medium

Store at +10 °C / +30 °C away from direct light in a dry place.

Selective supplements and ready-to-use plates

Store the products in the original package at +2 °C/ + 8°C away from direct light.

References

1. Buchanana RL *et al*. A review of Listeria monocytogenes: An update on outbreaks, virulence, dose-response, ecology, and risk assessments Food Control Volume 75, May 2017, Pages 1-13
2. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. FEMS Microbiol Rev. 2005 Nov;29(5):851-75
3. Curtis GDW, Mitchell RG, King AF, Emma J. A selective differential medium for the isolation of Listeria monocytogenes. Lett Appl Microbiol1989; 8:95-98.
4. U.S. Department of Health and Human Services, F.D.A. Bacteriological Analytical Manual, Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods, April 2022.
5. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
6. McClain D, Lee WH. Development of USDA-FSIS method for isolation of Listeria monocytogenes from raw meat and poultry J Ass Off Ass Chem. 1988; 71: 660

7. USDA-FSIS. Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat, Siluriformes (Fish) and Egg Products, and Environmental Samples. MLG 8.13, 10/01/2021
8. Laboratory Guidebook, Notice of Change: Media and Reagents. USDA-FSIS, Chapter MLG Appendix 1.09, 12/29/201
9. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:222-4.

PACKAGING

Product	Type	REF	Pack
Listeria Oxford Agar Base	Dehydrated medium	4016002	500 g (8.7 L)
Listeria Oxford Agar Base	Dehydrated medium	4016004	5 kg (87 L)
Listeria Oxford Antimicrobic Supplement	Freeze-dried supplement	4240038	10 vials, each for 500 mL of medium
Listeria MOX-COL Antimicrobic Supplement	Freeze-dried supplement	4240039	10 vials, each for 500 mL of medium
Listeria Oxford Selective Agar	Ready-to-use plates	541600	2 x 10 plates ø 90 mm
Listeria MOX-COL Agar	Ready-to-use plates	541600M	2 x 10 plates ø 90 mm

IFU rev 3, 2022/08

LISTERIA PALCAM AGAR BASE
LISTERIA PALCAM ANTIMICROBIC SUPPLEMENT
LISTERIA SELECTIVE AGAR (PALCAM)

Dehydrated culture medium, selective supplement, ready-to use plates

INTENDED USE

Selective and differential basal medium, selective supplement and ready-to-use plates for the isolation and enumeration of *Listeria* spp. from foodstuffs.

COMPOSITIONS*

LISTERIA PALCAM AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptocomplex	10.00 g
Tryptose	10.00 g
Peptone	3.00 g
Yeast extract	3.00 g
Maize starch	1.00 g
Sodium chloride	5.00 g
Glucose	0.50 g
Mannitol	10.00 g
Aesculin	0.80 g
Ferric ammonium citrate	0.50 g
Lithium chloride	15.00 g
Phenol red	0.08 g
Agar	12.00 g

LISTERIA SELECTIVE AGAR (PALCAM)
READY-TO-USE PLATES

Listeria PALCAM Agar Base	70.8 g
Polymyxin B sulphate	10.0 mg
Ceftazidime	20.0 mg
Acriflavine HCl	5.0 mg
Purified water	1000 mL

LISTERIA PALCAM SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Polymyxin B sulphate	5 mg
Ceftazidime	10 mg
Acriflavine HCl	2.5 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Identification of *Listeria monocytogenes* traditionally involves culture methods based on selective enrichment and plating on chromogenic media and aesculin containing media followed by the characterization of *Listeria* spp. based on colony morphology, sugar fermentation and haemolytic properties.[1]
Listeria PALCAM Agar Base is an aesculin based medium prepared without antibiotics and acriflavine; it is used with Listeria PALCAM Antimicrobic Supplement for the isolation and enumeration of *Listeria* spp. in foodstuffs.
The complete medium, prepared according to the formula developed by Van Netten et al.[2], is recommended by FDA-BAM[3] as one of the aesculin based Listeria selective agars and may be used as second isolation medium as recommended by ISO 11290-1.[4]
PALCAM is the abbreviation for Polymyxin-Acriflavine-Lithium Chloride-Ceftazidime-Aesculin-Mannitol.
The complete PALCAM medium contains peptones which provide nitrogen, carbon and minerals for microbial growth. Selectivity is provided by the presence of lithium chloride, polymyxin B, ceftazidime and acriflavine. The medium uses two indicator systems: hydrolysis of aesculin and fermentation of mannitol. *Listeria* spp. hydrolyse aesculin, producing black zones around the colonies because of the formation of black iron phenolic compounds derived from the aglucon. *Listeria* spp. do not ferment mannitol while the most common contaminants, staphylococci and streptococci ferment it, causing the phenol red to change colour and the growth with colonies surrounded by a yellow halo.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 35.4 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of Listeria PALCAM Antimicrobic Supplement reconstituted with 5 mL of sterile purified water under aseptic conditions. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Listeria PALCAM Agar	
Dehydrated medium appearance	Light pink, fine, free-flowing powder
Solution and prepared plates appearance	Dark red, slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2
Listeria PALCAM Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, yellow-orange pellet
Reconstituted supplement appearance	yellow, limpid

SPECIMENS

Food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[3,4]

Test procedure

Perform selective enrichment of the sample with broths recommended by the chosen method of analysis.
The enrichment broths recommended by ISO 11290-1 are Half Fraser Broth and Fraser Broth, incubated at 30°C and 37°C for 24 hours, respectively, while FDA-BAM includes only one medium, Buffered Listeria Enrichment Broth without and with selective agents, with incubation at 30°C for 48 hours.
Streak a loopful of the incubated enriched broth on the surface of a PALCAM Medium plate and an ALOA plate to obtain well isolated colonies.
Examine the plates after incubation at 37°C for 24 ± 2 hours and after 48 ± 4 hours.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
After 24 h incubation at 35° C, typical *Listeria* spp. colonies are approximately 1 mm diameter, greyish green or olive-green colonies sometimes with black centres, surrounded by a black halo. Following 48 h incubation, typical *Listeria* species colonies are approximately 1.5-2 mm diameter, black with a black halo and sunken centre.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains		Incubation T°/ t / ATM	Expected results
L. monocytogenes	ATCC 19111	37°C / 48 H / A	grey-green colonies with black-brown halo
E. faecalis	ATCC 19433	37°C / 48 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- As *L. grayi* ferments mannitol the colonies may have different characteristics from other species of *Listeria.*
- The PALCAM medium does not allow the differentiation of *L. monocytogenes* from other species of the genus *Listeria*.
- The complete identification of *L. monocytogenes* must be confirmed by suitable tests.

Storage conditions

Dehydrated medium
Store at +10 °C / +30 °C away from direct light in a dry place.
Selective supplement and ready-to-use plates
Store the products in the original package at +2 °C/ + 8°C away from direct light.

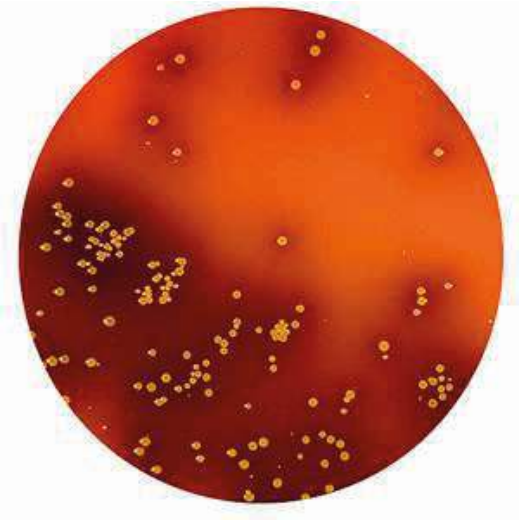
References

1. Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: a review. FEMS Microbiol Rev. 2005 Nov;29(5):851-75
2. Van Netten. et al. Liquid and solid selective differential media for the detection and enumeration of L. monocytogenes and other Listeria spp. - Int. J. Food Microbiol., 1989, vol. 8, p. 299-316.
3. ISO 11290:2017 Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. —Part 1: Detection method
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods. Rev 10/2017

Packaging

Product	Type	REF	Pack
Listeria PALCAM Agar Base	Dehydrated medium	4016042	500 g (7.1L)
Listeria PALCAM Agar Base	Dehydrated medium	4016044	5 kg (71 L)
Listeria PALCAM Antimicrobic Supplement	Freeze-dried supplement	4240042	10 vials, each for 500 mL of medium
Listeria Selective Agar (PALCAM)	Ready-to-use plates	541604	2 x 10 plates ø 90 mm

IFU rev 3, 2023/01



PALCAM Agar: colonies of *Listeria monocytogenes*

LITMUS MILK

Dehydrated culture medium

INTENDED USE

Liquid medium for the differentiation of microorganism based on multiple metabolic reactions and for the maintenance of lactic acid bacteria.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Skim milk	100.00 g
Litmus	0.75 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Litmus Milk has been used for many years as a help in the differentiation of organisms (especially within the genus *Clostridium*) based on multiple metabolic reactions on milk, including fermentation, reduction, clot formation, digestion, and the formation of gas.[1] It is also useful in the maintenance and propagation of lactic acid bacteria.

Litmus incorporated in the medium is both a pH and an oxidation-reduction indicator. Milk contains lactose and three main proteins: casein, lactalbumin and lactoglobulin. At pH 6.5 the medium is pale blue coloured; when inoculated with lactose-fermenting microorganisms, which produce lactic acid and occasionally butyric acid, it becomes pinkish-red through the litmus reaction. Some bacteria, which do not ferment lactose but hydrolyse the casein make the medium alkaline with a fouls smell, turning the medium into a purplish-blue colour. Some organisms remove the oxygen in the medium by means of a reductase, with reduction of the litmus to the white leuco-base.

The peptonisation phenomenon is due to digestion of the casein, which manifests by clearing of the medium. Breakage of the coagulum indicates gas production by the inoculated strain.

Acid production from the fermentation of lactose is shown by a change in colour of the indicator, and, when much acid is produced, by the formation of a clot. But another form of clot may be produced by rennet; in this case the clot forms first and later, like the fibrin clot in blood, contracts and expresses a clear whey. In contrast the acid clot does not contract. When the bacterium also produces proteolytic enzymes, the clot may be peptonized.[2]

DIRECTIONS FOR MEDIUM PREPARATION

Mix 100 g with a small quantity of cold purified water, making a smooth paste and add more purified water until a 10% mixture is obtained (100 g/L). Agitate the mixture continuously and dispense 5-10 mL amounts into suitable screwcap tubes. Sterilise by steaming (100°C) on three successive days for 60, 45 and 80 minutes.

Alternatively, autoclave at 121°C for 5 minutes or at 110°C for 10 minutes.

Overheating must be avoided to prevent caramelization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-grey, fine, homogeneous, free-flowing powder.
Solution appearance	pale blue with pink-blue precipitate, opaque. During autoclaving litmus is reduced to a white coloured base however, upon cooling, oxygen is absorbed and the original colour returns.
Final pH at 20-25 °C	6.5 ± 0.2

SPECIMENS

18-24 h pure broth culture.

TEST PROCEDURE

1. Inoculate with 4 drops of a 18-24-hour pure broth culture.
1. Incubate at 35-37°C in aerobic atmosphere.
2. If *Clostridium* is suspected or anaerobiosis is desired, pour a layer of sterile mineral oil over medium surface immediately after inoculation.[1]
3. Observe daily for seven days for alkaline reaction (litmus turns blue), indicator reduction, acid clot, acid reaction (litmus turns pink), rennet clot, and peptonisation. Longer periods up to 14 days may be necessary.[1,2]
4. Record all changes.

READING AND INTERPRETATION

Multiple changes can occur over the observation period.[1]

1. Pinkish-red: acid reaction; lactose and/or glucose fermented; red: lactose fermented, pink: glucose fermented
2. Purplish-blue: no fermentation of lactose, no change of litmus, same colour of uninoculated tube.
3. Blue: alkaline reaction; no fermentation of lactose, organism attacks nitrogenous substances to form ammonia or basic amines.
4. White: reduction of litmus to a white base by enzyme reductase.
5. Clot or curd formation: milk proteins coagulation due to either a precipitation of casein by acid formation or the conversion of casein in paracasein by the enzyme rennin resulting in a clear watery fluid called “whey”.
6. Digestion (peptonisation): milk protein digested; clearing of medium and dissolution of clot by digestion of casein
7. Gas production (H_2 and CO_2): bubbles in the medium and clot may be broken up.
8. Stormy clot: acid clot disrupted by an abundance of gas production

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C / 3-5 days / AN	acid, “stormy clot” coagulation
P. aeruginosa ATCC 27853	37°C / 3-5 days / A	peptonisation (clearing)
L. acidophilus ATCC 314	37°C / 3-5 days / A	acid, clot or curd

A: aerobic incubation; AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- A clot formation must be simply recorded as “clot”. The differentiation between a clot and curd formation is not useful.
- Reactions observed in Litmus Milk are not sufficient to speciate; additional biochemical and serological tests must be performed.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the tubed medium may be stored in screwcap tubes at 2-8°C for 2-4 weeks.[1]

REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Cowan ST. Cowan and Steel’s manual for the identification of medical bacteria. 3rd edition, edited and revised by Barrow GI and Feltham RKA. Cambridge University Press, 1993.

PACKAGING

Product	Type	REF	Pack
Litmus Milk	Dehydrated medium	4016112	500 g (5 L)

IFU rev 1, 2022/09

LÖWENSTEIN-JENSEN MEDIUM BASE
LÖWENSTEIN-JENSEN MEDIUM
Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. For the cultivation and isolation of *Mycobacterium* species, especially *M. tuberculosis*.

COMPOSITION*

LOWENSTEIN-JENSEN MEDIUM BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Magnesium sulphate	0.24 g
Magnesium citrate	0.60 g
Monopotassium phosphate	2.50 g
L-asparagine	3.60 g
Potato flour	30.00 g
Malachite green	0.40 g

LOWENSTEIN-JENSEN MEDIUM, READY-TO-USE TUBES
TYPICAL FORMULA

Lowenstein Jansen Medium Base	37.4 g
Glycerol	12.0 mL
Purified water	600 mL
Homogenized whole eggs	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The medium originally described by Löwenstein in 1931[1] contained congo red and malachite green to limit the growth of unwanted bacteria. In 1932 Jensen[2], modified the medium by suppressing the congo red, modifying the concentration of magnesium citrate and potassium phosphate and increasing malachite green concentration. Today it is generally accepted that the use of an egg-based medium in combination with a liquid medium is essential for good laboratory practice in the isolation of mycobacteria;[3] among the egg-based media Lowenstein-Jensen Medium is the most commonly used in clinical laboratories.
In the Lowenstein-Jensen Medium, during the cooking process, the egg albumin coagulates thus providing a solid surface for bacterial growth. The concentration of malachite green is selected to maximize the growth of mycobacteria while inhibiting other microorganisms. L-asparagine and potato flour are sources of nitrogen and vitamins. Monopotassium phosphate and magnesium sulphate enhance organism growth and act as buffers. Egg suspension provides fatty acids and proteins required for the metabolism of mycobacteria. Glycerol is a carbon source and is favourable to the growth of the human type tubercle bacillus while being unfavourable to the bovine type.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37.4 g in 600 ml of purified water, add 12 mL of glycerol and heat to boiling. Autoclave at 121°C for 15 minutes. Cool to 50°C and add 1000 mL homogenised whole eggs aseptically collected. Distribute into sterile tubes and heat to 85°C in a slanting position for 45 minutes or more until the medium solidifies due to coagulation of the egg (long slant, short butt).

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	from pale to dark green-blue, fine, homogeneous, free-flowing powder
Solution appearance	green, opaque
Final pH at 20-25°C	not applicable

SPECIMENS

Specimens submitted for mycobacterial culture fall into two categories:
1- specimens normally contaminated with resident flora: the majority originates from respiratory tract, including sputum, tracheal and bronchial aspirates, and bronchoalveolar lavage specimens; other commonly submitted specimens types include urine, gastric aspirates, tissues, biopsy specimens.
2- specimens from normally sterile sites such as pleural and pericardial aspirates.
Contaminated specimens require a decontamination step before culture to reduce the likelihood of overgrowth by organisms other than mycobacteria. Specimens from normally sterile sites should be concentrated by centrifugation. Consult appropriate references for the applicable techniques[3,4] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.[3,4]

TEST PROCEDURE

Remove any condensation water present at the bottom of the slope and inoculate the surface of the slope with 0.2 mL (3-5 drops) of decontaminated and/or concentrated specimen.
Briefly angle slopes to allow the specimen to inoculate the entire surface. Ensure that the caps are tightly closed.
Incubate at 35 to 37°C for 6-8 weeks, extending to 12 weeks if necessary. 5-10% CO_2 in air stimulates the growth of mycobacteria in primary isolation cultures. It is necessary to incubate under CO_2, with loosening the caps to promote the circulation of carbon dioxide, for only the first 7 to 10 days after inoculation, subsequently L-J cultures can be removed to ambient air incubators if space is limited and incubated with the caps tightly screwed to prevent dehydration of the medium.[3]
Specimens with positive smear that are culture negative should be held for an additional 4 weeks. The same should be done for culture negative specimens that were positive for mycobacteria by nucleic acid-based amplification assays.[3]
The cultures should be examined within 2 to 5 days after inoculation to permit early detection of rapidly growing mycobacteria. Young cultures (up to 4 weeks of age) should be examined twice a week, whereas older cultures could be examined at weekly intervals.[3]
For samples obtained from surface sites, such as skin, or when the clinician suspects the presence of particular mycobacterial species (*M. marinum, M. ulcerans, M. chelonae,* or *M. haemophilum*), it is recommended to inoculate two sets of media, one of which incubated at 35-37°C and one at a lower temperature (30-32°C). Consult appropriate references for the detailed procedures about the treatment, inoculation and incubation of clinical specimens.[3,4]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies
M. tuberculosis appears as granular, rough, dry colonies; *M. kansasii* appears as smooth to rough photochromogenic colonies; *M. gordonae* appears as smooth yellow-orange colonies; *M. avium* appears as smooth, colourless colonies; *M. smegmatis* appears as wrinkled, creamy white colonies.[5]
Confirm the presence of Acid-Fast Bacilli in positive cultures with the Ziehl-Nielsen or auramine-phenol stain.[4]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[6]

Control strains	Incubation T°/ t / ATM	Expected results
M. tuberculosis H37Ra ATCC 25177	35-37°C / <21 days / CO_2	growth
M. kansasii Group I ATCC 12478	35-37°C / <21 days / CO_2	growth
M. intracellulare Group III ATCC 13950	35-37°C / <21 days / CO_2	growth
M. fortuitum Group IV ATCC 6841	35-37°C / <21 days / CO_2	growth

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- To shorten the isolation time as much as possible and to obtain a faster identification, the combination of a solid medium and a liquid medium is strongly recommended. The latter allows to reduce the incubation time and egg-based media allow the growth of some strains of *M. tuberculosis* complex and some non-tuberculous species that are unable to develop in liquid media.[7]
- It should be noted that if there is not enough specimen volume for PCR and culture, then only culture should be done. All samples, even if PCR positive, should be submitted for culture.[4]
- M. *bovis* grows poorly, or not at all on L-J medium but grows much better in media where glycerol is substituted by sodium pyruvate.[8]
- *M. leprae* and *M. genavense* fail to grow on L-J Medium.[3,8]
- A negative culture does not exclude an ongoing mycobacterial infection. There are several factors that can be responsible for negative cultures even in the presence of an infection: unrepresentative sample, mycobacteria destroyed during digestion and decontamination of the sample, presence of contaminants that mask or inhibit the growth of mycobacteria, inadequate incubation conditions.
- False positive cultures may result from mislabelling, specimen switching during handling, specimen carryover, contaminated reagents, or cross-contamination between cultures tubes.[3]
- L-J medium contains malachite green and is photosensitive and should not be exposed to light during storage.[8]
- L-J Medium may display some variation in the light-green colour throughout the tube. This doesn't interfere with the growth of mycobacteria; however, colour changes showing bright yellow or dark blue zones may indicate contamination.[8]
- The presence of yellow granules due to the lipid part of the egg, does not interfere with the performance of the medium.
- It is recommended that suitable identification and susceptibility tests be performed on isolates. For the detailed procedures consult appropriate references.[3,4,9]

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 4 weeks.[8]
Ready-to-use medium in tubes
Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Lowenstein E. Die Zachtung der Tuberkelba zillen aus dem stramenden Blute. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg Abt I Orig 1931; 120:127.
2. Jensen KA. Rinzuchtung und Typenbestim mung von Tuberkelbazillentammen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg Abt I Orig 1932; 125:222-239.
3. Martin I, Pfyffer GE, Parrish N. Mycobacterium: general characteristics, laboratory detection and staining procedures. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. Public Health England. Investigation of specimens for Mycobacterium species. UK Standards for Microbiology Investigations. B 40, Issue 7.3, 2020.
5. Atlas R, Snyder J. Media Reagents and Stains. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
6. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
7. Manuale tecnico per la diagnosi microbiologica della tubercolosi: http://www.salute.gov.it/imgs/C_17_pubblicazioni_614_allegato.pdf.
8. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
9. Warshauder DM, Salfinger M, Desmond E, Grace Lin SY. *Mycobaterium tuberculosis* complex. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
Lowenstein-Jensen Medium Base	Dehydrated medium	4016352 4016354	500 g (8 L) 21,4 5 Kg (80 L) 214
Lowenstein-Jensen Medium	Ready-to-use tubes	551635	20 glass tubes with slanted medium, 18 x145 mm

IFU rev 2, 2022/04

M. kansasii
on Lowenstein-Jensen Medium

LPT DILUTION BROTH

Dehydrated and ready-to-use culture medium

INTENDED USE

For the samples suspension and dilution in the microbiological examination of cosmetics.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE FLASKS

Lecithin	3.0
Sodium thiosulphate	5.0
Tryptic digest of casein	1.0
Sodium chloride	8.5
Disodium hydrogen phosphate	8.0
Potassium dihydrogen phosphate	1.5
L-histidine HCl	1.0

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Preservatives are generally present in cosmetics to reduce the risk of microbial contamination and to ensure that the product remains suitable and safe during the period of storage and consumer use.
LPT Dilution Broth is a universal diluent for the homogenisation of cosmetic samples and the preparation of decimal dilutions, with neutralizing properties against antimicrobial agents, produced according to the formulation proposed by UNIPRO.[1]
Lecithin and Tween® 80 remove the antimicrobial activity of quaternary ammonium compounds and parabens, while sodium thiosulphate and L-histidine neutralize microbicidal active halogen compounds.
The low concentration of peptone, a phosphate buffer and sodium chloride, provide osmotic stability, a stable pH value and maintain the viability of microorganisms without supporting growth during sample preparation.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 28 g in 1000 mL of cold purified water. Mix well and add 30 mL of Tween® 80 (REF 42120502). Heat to boiling with frequent agitation, distribute into flasks or tubes and sterilise by autoclaving at 121°C for 15 minutes. Mix well after sterilisation.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale yellow, slightly opalescent
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Cosmetic products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE, READING AND INTERPRETATION

The initial suspension is prepared from a sample of at least 1 g-mL or 10 g-mL of the well-mixed product under test, depending of the expected level of contamination. Transfer the sample of product to an appropriate volume (e.g. 9 mL or 90 mL) of LPT Dilution Broth.
If needed, additional serial dilutions (e.g. 1:10 dilution) may be performed from the initial suspension using the same diluent.
For pour-plate enumeration method, within 45 minutes add 1 mL of the initial suspension and/or sample dilutions into two sterile Petri dishes and pour 15 mL to 20 mL of the melted agar medium.
If an enrichment procedure is needed, incubate the initial suspension and the dilutions at 30-35 °C for at least 20 hours and observe for growth. Using a sterile loop, streak an aliquot of the incubated LPT Dilution Broth on the surface of a Petri dish with the specific medium for the target organism.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538	35-37°C / 18-24H / A	good growth
P. aeruginosa ATCC 27853	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use medium in flasks
Store flasks in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. La Microbiologia nell'Industria Cosmetica. UNIPRO, aprile 1990, vol. 1. Ricerche e Tecnologie Cosmetiche.

PACKAGING

Product	Type	REF	Pack
LPT Dilution Broth	Dehydrated medium	4016392 4016394	500 g (17.8 L) 5 kg (178 L)
LPT Dilution Broth	Ready-to-use flasks	5116392	6 x 90 mL

®Tween is a trademark of ICI Americas Inc.
IFU rev 1, 2022/08

LYSINE IRON AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. For the differentiation of some members of *Enterobacteriaceae*, especially *Salmonella*, isolated from clinical and non- clinical specimens.

COMPOSITION TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Peptone	5.00 g
Yeast Extract	3.00 g
Glucose	1.00 g
L-Lysine	10.00 g
Fe-Ammonium Citrate	0.50 g
Sodium Thiosulphate	0.04 g
Bromocresol Purple	0.02 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Edwards and Fife[1] in 1961 devised a medium to solve the problem of misidentification of strong lactose-fermenters strains of *Arizona (*now *Salmonella enterica* subsp. *arizonae*) that didn't produce blackening of TSI or KIA tubes.
Johnson et al.[2] in 1965 described a method based on primary differentiation of various groups of bacteria by the use of Kligler iron agar and lysine iron agar, and triple sugar iron agar and lysine iron agar for the identification of *Salmonella*, *Shigella*, and *Arizona* group isolated from stool.
Lysine Iron Agar (LIA), prepared according to the formula proposed by Edwards and Fife[1], aids in the differentiation of some members of rapid lactose-fermenter *Enterobacteriaceae*, especially *S. arizonae*, isolated from clinical and non-clinical specimens, by means of deamination or decarboxylation of lysine and production of hydrogen sulphide.[3] The medium is included in the FDA-BAM[4] schemes for the identification of *Salmonella* from food, together with other biochemical tests.
Lysine iron agar contains lysine, peptones, a small amount of glucose, a pH indicator, ferric ammonium citrate, and sodium thiosulfate.
Peptone and yeast extract provide nitrogen, carbon, vitamins and trace elements for bacterial growth. Glucose is the fermentable carbohydrate. Bromocresol purple is a pH indicator that changes to a yellow colour at or below pH 5.2 and is purple at or above 6.8. Sodium thiosulfate and ferric ammonium citrate allow for hydrogen sulphide detection: strains that produce hydrogen sulphide cause blackening of the medium due to ferrous sulphide production. Lysine is included for the detection of decarboxylase and deaminase enzymes.
Lysine decarboxylation is an anaerobic process which occurs in the butt of the medium; lysine deamination is an aerobic process which occurs on the slant.
Lysine decarboxylase removes the COOH group from lysine to produce CO_2 and cadaverine, an alkaline polyamine which neutralizes the organic acids formed by glucose fermentation, and the butt of the medium reverts to the alkaline state (purple). If the decarboxylase is not produced, the butt remains acidic (yellow). If oxidative deamination of lysine occurs, α-ketocarboxylic acid is formed that reacts with ferric ions near surface of

medium under influence of oxygen, to form a reddish-orange compound; the combination of this compound with bromocresol purple produces a distinct red colour on the slant. If deamination does not occur, the slant remains purple.[3]
Within *Enterobacteriaceae*, *Salmonella*, with the sole exception of *S*. Paratyphi A, is the only genus that rapidly decarboxylates lysine and produces hydrogen sulphide: on Lysine Iron Agar these two characteristics are clearly visible both for the lactose-fermenting strains and for the lactose non-fermenting strains.
Deamination of lysine is a characteristic of *Proteus*, *Providencia* and *M. morganii*, the only members of *Enterobacteriaceae* that produce lysine deaminase enzyme.

Directions for medium preparation
Suspend 34.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute in tubes, and sterilize by autoclaving at 121°C for 15 minutes. Cool in slanted position to obtain a deep butt.

Physical characteristics
Dehydrated medium appearance	grey-purple, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	purple, limpid
Final pH at 20-25 °C	6.7 ± 0.2

Specimens
The specimens consist of bacteria strains isolated from clinical specimens or other samples, purified on appropriate medium (e.g. Tryptic Soy Agar or Blood Agar).

Test procedure
With a straight inoculating needle, inoculate by stabbing through the centre of the medium to the bottom of the tube and then streaking the slant.
Incubate the tubes aerobically, with the loosened caps so that aerobic conditions prevail on the slant, at 35 ± 2°C for 24 ± 2 hours.
Unpublished data have demonstrated that 48 hours reading of LIA slants has no diagnostic value.[4]

Reading and interpretation
After incubation, observe the colour changes in the butt and on the slant.
Lysine decarboxylation (detected in the butt):
Positive test: purple slant/purple butt (alkaline), the butt reaction may be masked by H_2S production.
Negative test: purple slant/yellow butt (acid), fermentation of glucose only.
Lysine deamination (detected on the slant):
Positive test: red slant
Negative test: slant remains purple
H_2S production:
Positive test: black precipitate
Negative test: absence of black precipitate

Typical reactions by members of the *Enterobacteriaceae*

Organism	Slant	Butt	H_2S
Salmonella	AK	AK	+
S. arizonae	AK	AK/N	+
S. Paratyphi A	AK	A	-
Shigella	AK	A	-
Escherichia	AK	A/N	-
Klebsiella	AK/N	AK/N	-
Citrobacter	AK	A	+ or -
Proteus	R	A	-
Providencia	R	A	-

AK: alkaline reaction, purple colour; A: acid reaction, yellow colour; R: red colour (lysine deamination); N: neutral reaction, no colour change; +: positive reaction; -: negative reaction

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

S. Typhimurium ATCC 14028	growth, purple slant, purple butt, H_2S +
P. mirabilis ATCC 12453	growth, red slant, yellow butt, H_2S -
S. flexneri ATCC 12022	growth, purple slant, yellow butt, H_2S -

Aerobic incubation at 35 ± 2°C for 18-24 hours.
ATCC is a trademark of American Type Culture Collection

Limitations of the method
- It is necessary to inoculate the medium with a microbiological needle without breaking the agar (do not use loops).
- H_2S producing *Proteus* spp. do not blacken LIA.[3]
- Ferrous sulphide may not be seen with organisms that do not decarboxylate lysine because acid in the butt may suppress its formation; for this reason and for distinguishing coliforms from Shigella, it is recommended to use LIA in conjunction with TSI or KIA media.[3]
- Red slant reaction with *M. morganii* may be variable after 24 hours of incubation; complete deamination of lysine usually requires longer incubation (up to 48 hours).[3]
- On Lysine Iron Agar, gas production is normally irregular or suppressed, with the sole exception of *Citrobacter*.[3]
- *Salmonella enterica* ser. Paratyphi A does not decarboxylate lysine and the reactions are: K / A, H_2S -.
- Lysine Iron Agar is not a substitute for the lysine decarboxylation test on Moeller Decarboxylase Medium.
- The lysine decarboxylation/deamination is one of the tests necessary for the identification of *Enterobacteriaceae*. The results on LIA must be interpreted together with other tests for a correct identification of the strains. Therefore, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6.8 weeks.[3]

Ready-to-use medium in tubes

Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Edwards, P.R., and M.A. Fife. 1961. Lysine-Iron Agar in the detection of Arizona cultures. Appl. Microbiol. 9:478-480.
2. Johnson, J.G., L.J. Kunz, W. Barron, and W.H. Ewing. 1966. Biochemical differentiation of the Enterobacteriaceae with the aid of Lysine-Iron-Agar. Appl. Microbiol. 14:212-217.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019

PACKAGING

Product	Type	REF	Pack
Lysine Iron Agar	Dehydrated medium	4016362	500 g (14,5 L)
Lysine Iron Agar	Ready-to-use tubes	551636	20 glass tubes with slanted medium, 17x125 mm,

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Lysine Iron Agar- from left: uninoculated tube, *S. flexneri*, *S. arizonae*, *P. mirabilis*, *E. coli*

M17 AGAR

Dehydrated culture medium

INTENDED USE

For the cultivation and enumeration of lactic streptococci in milk and dairy products; for the enumeration of *Streptococcus thermophilus* in yogurt.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptic digest of casein	2.50 g
Peptone	2.50 g
Soy peptone	5.00 g
Yeast extract	2.50 g
Beef extract	5.00 g
Sodium glycerophosphate	19.00 g
Magnesium sulphate	0.25 g
Ascorbic acid	0.50 g
Lactose	5.00 g
Agar	13.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactic streptococci are acid-producing bacteria, nutritionally fastidious, requiring complex media for optimum growth. They are used extensively as starter cultures in the dairy industry.

M17 Agar was developed by Terzaghi and Sandine[1] for the improved growth of lactic streptococci and their bacteriophages by adding disodium-β-glycerophosphate as a buffer system to the medium M16 devised by Lowrie RJ et al.[2] It was later found that the addition of glycerophosphate buffer inhibits the growth of many *Lactobacillus* species.[3]

M17 Agar is recommended by ISO 7889 (IDF 117) for the enumeration of *Streptococcus thermophilus* from yogurt.[4.] Terzaghi and Sandine recommend M17 Agar also for the demonstration of lactic bacteriophage activity; when this method is adopted, 100mL of medium is supplemented with 10 mL $CaCl.6H_2O$ 1.0 M.[1] The medium also proved useful for isolation of bacterial mutants lacking the ability to ferment lactose; such mutants formed minute colonies on M17 agar plates, whereas wild-type cells formed colonies 3 to 4 mm in diameter.[1]

The plant protein extract (soy peptone) and other peptones provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is the fermentable carbohydrate and a source of carbon and energy. Sodium glycerophosphate buffers the acidity produced by lactose fermentation and maintains the pH above 5.7 during the active microbial growth, allowing the optimal recovery of lactic streptococci and the inhibition of many lactobacilli. Ascorbic acid stimulates growth of lactic streptococci while magnesium sulphate provides essential ions for growth. The calcium-containing medium is used for the assay of bacteriophages of lactic streptococci.[1]

As indicated by the Pharmacopoeia of Culture Media for Food Microbiology[5] lactose is included in the formulation, however Biolife makes lactose-free medium available (M17 Agar w/o Lactose REF 401719S2).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 55.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Milk and dairy products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

For the enumeration of *S. thermophilus* in yogurt, ISO 7889[4] recommends the following technique:

1. Accurately mix the contents of the yoghurt pot by using a sterile spatula. In the case of fruit-yogurt homogenise the contents of the pot for one minute.
2. Weigh 10 g of product in a 200 mL bottle and bring to 50 g with an autoclaved diluent as specified in ISO 6887-1[6] and ISO 6887-5[7](e.g., 0.1% peptone solution).
3. Blend for 1 min with the blender. Dilute to 100 g with the diluent to obtain a 10^{-1} dilution.
4. Prepare a suitable series of decimal dilutions of the sample suspension in 9 mL of diluent.
5. From each tube, pipette 1 mL of the appropriate dilution in a 90 or 100mm Petri dish in duplicate.
6. Pour 15 mL of M 17 Agar, cooled to 45°C to each dish. Mix the inoculum with the medium and allow the mixture to solidify.
7. Incubate at 37°C for 48 hours.

READING AND INTERPRETATION

After incubation, examine the plates under subdued light. Count the colonies on plates containing between 15 and 300 colonies.

S. thermophilus forms lenticular colonies of diameter 1 mm to 2 mm. Under a microscope, these microorganisms appear as spherical or ovoid cells (of diameter 0.7 µm to 0.9 µm) in pairs or in long chains. They are Gram-positive and catalase-negative.

Take care not to mistake particles of undissolved sample or precipitated matter for pinpoint colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T – ATM	EXPECTED RESULTS
S. thermophilus ATCC 19258	37°C / 48 hours /A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Some strains of *L. delbrueckii* subsp. *bulgaricus* may form small pinpoint colonies on the M17 Agar, especially with samples of yogurt presenting a much higher number of lactobacilli compared to the number of streptococci.[4]

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to Curtis GDV and Baird RM,[5] the self-prepared medium may be stored at +2°C/+8°C for 7 days.

REFERENCES

1. Terzaghi BE, Sandine WE. Improved medium for lactic streptococci and their bacteriophages. App Microbiol 1975;29 (6): 807–813.
2. Lowrie RJ, Pearce LE. The plating efficiency of bacteriophages of lactic streptococci. NZ J Dairy Sci Technol 1971; 6: 166–171.
3. Shankar PA, Davies FL. A note on the suppression of Lactobacillus bulgaricus in media containing β-glycerophosphate and application of the media to selective isolation of Streptococcus thermophilus from yoghurt. Int J Dairy Technol 1977;30 (1):28–30.
4. ISO 7889 (IDF 117): 2003. Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 °C.
5. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:214-15.
6. ISO 6887-1:2017 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions.
7. ISO 6887-5:2020 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 5: Specific rules for the preparation of milk and milk products.

PACKAGING

Product	Type	REF	Pack
M17 Agar	Dehydrated medium	4017192	500 g (9.1 L)

The medium is also available without lactose at the customer's request: M17 Agar w/o Lactose REF 401719S2, 500 g.

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M17 BROTH

Dehydrated culture medium

INTENDED USE

For the cultivation of lactic streptococci in milk and dairy products.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptic digest of casein	2.50 g
Peptone	2.50 g
Soy peptone	5.00 g
Yeast extract	2.50 g
Beef extract	5.00 g
Sodium glycerophosphate	19.00 g
Magnesium sulphate	0.25 g
Ascorbic acid	0.50 g
Lactose	5.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactic streptococci are acid-producing bacteria, nutritionally fastidious, requiring complex media for optimum growth. They are used extensively as starter cultures in the dairy industry.
M17 Broth is based on the formulation developed by Terzaghi and Sandine[1] for the improved growth of lactic streptococci and their bacteriophages by adding disodium-β-glycerophosphate as a buffer system to the medium M16 devised by Lowrie RJ et al.[2] It was later found that the addition of glycerophosphate buffer inhibits the growth of many *Lactobacillus* species.[3]
M17 Broth use in conjunction with M17 Agar in bacteriophage assays has been described by Terzaghi and Sandine[1] who also suggested that M17 Broth would be a suitable medium for the maintenance of starter cultures.
The plant protein extract (soy peptone) and other peptones provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is the fermentable carbohydrate and a source of carbon and energy. Sodium glycerophosphate buffers the acidity produced by lactose fermentation and maintains the pH above 5.7 during the active microbial growth, allowing the optimal recovery of lactic streptococci and the inhibition of many lactobacilli. Ascorbic acid stimulates growth of lactic streptococci while magnesium sulphate provides essential ions for growth.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute and sterilise in the autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellowish, limpid
Final pH at 20-25 °C	7.1 ± 0.1

SPECIMENS

Milk and dairy products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations

TEST PROCEDURE

Inoculate the tubes and incubate 24-48 hours at 37 °C (or other suitable temperature) aerobically.

READING AND INTERPRETATION
After incubation, the growth is evidenced by turbidity in the broth.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. thermophilus ATCC 19258	37°C / 48 ore /A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Some strains of *L. delbrueckii* subsp. *bulgaricus* may exhibit a slight growth on M17 Broth.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Terzaghi BE, Sandine WE. Improved medium for lactic streptococci and their bacteriophages. App. Microbiol 1975;29 (6): 807–813.
2. Lowrie RJ, Pearce LE. The plating efficiency of bacteriophages of lactic streptococci. N.Z. J. Dairy Sci. Technol 1971; 6: 166–171.
3. Shankar PA, Davies FL. A note on the suppression of Lactobacillus bulgaricus in media containing β-glycerophosphate and application of the media to selective isolation of Streptococcus thermophilus from yoghurt. Int J Dairy Technol 1977;30 (1):28–30.

PACKAGING
Product	Type	REF	Pack
M17 Broth	Dehydrated medium	4017202	500 g (11.9 L)

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MAC CONKEY AGAR
Dehydrated and ready-to-use culture medium

Mac Conkey Agar: *E. coli* (colonies with red halo) and *Pseudomonas aeruginosa* (greenish colonies)

INTENDED USE
In vitro diagnostics. Selective and differential medium for the isolation and differentiation of *Enterobacteriaceae* and other Gram-negative bacilli from clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE PLATES AND FLASKS

Gelatin peptone	17.000 g
Peptones (meat and casein)	3.000 g
Lactose	10.000 g
Bile salts n°3	1.500 g
Sodium chloride	5.000 g
Neutral red	0.030 g
Crystal violet	0.001 g
Agar	13.500 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Mac Conkey Agar is a selective, differential medium based on the formulation described by Alfred Theodore MacConkey in 1900[1] and later modified by Albert Grunbaum ed Edward Hume in 1902[2] with the inclusion of neutral red and crystal violet.
By 1930, ten modifications of "MacConkey's Basal Bile Salt Peptone" agar were published in a compendium of microbiological media, but among all of these, it was Grunbaum and Hume's formula that stood the test of time and is (with minor modifications) the basis of modern Mac Conkey agar; 120 years later, MacConkey agar remains ubiquitous in clinical and industrial laboratories, where it is used routinely to detect non-fastidious Gram-negative organisms in a variety of human specimens and non-clinical materials.[3]
Mac Conkey Agar is intended for the isolation of *Enterobacteriaceae* and other Gram-negative bacilli and for the differentiation of lactose-fermenting from lactose-nonfermenting Gram-negative enteric bacilli. Mac Conkey Agar is used for the microbiological examination of human clinical specimens[5,6], is included in the FDA-BAM[7] for the primary isolation of Enteropathogenic *E. coli* in food, meets harmonized EP, USP, JP specifications[8] for *E. coli* detection in non-sterile pharmaceutical products and is recommended by ISO 21150 for *E. coli* detection in cosmetics[9].
The original MacConkey medium has been modified in the present preparation: the agar content is lower, 5g/L of sodium chloride have been added, the concentration of bile salts and neutral red has been modified.[4] These modifications support excellent growth of most strains of *Salmonella* and *Shigella*, and permit better differentiation of these pathogens from coliform bacteria. The selective action of Mac Conkey Agar is due to the presence of bile salts no. 3, which inhibits the growth of Gram-positive bacteria; this inhibitory activity is enhanced by the addition of crystal violet. The peptones provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The fermentation of lactose by coliforms causes acidification of the medium, with the consequent precipitation of the bile salts and absorption of the neutral red.[4] The coliform bacteria grow with red-pink to red-violet colonies surrounded by a red precipitation zone. Lactose non-fermenters strains (e.g. *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Alkaligenes* etc.) develop transparent, colourless colonies without precipitation zone. The swarming of *Proteus* spp. is partially controlled on Mac Conkey Agar by using selected raw materials.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)
Suspend 50 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C. Mix well and pour into sterile Petri dishes.

Directions for medium preparation (ready-to-use medium in flasks)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

Physical characteristics

Dehydrated medium appearance	pale pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 25 °C	7.1 ± 0.2

Specimens

Mac Conkey Agar is intended for the bacteriological examination of several human clinical specimens with mixed flora (e.g. urine, stool, materials from respiratory tract, wounds and abscesses etc.)[5,6] and non-clinical specimens, as food, non-sterile pharmaceutical products, cosmetics[7,8,9]. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, storage and transport of the specimens to the Laboratory should be applied.

Test procedure

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate in aerobic atmosphere at 35-37°C for 18-24 hours or longer if necessary (maybe up to 48 h for late lactose fermenters: *Citrobacter, Providencia*, *Serratia*, *Hafnia*).[4]
For the detection of *E. coli* in non-sterile pharmaceuticals products, the technique recommended by European Pharmacopoeia[8] and summarized below, should be followed:

- Prepare a sample using a 1:10 dilution of not less than 1 g of the product to be examined and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate the suitable amount of Tryptic Soy Broth. Mix and incubate at 30-35°C for 18-24 h.
- Shake the container, transfer 1 mL of Tryptic Soy Broth to 100 mL of Mac Conkey Broth EP and incubate at 42-44 °C for 24-48 h.
- Subculture on a plate of Mac Conkey Agar and incubate at 30-35 °C for 18-72 h.

Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Colonies of lactose fermenters are red-pink to red-violet and may be surrounded by red zones of precipitated bile.
Colonies of lactose non-fermenters are colourless or white or light yellow or with a natural pigmentation (e.g. green for *P. aeruginosa*).

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[10]

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 8739	35-37°C / 18-24 h / A	red-violet colonies with red opaque halo
P. mirabilis ATCC 12453	35-37°C / 18-24 h / A	non-swarming colourless colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 h / A	colourless colonies
E. faecalis ATCC 29212	35-37°C / 18-24 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Prolonged incubation may lead to confusion of results; do not incubate longer than 48 hours.[4]
- Due to selective properties of this medium some strains of Gram-negative enteric bacteria fail to grow or grow poorly; similarly, some Gram-positive organisms may not be inhibited or are partially inhibited.[4]
- Some enterococci strains may exhibit growth after prolonged incubation.[4]
- Mac Conkey agar is not a satisfactory medium for the detection and enumeration of coliform organisms in food. One of the most reliable methods uses violet red bile agar with pour plate counts.[11]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 6-8 weeks.[4]
Ready-to-use plates and flasks
Store in their original pack at +2°C/+8°C away from direct light.

References

1. MacConkey AT. Note on a new medium for the growth and differentiation of the Bacillus coli communis and the Bacillus Typhi abdominalis. The Lancet, July 07, 1900; vol 156, Issue 4010, P20.
2. Grunbaum AS, Hume EH. Note on media for distinguishing B.coli, B.typhosus and related species. Brit Med J, June 14 1902; p 1473-1474
3. Smith KP, The origin of MacConkey Agar. American Society for Microbiology: Articles, Oct. 14, 2019.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
6. Vandepitte J, Verhaegen J, P. Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd edition Geneve: World Health Organization Geneva; 2003.
7. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Bacteriological Analytical Manual Chapter 4A Diarrheagenic *Escherichia coli.* Content current as of:11/03/2017
8. European Pharmacopoeia, current edition.
9. ISO 21150:2015. Cosmetics- Microbiology -Detection of Escherichia coli.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
11. Harrigan WF, McCance ME. The Microbiological examination of foods. in Laboratory Methods in Microbiology, Elsevier B.V. 1966.

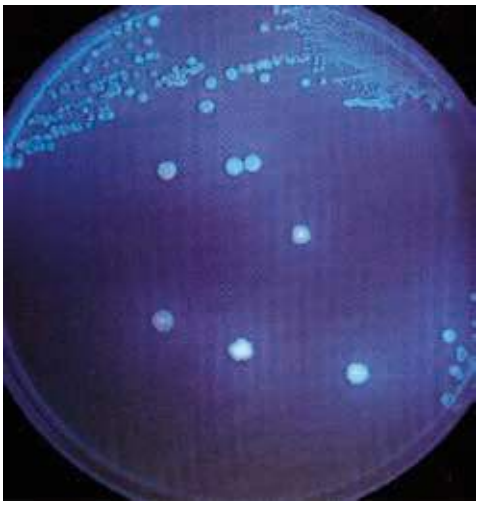
PACKAGING

Product	Type	REF	Pack
Mac Conkey Agar	Dehydrated medium	4016702 4016704	500 g (10 L) 5 kg (100 L)
Mac Conkey Agar	Ready-to-use plates	541670	2 x 10 plates ø 90 mm
Mac Conkey Agar	Ready-to-use flasks	5116702 5116703	6 x 100 mL 6 x 200 mL

IFU rev 3, 2022/01

MAC CONKEY AGAR MUG

Dehydrated and ready-to-use culture medium



Mac Conkey Agar MUG: *E. coli* colonies, fluorescent under Wood's lamp

INTENDED USE

In vitro diagnostics. Selective and differential medium for the isolation and differentiation of *Enterobacteriaceae* and other Gram-negative bacilli and for the presumptive identification of *Escherichia coli*, from clinical specimens.

COMPOSITION -TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Gelatin peptone	17.000 g
Peptocomplex	3.000 g
Lactose	10.000 g
Bile salts n° 3	1.500 g
Sodium chloride	5.000 g
Neutral red	0.003 g
Crystal violet	0.001 g
Agar	13.500 g
4-Methylumbelliferyl- β-D-glucuronide (MUG)	0.100 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Mac Conkey Agar MUG is a selective and differential medium based on the formula described by Trepeta and Edberg[1] who modified the classical Mac Conkey Agar [by] incorporation of the fluorogenic compound 4-methylumbelliferyl- β-D-glucuronide (MUG), according to the preliminary studies of Dahlen and Linden[2] and of Kilian and Bulow[3]. Inclusion of MUG doesn't change Mac Conkey Agar characteristics of selectivity or lactose fermentation.[1]

Mac Conkey Agar MUG is intended for the isolation of *Enterobacteriaceae* and other Gram-negative bacilli from clinical specimens and for the differentiation of lactose-fermenting from lactose-nonfermenting Gram-negative enteric bacilli and for the rapid, presumptive identification of *E. coli* by the detection of β-glucuronidase enzyme.[1,4]

MUG is cleaved by β-D-glucuronidase produced by *E. coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be determined directly by using a long-wave ultraviolet light.

The selective action of Mac Conkey Agar MUG is due to the presence of bile salts no. 3, which inhibits the growth of Gram-positive bacteria; this inhibitory activity is enhanced by the addition of crystal violet. The peptones provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The fermentation of lactose by coliforms causes acidification of the medium, with precipitation of the bile salts and absorption of the neutral red.[4] The coliform bacteria grow with red-pink to red-violet colonies surrounded by a red precipitation zone. Lactose non-fermenters strains (e.g. *Salmonella, Shigella, Proteus, Pseudomonas, Alkaligenes* etc.) develop transparent, colourless colonies without precipitation zone. *Proteus* swarming is partially controlled by using selected raw materials. β-glucuronidase positive and lactose positive *E. coli* strains grow with red-pink to red-violet colonies surrounded by a red precipitation zone, with a slight blue fluorescence under long-wave ultraviolet light.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 50 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

Mac Conkey Agar MUG is intended for the bacteriological processing of the same specimens examinable with Mac Conkey Agar: urine and several human clinical specimens with mixed flora (e.g. stool, materials from respiratory tract, wounds, abscesses, etc.)[5,6,7]. Good laboratory practices for collection, transport and storage of the specimens should be applied.[5]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic conditions at 35-37°C for 18-24 hours or longer if necessary (up to 48 h for late lactose fermenters: *Citrobacter, Serratia*, *Hafnia*).[4]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies at the normal light. Check the plates by using a hand-held 366-nm light or by placing the plate under a long-wave UV lightbox (Wood's Lamp).
Colonies of lactose fermenters are red-pink to red-violet and may be surrounded by red zones of precipitated bile.
Colonies of *E. coli* develop a slight blue fluorescence under Wood's lamp.
Colonies of lactose non-fermenters are colourless or white or light yellow or with a natural pigmentation (e.g. green for *P. aeruginosa*).
The confirmation of *E. coli* identification can be done by indole test (+), directly on the plate.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 8739	35-37°C / 18-24 h / A	red violet colonies with red opaque halo, fluorescent under Wood's lamp
P. mirabilis ATCC 12453	35-37°C / 18-24 h / A	non-swarming colourless colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 h / A	colourless colonies
E. faecalis ATCC 29212	35-37°C / 44-48 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

PERFORMANCES CHARACTERISTICS

Biolife Mac Conkey Agar MUG was evaluated by Goglio *et al*.[9] using 1534 urine samples and this product was compared with the routine method of calibrated loop with Mac Conkey Agar and blood agar for screening for uropathogens. Combining positivity to β-glucuronidase, positivity to lactose fermentation and the indole test, the identification of *E. coli* colonies has a sensitivity of 100% and a specificity of 85%. The authors' conclusion was: the detection of β-glucuronidase with Mac Conkey Agar MUG allows to anticipate the identification of *E. coli* and the information to the clinician by 24 hours with a possible impact on the therapeutic strategy.
According to the data of Trepeta and Edberg[1] MUG supplemented Mac Conkey agar (MCM) proved to be sensitive in elucidating β-glucuronidase positive microbes directly from clinical specimens. Compared to Mac Conkey agar (MCA), MCM showed enhanced recovery of *E. coli* organisms: 255 clinical specimens were processed with both media and *E. coli* has been isolated in 82 specimens with MCM and in 77 specimens with MCA. MCM proved especially useful in establishing the presence of *E. coli* mixed with other pathogens.

LIMITATIONS OF THE METHOD

- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive and fluorescent under Wood's Lamp; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[1,10,11]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains[10].
- Up to 10% of clinical *E. coli* isolates have been reported to be slow or non-lactose fermenting.[12]
- Prolonged incubation may lead to confusion of results[4]; do not incubate plates longer than 48 hours.
- Due to selective properties of this medium some strains of Gram-negative enteric bacteria fail to grow or grow poorly.[4]
- Some enterococci strains may exhibit growth after prolonged incubation.[4]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +2°C/+8°C away from direct light in a dry place.
Ready-to-use plates
Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
2. Dahlén G, Linde A. Screening plate method for detection of bacterial beta-glucuronidase. Appl Microbiol 1973;26 (6): 863-6
3. Killian M, Bulow P. 1976. Rapid diagnosis of Enterobacteriaceae. Detection of bacterial glycosidases. Acta Pathol Microbiol Scand Sec B 1976; 84:245-251.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
6. Vandepitte J, Verhaegen J, P. Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd edition Geneve: World Health Organization Geneva; 2003.
7. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
8. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
9. Goglio A, Vaiani R, Centemeri A, Passerini-Tosi C. Utilizzo del metilumbelliferil glucuronide nella valutazione rapida di E. coli. Valutazione in microbiologia clinica nella diagnostica delle urino colture. Comunicazione Convegno AMCLI Lombarda, Varenna, 21/06/1985; Ed Biolife Italiana: Documenta; 1985
10. Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 48:285-288
11. Kaluzewski S, D Tomczuk D. Evaluation of the Usefulness of Tests for Productionof Beta-D-glucuronidase and Propylene Glycol Utilization for the Differentiation of Enterobacteriaceae Rods. Med Dosw Mikrobiol, 1995; 47:155-68.
12. Gokul Yaratha, MD, Sarah Perloff, DO, Kinesh Changala, MBBS. Lactose vs non-lactose fermenting E. coli: Epidemiology, Clinical Outcomes, and Resistance. Open Forum Infect Dis 2017; V4 (Suppl 1)

PACKAGING

Product	Type	REF	Pack
Mac Conkey Agar MUG	Dehydrated medium	4016722	500 g (10L)
Mac Conkey Agar MUG	Dehydrated medium	4016724	5 kg (100 L)
Mac Conkey Agar MUG	Ready-to-use plates	541672	2 x 10 plates ø 90 mm

IFU rev 2, 2022/01

MAC CONKEY AGAR N° 2

Dehydrated culture medium

Mac Conkey Agar n° 2: colonies of *E. coli*

INTENDED USE

For the recognition of enterococci in the presence of coliforms and non-lactose fermenters from water, sewage and food products.

COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20.00 g
Lactose	10.00 g
Bile salts	1.500 g
Sodium chloride	5.000 g
Neutral red	0.050 g
Crystal violet	0.001 g
Agar	15.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Alfred Theodore MacConkey, working at the University of Liverpool under the auspices of the Royal Commission on Sewage Disposal, in 1900[1] published in Lancet the formulation of MacConkey Agar. The use of the medium caught on rapidly amongst those interested in water microbiology. Later in 1902, Albert Grunbaum ed Edward Hume[2] modified the MacConkey's formulation with the inclusion of neutral red and crystal violet and by 1930, ten modifications of "MacConkey's Basal Bile Salt Peptone" agar were published.[3]

Mac Conkey Agar n° 2, compared to the classic formula of Mac Conkey Agar (REF 401670), includes the less selective bile salts instead of bile salts n° 3, which partially inhibits the growth of some Gram-positive bacteria; this inhibitory activity is enhanced by the inclusion of crystal violet that does not interfere with the growth of enterococci. The peptone provides carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The fermentation of lactose by coliforms and enterococci causes acidification of the medium and the formation of red-pink to red-violet colonies. Non-lactose fermenter strains (e.g. *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Alkaligenes* etc.) develop transparent, colourless colonies. Enterococci produce small red colonies whereas staphylococci and a and non-faecal streptococci are inhibited.

Mac Conkey Agar n° 2 is used for the recognition of enterococci in the presence of coliforms and non-lactose fermenters from water, sewage and food products.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 51.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	orange-red, limpid or slightly opalescent
Final pH at 25 °C	7.2 ± 0.2

SPECIMENS

Foodstuffs and waters. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.

Incubate in aerobic atmosphere at 35-37°C for 18-24 hours or longer if necessary (maybe up to 48 h for late lactose fermenters: *Citrobacter, Providencia*, *Serratia*, *Hafnia*).[4]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Enterococci produce small red colonies.

Colonies of coliforms are red-pink to red-violet.

Colonies of non-lactose fermenters are colourless or white or light yellow or with a natural pigmentation (e.g. green for *P. aeruginosa*).

Staphylococci are totally inhibited.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37°C / 18-24 h / A	red-violet colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 h / A	colourless colonies
E. faecalis ATCC 29212	35-37°C / 18-24 h / A	small red colonies
S. aureus ATCC 25923	35-37°C / 18-24 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Prolonged incubation may lead to confusion of results; do not incubate longer than 48 hours.[4]
- Due to selective properties of this medium some strains of Gram-negative enteric bacteria fail to grow or grow poorly; similarly, some Gram-positive organisms may not be inhibited or are partially inhibited.[4]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C/+8°C in the dark and protected against evaporation for up to 6-8 weeks.[4]

REFERENCES

1. MacConkey AT. Note on a new medium for the growth and differentiation of the Bacillus coli communis and the Bacillus Typhi abdominalis. The Lancet, July 07, 1900; vol 156, Issue 4010, P20.
2. Grunbaum AS, Hume EH. Note on media for distinguishing B.coli, B.typhosus and related species. Brit Med J, June 14 1902; p 1473-1474
3. Smith KP. The origin of MacConkey Agar. American Society for Microbiology: Articles, Oct. 14, 2019.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Mac Conkey Agar n° 2	Dehydrated medium	4016732	500 g (9.7 L)

IFU rev 1, 2022/09

MAC CONKEY AGAR OMS W/O CRYSTAL VIOLET (CV)

Dehydrated culture medium

INTENDED USE

A moderately selective medium used for the isolation and differentiation of Gram-negative organisms from food and water samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	17.00 g
Peptocomplex	3.00 g
Lactose	10.00 g
Bile salts	5.00 g
Sodium chloride	5.00 g
Neutral red	0.05 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Alfred Theodore MacConkey, working at the University of Liverpool under the auspices of the Royal Commission on Sewage Disposal, in 1900[1] published in Lancet the formulation of MacConkey Agar. The use of the medium caught on rapidly amongst those interested in water microbiology. Later in 1902, Albert Grunbaum ed Edward Hume[2] modified the MacConkey's formulation with the inclusion of neutral red and crystal violet. By 1930, ten modifications of "MacConkey's Basal Bile Salt Peptone" agar were published and among these MacConkey Agar without crystal violet.[3] Mac Conkey Agar OMS w/o CV compared to the classic formula of Mac Conkey Agar (REF 401670) does not contain crystal violet, includes bile salts instead of bile salts n° 3 and has lower selective properties. Mac Conkey Agar OMS w/o CV is a moderately selective medium used for the isolation and differentiation of Gram-negative organisms from a variety of food, water samples and industrial sources; the lack of crystal violet permits the growth of enterococci and mycobacteria. This formula has been recommended by WHO and Windle-Taylor for the examination of waters.[4,5] The moderate selective action of the medium is due to the presence of bile salts, which inhibits the growth of some Gram-positive bacteria. The peptones provide carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The fermentation of lactose by coliforms causes acidification of the medium and the formation of red-pink to red-violet colonies. Non-lactose fermenter strains (e.g., *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Alkaligenes* etc.) develop transparent, colourless colonies. Staphylococci are partially inhibited and produce pale pink to red colonies and enterococci produce compact tiny red colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 55 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red, limpid or slightly opalescent
Final pH at 25 °C	7.3 ± 0.2

SPECIMENS

Foodstuffs and waters. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Incubate in aerobic atmosphere at 35-37°C for 18-24 hours or longer if necessary (maybe up to 48 h for late lactose fermenters: *Citrobacter, Providencia*, *Serratia*, *Hafnia*).[6]

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Colonies of lactose fermenters are red-pink to red-violet.
Colonies of non-lactose fermenters are colourless or white or light yellow or with a natural pigmentation (e.g. green for *P. aeruginosa*).
Enterococci produce compact tiny red colonies.
Staphylococci are partially inhibited and produce pale pink to red colonies.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 8739	35-37°C / 18-24 h / A	red-violet colonies
S. Typhimurium ATCC 14028	35-37°C / 18-24 h / A	colourless colonies
E. faecalis ATCC 29212	35-37°C / 18-24 h / A	small red colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Prolonged incubation may lead to confusion of results; do not incubate longer than 48 hours.[6]
- Due to selective properties of this medium some strains of Gram-negative enteric bacteria fail to grow or grow poorly; similarly, some Gram-positive organisms may not be inhibited or are partially inhibited.[6]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up to 6-8 weeks.[6]

REFERENCES

1. MacConkey AT. Note on a new medium for the growth and differentiation of the Bacillus coli communis and the Bacillus Typhi abdominalis. The Lancet, July 07, 1900; vol 156, Issue 4010, P20.
2. Grunbaum AS, Hume EH. Note on media for distinguishing B.coli, B.typhosus and related species. Brit Med J, June 14 1902; p 1473-1474
3. Smith KP. The origin of MacConkey Agar. American Society for Microbiology: Articles, Oct. 14, 2019.
4. World Health Organisation. International Standards for Drinking Water, 2nd Ed. 1963. WHO, Geneve.
5. Windle-Taylor E. The examination of waters and water supplies. 7th Ed. 1958. Churchill Ud., London.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
Mac Conkey Agar OMS w/o Crystal Violet	Dehydrated medium	4016712	500 g (9.1 L)

IFU rev 2, 2022/09

MAC CONKEY BROTH EP
Dehydrated culture medium

INTENDED USE
Liquid medium for the detection of *Escherichia coli* in non-sterile pharmaceutical products, according to harmonized EP method and coliform organisms in foodstuffs and water samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Pancreatic digest of gelatin	20.00 g
Lactose monohydrate	10.00 g
Dehydrated ox bile	5.00 g
Bromocresol purple	0.01 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Alfred Theodore MacConkey[1] in 1901 devised a liquid medium for the cultivation of "*Bacillus coli",* containing sodium taurocholate as a selective agent and litmus as an indicator. The medium was later modified by MacConkey[2,3] by replacing litmus with phenol red and by Childs and Allen[4] who introduced the less inhibitory bromocresol purple as a pH indicator.
Mac Conkey Broth EP, in its current formulation, complies with the recommendations of the harmonized method in the European Pharmacopeia.[5]
MacConkey Broth is used for cultivating Gram-negative, lactose-fermenting bacilli, as a presumptive test for coliform organisms and for detecting *E coli* in non-sterile pharmaceutical products.
Essential growth factors are provided by pancreatic digest of gelatin which is a source of nitrogen, carbon and minerals. Lactose is the fermentable carbohydrate and a source of carbon and energy. Bromocresol purple is the pH indicator. Ox bile inhibits the growth of Gram-positive organisms. Compared to the classic formula, Mac Conkey Broth EP does not contain sodium chloride.
Acids and gases are produced from lactose fermentation: the acidity of the medium is detected by the pH indicator, which turns yellow, while the gas is evidenced by the formation of bubbles that are collected in Durham tubes.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 35 g in 1000 mL of cold purified water; heat to completely dissolve the powder, mix well and distribute 10 mL into test tubes containing inverted Durham tube or 100 mL into flasks. Sterilise by autoclaving at 121°C for 15 minutes. The Durham tubes shall not contain air bubbles after sterilization.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Non-sterile pharmaceutical products, foodstuffs and water samples. Refer to applicable International Standards and regulations for the collection of samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

For the detection of *E. coli* in non-sterile pharmaceuticals products the technique recommended by European Pharmacopoeia should be followed.[5]
Prepare a sample using a 1 in 10 dilution in Pharmacopoeia Diluent (REF 401395) of not less than 1 g of the product to be examined. Use 10 mL or the quantity corresponding to 1 g or 1 mL of sample to inoculate the suitable amount of Tryptic Soy Broth (REF 402155). When testing orodispersible films dissolve 10 films in Pharmacopoeia Diluent. Filter the volume corresponding to 1 film through a sterile filter membrane and place in 100 mL of Tryptic Soy Broth. Mix and incubate at 30-35 °C for 18-24 h.
Shake the container, transfer 1 mL of Tryptic Soy Broth to 100 mL of Mac Conkey Broth EP and incubate at 42-44 °C for 24-48 h.
Subculture on a plate of Mac Conkey Agar (REF 401670) at 30-35 °C for 18-72 h.
For the procedure for the determination of coliforms in samples other than non-sterile pharmaceutical products see 401675 Mac Conkey Broth (Purple).

READING AND INTERPRETATION

After incubation the microbial growth is evidenced by turbidity in the broth. The yellowing of the broth and the production of gas suggest the presence of *E. coli* and possibly of other coliform bacteria. Yellowing alone suggests the presence of coliforms other than *E. coli.*
Growth of colonies on MacConkey Agar indicates the possible presence of *E. coli*. This is confirmed by identification tests. According to European Pharmacopoeia the product complies with the test if no colonies are present on MacConkey Agar plates or if the identification testes are negative.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 8739	42-44°/ 24 H-A	good growth with gas, the medium turns yellow
S. aureus ATCC 6538	42-44°/ 48 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. MacConkey A. Zentralbl. Bakteriol. 1901; 29:740.
2. MacConkey A. Lactose-Fermenting Bacteria in Faeces. J Hyg (Lond) 1905; Jul;5(3):333-79
3. MacConkey A. Bile Salt Media and their advantages in some Bacteriological Examinations. J Hyg (Lond) 1905; 8:322.
4. Childs E, Allen LA. Improved methods for determining the most probable number of Bacterium coli and of Streptococcus faecalis. J Hyg Camb 1953; 51:468.
5. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.

PACKAGING

Product	Type	REF	Pack
Mac Conkey Broth EP	Dehydrated medium	4016792	500 g (14.3 L)

IFU rev 2, 2022/09

MAC CONKEY BROTH (PURPLE)

Dehydrated culture medium

INTENDED USE

Liquid medium for the detection of coliform organisms in foodstuffs and water samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20.00 g
Lactose	10.00 g
Bile salts	5.00 g
Sodium chloride	5.00 g
Bromocresol purple	0.01 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Alfred Theodore MacConkey[1] in 1901 devised a liquid medium for the cultivation of "*Bacillus coli",* containing sodium taurocholate as a selective agent and litmus as an indicator. The medium was later modified by MacConkey[2,3] by replacing litmus with phenol red and by Childs and Allen[4] who introduced the less inhibitory bromocresol purple as a pH indicator. Mac Conkey Broth (Purple), in its current formulation, has been used for decades for the presumptive determination of coliforms by the MPN method in foods, water and dairy products.[5-7]
Essential growth factors are provided by peptone which is a source of nitrogen, carbon and minerals. Lactose is the fermentable carbohydrate and a source of carbon and energy. Bromocresol purple is the pH indicator. Bile salts inhibit the growth of Gram-positive organisms. Compared to the formula included in the European Pharmacopoeia, Mac Conkey Broth (Purple) additionally contains sodium chloride. Acids and gas are produced from lactose fermentation: the acidity of the medium is detected by the pH indicator, which turns yellow, whereas the gas is evidenced by the formation of bubbles that are collected in the Durham tubes.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 10 mL into test tubes containing inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. The Durham tubes shall not contain air bubbles after sterilization. If required, prepare the double strength medium by weighing 80 g/L.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	purple, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	purple, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Food and water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

- Transfer 1 mL of the test sample and its serial decimal dilutions into the tubes.
- Alternatively transfer 10 mL of the test sample into the tubes with 10 mL of double strength medium.
- For coliform bacteria, incubate for 24-48 hours at 30 or 37°C, depending on the analytical protocol.
- For faecal coliform bacteria, incubate at 44.5°C for 24-48 hours.

READING AND INTERPRETATION

After incubation the microbial growth is evidenced by turbidity in the broth. The yellowing of the broth and the production of gas suggest the presence of *E. coli* and possibly of other coliform bacteria. Yellowing alone suggests the presence of coliforms other than *E. coli.*
Bacteria in gas-positive tubes can be identified by subculturing on suitable media such as Levine EMB Agar (REF 401595).

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 8739	37°/ 24 H-A	good growth with gas, the medium turns yellow
S. aureus ATCC 6538	37°/ 24 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. MacConkey A. Zentralbl Bakteriol 1901; 29:740.
2. MacConkey A. Lactose-Fermenting Bacteria in Faeces. J Hyg (Lond) 1905; Jul;5(3):333-79
3. MacConkey A. Bile Salt Media and their advantages in some Bacteriological Examinations. J Hyg (Lond) 1905; 8:322.
4. Childs E, Allen LA. Improved methods for determining the most probable number of Bacterium coli and of Streptococcus faecalis. J Hyg Camb 1953; 51:468
5. Departments of the Environment, Health, Social Security and Public Health Laboratory Service. The Bacteriological Examination of Drinking Water Supplies. Report No. 71. 1982. HMSO London.
6. World Health Organization. International Standards for Drinking Water' 2nd ed., 1963. WHO, Geneva.
7. Davis JG. `Milk Testing' 2nd ed. Dairy Industries Ltd., London,1959.

PACKAGING

Product	Type	REF	Pack
Mac Conkey Broth (Purple)	Dehydrated medium	4016752	500 g (12.5 L)

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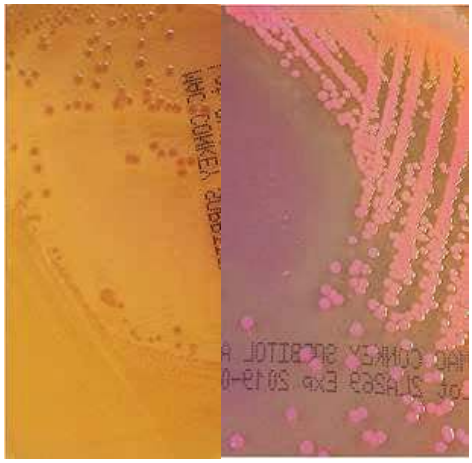
MAC CONKEY SORBITOL AGAR
CEFIXIME TELLURITE O157 SUPPLEMENT
Dehydrated and ready-to-use culture medium, selective supplement



Mac Conkey Sorbitol Agar*:* at left sorbitol non-fermenting *E. coli* O157, at right sorbitol fermenting *E. coli*

INTENDED USE
Selective and differential medium and selective supplement for the isolation of *Escherichia coli* O157:H7 from food and other materials.

COMPOSITION
MAC CONKEY SORBITOL AGAR, DEHYDRATED MEDIUM AND READY-TO-USE PLATES TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Tryptone	17.000 g
Peptocomplex	3.000 g
D-sorbitol	10.000 g
Bile salts No. 3	1.500 g
Sodium chloride	5.000 g
Neutral red	0.030 g
Crystal violet	0.001 g
Agar	14.500 g

CEFIXIME TELLURITE O157 SUPPLEMENT (FOR 500 mL OF MEDIUM)
VIAL CONTENTS

Cefixime	0.025 mg
Potassium tellurite	1.250 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
E. coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak investigation of haemorrhagic colitis.[1] Although more than 300 verotoxins or Shiga toxins producing serotypes are known, the infection is mainly caused by the motile serotype *E. coli* O157:H7 and its non-motile variant O157:NM (O157:H-).[2] The severity of illness presents different degrees, from uncomplicated diarrhoea to haemorrhagic colitis, up to haemolytic-uremic syndrome and thrombotic thrombocytopenic purpura; the infectious dose for O157:H7 is estimated to be 10-100 cells; the infection is particularly serious for the most vulnerable subjects, such as children and the elderly.[3] The strain virulence is substantially due to the production of one or both of the Shiga toxins Stx1 and Stx2 and, more rarely, of their variants. Infections are mostly food or water borne and have implicated undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice, sprouts and vegetables.[4] Direct contact with animals belonging to the reservoir species and person to person transmission may play a role in the spread of infection.[5]
E. coli O157:H7 strains are phenotypically distinct from *E. coli* as they exhibit slow or no fermentation of sorbitol and do not have glucuronidase activity; these characteristics led to the design of various culture media for primary isolation.[6]
Mac Conkey Sorbitol Agar is prepared according to a modification of the formula described by Rappaport and Henig[7]; the selective supplement Cefixime Tellurite O157 Supplement is prepared on the basis of the observations published by Zadik[8].
Mac Conkey Sorbitol Agar, supplemented with cefixime and potassium tellurite (CT-SMAC), complies with the formulation reported in ISO 16654[9] for the isolation of *E. coli* O157, as well as with the FDA-BAM requirements[2].
Mac Conkey Sorbitol Agar is identical to Mac Conkey Agar except that lactose has been replaced with sorbitol. *E. coli* 0157:H7 does not ferment sorbitol or ferments it beyond 24 hours of incubation and grows with colourless colonies, lactose fermenter non-O157 strains grow with red-purple colonies, often surrounded by an opaque pink-red halo.
The determination of *E. coli* 0157:H7 on faecal samples with MacConkey Agar with sorbitol, according to the data of March[10], has a sensitivity of 100%, a specificity of 85% and an accuracy of 86%.
The selective action of Mac Conkey Sorbitol Agar is due to the presence of bile salts n°3, which inhibit the growth of Gram-positive bacteria; this inhibitory activity is enhanced by the addition of crystal violet. To increase the selective properties and the specificity of the results, potassium tellurite and cefixime can be added to the medium: according to the data of Zadik[10] this addition completely or partially inhibits the growth of 67% of *E. coli* non-O157 and almost completely the growth of others sorbitol non-fermenting Gram-negative bacteria.
Mac Conkey Sorbitol Agar, supplemented with cefixime and potassium tellurite, is intended for the isolation and differentiation of E. *coli* O157:H7 and from food and other materials[2,9].

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 25.5 g in 500 mL of cold purified water. Heat to boiling, stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C and distribute into sterile Petri dishes. If cefixime-tellurite addition is required, reconstitute one vial of the lyophilised supplement with 5 mL of sterile purified water and, under aseptic conditions, add to 500 mL of pre-cooled medium base. Mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greyish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS
Mac Conkey Sorbitol Agar, with or without Cefixime Tellurite O157 Supplement, is intended for the bacteriological processing of food and other materials. For the sample's preparation, refer to the applicable international standards.[2,9]

TEST PROCEDURE
Allow plates to come to room temperature and to dry the surface of the medium.
- A test amount is enriched in nine times the weight of pre-warmed Modified Tryptic Soy Broth (REF 402155M2) plus novobiocin 20mg/L (Novobiocin Antimicrobic Supplement -REF 4240045) at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h.
- *E. coli* O157 cells are separated and concentrated using immunomagnetic beads coated with antibodies to *E. coli* O157 after 6 h and again, if necessary, after a further 12 to 18 h incubation.

- 50 µl of immunomagnetic concentrated broth are sub-cultured onto CT-SMAC and onto a second selective isolation agar of laboratory choice (e.g. Mac Conkey Sorbitol MUG Agar REF 401669 or Chromogenic *E. coli* O157 Agar REF 405581). CT-SMAC is incubated at 37±1°C for 18 to 24 h. The second agar of choice should be incubated following the IFU's recommended procedures.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Colourless colonies (sorbitol negative) can be presumptively identified as *E. coli* O157.
Purify the sorbitol negative colonies from CT-SMAC by streaking onto Nutrient Agar and incubate at 35-37°C for 18 to 24 h.
For confirmation, ISO16654[9] requires indole test (+) and agglutination with *E. coli* O157 antiserum.
FDA BAM[2] requires β-galactosidase (+), β-glucuronidase (-) and indole (+) tests and the presence of the O157 and H7 antigens.
The sorbitol negative colony with the biochemical profile of *E. coli* and positive for the antisera O157 and H7 is confirmed as *E. coli* O157:H7.
If the isolate is O157 positive but H7 negative it may be a non-motile variant (O157: NM) and therefore requires a confirmation test of its toxigenic potential (for example with PCR technique). The colony can also be sub-cultured to blood agar plate to induce mobility and re-tested with H7 antiserum.
O157:H7 and O157: NM isolates that produce verocytotoxin are considered pathogenic. However, an O157: NM strain that does not produce shiga toxins or other EHEC (Enterohaemorrhagic *E. coli*) virulence factors is probably non-pathogenic. There are many E. coli O157 serotypes that carry other than H7 antigens (e.g.: H3, H12, H16, H38, H45, etc), and these often do not carry EHEC virulence factors.[2]
For a complete explanation of the identification criteria and methods, refer to the literature for food samples.[2,12]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Escherichia coli O157 ATCC 43894	35-37°C / 18-24 H / A	growth, colourless colonies
Escherichia coli ATCC 25922	35-37°C / 18-24 H / A	growth partially inhibited, red colonies
S. aureus ATCC 25923	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- There are several well-known EHEC strains that have caused illness worldwide, e.g.: O26, O111, O121, O103, O145, O45, etc. However, these strains ferment sorbitol and are not distinguishable on CT-SMAC. For the determination of these strains in food, refer to the cited literature.[2]
- *E. coli* O157 sorbitol positive and β-glucuronidase positive strains and strains that do not grow on CT-SMAC have been reported.[13,14] For the management of these strains refer to the cited literature.[11,12]
- Follow the recommended times and temperatures as *E. coli* O157 does not grow at 44-45 ° C and because delayed observation of the colonies can lead to errors of interpretation.
- Some enterococci can develop small colonies with prolonged incubation beyond 24 hours.
- The presence of colourless colonies on the medium is not in itself indicative of the presence of *E. coli* O157 as other sorbitol negative bacteria can grow with colourless colonies (*Escherichia hermannii, Proteus, Pseudomonas, Acinetobacter* etc.).
- Although the intended use and the test procedure of the medium refers to the detection of *E. coli* O157:H7 in food and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of the medium for human clinical specimens.[11,12] Clinical applications should be validated by the user.
- It is recommended that suitable testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium: store at +10°C /+30°C away from direct light in a dry place.
Selective supplement: store the product in the original package at +2°C /+8°C away from direct light.
According to ISO 16654 the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to two weeks.[9]
Ready-to-use plates: store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Centers for Disease Control. 1984. Update: sporadic hemorrhagic colitis. Morbid. Mortal. Weekly Rep. 33:28
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual. Chapter 4a Diarrheagenic Escherichia coli. Rev October 2018
3. Griffin, P. M., S. M. Ostroff, R. V. Tauxe, K. D. Greene, J. G. Wells, J. H. Lewis, and P. A. Blake. 1988. Illnesses associated with Escherichia coli O157:H7 infections. A broad clinical spectrum. Ann. Intern. Med. 109:705–712.
4. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis. 2005; 11:603–609.
5. Johnson RP, Wilson JB, Michel P, Rahn K, Renwick SA, Gyles CL, Spika JS. Human infection with verotoxigenic *Escherichia coli* associated with exposure to farms and rural environments. In: Stewart CS, Flints HJ, editors. Escherichia coli O157 in Farm Animals. CABI Publishing; Wallingford, U.K: 1999. pp. 147–168.
6. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of Escherichia coli serotype O157. J Clin Microbiol. 1990; 28:2165–2168.
7. Rappaport F, Henig E. Media for the isolation and differentiation of pathogenic Escherichia coli (serotypes 0111 and 055). J Clin Path 1952; 5:361-362.
8. Zadik PM, Chapman PA, and Siddons CA. Use of tellurite for the selection of verocytotoxigenic Escherichia coli O157. J. Med. Microbiol 1993; 39:155-158
9. ISO 16654:2001. Microbiology of food and animal feeding stuffs- Horizontal method for detection of E. coli O157
10. March SB, Ratnam S. Sorbitol-MacConkey Medium for detection of Escherichia coli 0157:H7 associated with hemorrhagic cholitis. J Clin Microbiol 1986; 23: 869-872
11. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. B38. Issue 8.1. 2014
12. Public Health England. Identification of Vero cytotoxin-producing Escherichia coli including Escherichia coli O157. ID22. Issue 4. 2015.
13. Díaz S, Vidal D, Herrera-León S, Sánchez S. Sorbitol-fermenting, β-Glucuronidase-positive, shiga toxin-negative Escherichia coli O157:H7 in free-ranging red deer in South-Central Spain. Foodborne Pathog Dis 2018; 8:1313-1315
14. CDR Weekly. CDR. Sorbitol-fermenting Vero cytotoxin-producing E. coli (VTEC 0157). CDR. 2006

PACKAGING

Product	Type	REF	Pack
Mac Conkey Sorbitol Agar	Dehydrated medium	401669S2	500 g (9,8 L)
Cefixime Tellurite O157 Supplement	Freeze-dried supplement	42ISEC	10 vials, each for 500 mL of medium
Mac Conkey Sorbitol Agar	Ready-to-use plates	541669S	2 x 10 plates ø 90 mm

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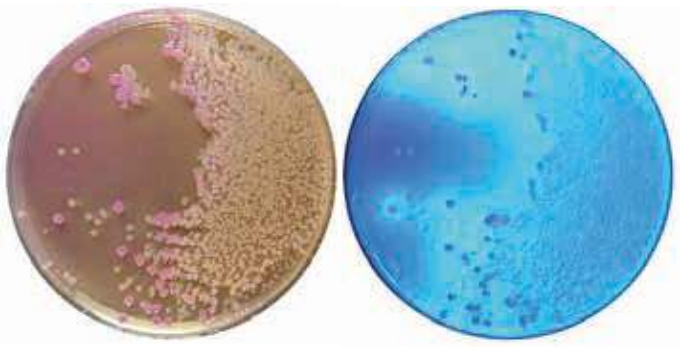
MAC CONKEY SORBITOL MUG AGAR
CEFIXIME TELLURITE O157 SUPPLEMENT
Dehydrated culture medium and selective supplement



Mac Conkey Sorbitol MUG Agar: *E. coli* and *E. coli* O157 colonies under normal light and Wood's lamp

INTENDED USE
Selective and differential medium and selective supplement for the isolation of *Escherichia coli* O157:H7.

COMPOSITION
MAC CONKEY SORBITOL MUG AGAR
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Tryptone	17.000 g
Peptocomplex	3.000 g
D-Sorbitol	10.000 g
Bile Salts No. 3	1.500 g
Sodium chloride	5.000 g
Neutral red	0.030 g
Crystal violet	0.001 g
4-Methylumbelliferyl- β-D-glucuronide (MUG)	0.100 g
Agar	14.500 g

CEFIXIME TELLURITE O157 SUPPLEMENT (FOR 500 ML OF MEDIUM)
VIAL CONTENTS

Cefixime	0.025 mg
Potassium tellurite	1.250 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
E. coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak investigation of haemorrhagic colitis.[1] Although more than 300 verotoxins or Shiga toxins producing serotypes are known, the infection is mainly caused by the motile serotype *E. coli* O157:H7 and its non-motile variant O157:NM (O157:H-).[2] The severity of illness presents different degrees, from uncomplicated diarrhoea to haemorrhagic colitis, up to haemolytic-uremic syndrome and thrombotic thrombocytopenic purpura; the infectious dose for O157:H7 is estimated to be 10-100 cells; the infection is particularly serious for the most vulnerable subjects, such as children and the elderly.[3] The strain virulence is substantially due to the production of one or both of the Shiga toxins Stx1 and Stx2 and, more rarely, of their variants. Infections are mostly food or water borne and have implicated undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice and sprouts and vegetables.[4] Direct contact with animals belonging to the reservoir species and person to person transmission may play a role in the spread of infection.[5]
E. coli O157:H7 strains are phenotypically distinct from *E. coli* as they exhibit slow or no fermentation of sorbitol and do not have glucuronidase activity; these characteristics led to the design of various culture media for primary isolation.[6]
Mac Conkey Sorbitol MUG Agar is prepared according to a modification of the MacConkey formula described by Rappaport and Henig[7] for substituting lactose with sorbitol and by Szabo[8], for the inclusion of 4-methylumbelliferyl- β-D-glucuronide (MUG). The selective supplement Cefixime Tellurite O157 Supplement is prepared on the basis of the observations published by Zadik[9].
E. coli O157:H7 does not ferment sorbitol or ferments it beyond 24 hours of incubation, does not hydrolyse MUG and grows with colourless colonies, not fluorescent under long-wave ultraviolet light (Wood's lamp), lactose fermenter non-O157 strains grow with red-purple colonies, often surrounded by an opaque pink-red halo and with a slightly blue fluorescence under Wood's lamp.
The determination of *E. coli* O157:H7 on faecal samples with MacConkey Agar with sorbitol, according to the data of March[10], has a sensitivity of 100%, a specificity of 85% and an accuracy of 86%.
According to Okrend[11], the addition of a substrate to determine the β-glucuronidase enzyme decreases falsely suspect colonies by 36% compared to Mac Conkey Sorbitol Agar.
The selective action of Mac Conkey Sorbitol Agar is due to the presence of bile salts n°3, which inhibit the growth of Gram-positive bacteria; this inhibitory activity is enhanced by the addition of crystal violet. To increase the selective properties and the specificity of the results, potassium tellurite and cefixime can be added to the medium: according to the data of Zadik[9] this addition completely or partially inhibits the growth of 67% of *E. coli* non-O157 and almost completely the growth of others sorbitol non-fermenting Gram-negative bacteria.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 25.5 g in 500 mL of cold purified water. Heat to boiling, stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to 44-47°C and distribute into sterile Petri dishes. If cefixime-tellurite addition is required, reconstitute one vial of the lyophilised supplement with 5 mL of sterile purified water and, under aseptic conditions, add to 500 mL of pre-cooled medium base. Mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greyish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid or slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS
Mac Conkey Sorbitol MUG Agar, with or without Cefixime Tellurite O157 Supplement, is intended for the bacteriological processing of non-clinical specimens; good laboratory practices for collection, transport and storage of the samples should be applied.[13] Refer to the applicable international standards.[2,12]

TEST PROCEDURE
- A test amount is enriched in nine times the weight of pre-warmed Modified Tryptic Soy Broth (REF 402155M2) plus novobiocin 20mg/L (Novobiocin Antimicrobic Supplement -REF 4240045) at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h.
- *E. coli* O157 cells are separated and concentrated using immunomagnetic beads coated with antibodies to *E. coli* O157 after 6 h and again, if necessary, after a further 12 to 18 h incubation.

- 50 µl of immunomagnetic concentrated broth are sub-cultured onto CT-SMAC MUG and onto a second selective isolation agar of laboratory choice (e.g. Chromogenic E. coli O157 Agar REF 4055812). CT-SMAC MUG is incubated at 37±1°C for 18 to 24 h. The second agar of choice should be incubated following the IFU recommended procedures.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies at the normal light; check the plates by using a hand-held 366-nm light or by placing the plate on a long-wave UV lightbox UV (Wood's Lamp).
Colourless and not fluorescent colonies (sorbitol negative, β-glucuronidase negative) can be presumptively identified as *E. coli* O157.
Purify the typical colonies from CT-SMAC MUG by streaking onto Nutrient Agar and incubate at 35-37°C for 18 to 24 h.
For confirmation, ISO16654[12] requires indole test (+) and agglutination with *E. coli* O157 antiserum.
In addition to β-glucuronidase negative test, FDA BAM[2] requires β-galactosidase (+), indole (+) tests and the presence of the O157 and H7 antigens.
The sorbitol negative and β-glucuronidase negative colony with the biochemical profile of *E. coli* and positive for the antisera O157 and H7 is confirmed as *E. coli* O157:H7.
If the isolate is O157 positive but H7 negative it may be a non-motile variant (O157: NM) and therefore requires a confirmation test of its toxigenic potential (for example with PCR technique). The colony can also be sub-cultured to blood agar plate to induce mobility and re-tested with H7 antiserum.
O157:H7 and O157: NM isolates that produce verocytotoxin are considered pathogenic. However, an O157: NM strain that does not produce shiga toxins or other EHEC (Enterohaemorrhagic *E. coli*) virulence factors is probably non-pathogenic. There are many *E. coli* O157 serotypes that carry other than H7 antigens (e.g.: H3, H12, H16, H38, H45, etc), and these often do not carry EHEC virulence factors.[2]
For a complete explanation of the identification criteria and methods, refer to the literature cited for clinical samples[14] and for food samples[2].

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Escherichia coli O157 ATCC 43894	35-37°C / 18-24 H / A	growth, colourless colonies not fluorescent under Wood's lamp
Escherichia coli ATCC 25922	35-37°C / 18-24 H / A	growth partially inhibited, red colonies fluorescent under Wood's lamp
S. aureus ATCC 25923	35-37°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- There are several well-known EHEC strains that have caused illness worldwide, e.g.: O26, O111, O121, O103, O145, O45, etc. However, these strains ferment sorbitol and are not distinguishable on CT-SMAC MUG. For the determination of these strains in food, refer to the cited literature.[2]
- *E. coli* O157 sorbitol positive and β-glucuronidase positive strains and strains that do not grow on CT-SMAC MUG have been reported.[15,16] For the management of these strains refer to the cited literature.[14]
- Follow the recommended times and temperatures as *E. coli* O157 does not grow at 44-45°C and because delayed observation of the colonies can lead to errors of interpretation.
- Some enterococci can develop small colonies with prolonged incubation beyond 24 hours.
- The presence of colourless colonies on the medium is not in itself indicative of the presence of *E. coli* O157 as other sorbitol negative bacteria can grow with colourless colonies (*Escherichia hermannii, Proteus, Pseudomonas, Acinetobacter* etc.).
- Although the intended use and the test procedure of the medium refers to the detection of E. coli O157:H7 in food and therefore the product should not be regarded as an *in vitro* diagnostic, the literature reports the use of the medium for human clinical specimens.[13,14] Clinical applications should be validated by the user.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Selective supplement
Store the product in the original package at 2-8°C away from direct light.

REFERENCES

1. Centers for Disease Control. 1984. Update: sporadic hemorrhagic colitis. Morbid. Mortal. Weekly Rep. 33:28
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual. Chapter 4a Diarrheagenic Escherichia coli. Rev October 2018
3. Griffin, P. M., S. M. Ostroff, R. V. Tauxe, K. D. Greene, J. G. Wells, J. H. Lewis, and P. A. Blake. 1988. Illnesses associated with Escherichia coli O157:H7 infections. A broad clinical spectrum. Ann. Intern. Med. 109:705–712.
4. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis. 2005; 11:603–609.
5. Bach SJ, McAllister TA, Veira DM, Gannon VPJ, Holley RA. Transmission and control of Escherichia coli O157:H7—a review. Can J Anim Sci 2002; 82:475–490.
6. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of Escherichia coli serotype O157. J Clin Microbiol. 1990; 28:2165–2168.
7. Rappaport F, Henig E. Media for the isolation and differentiation of pathogenic Escherichia coli (serotypes 0111 and 055). J Clin Path 1952; 5:361-362.
8. Szabo RA, Todd EC, Jean A., 1986. Method to isolate E. coli O157:H7 from food. J. Food Protect 1986; 10:768–772.
9. Zadik PM, Chapman PA, and Siddons CA. Use of tellurite for the selection of verocytotoxigenic Escherichia coli O157. J. Med. Microbiol 1993; 39:155-158
10. March SB, Ratnam S. Sorbitol-MacConkey Medium for detection of Escherichia coli 0157:H7 associated with hemorrhagic cholitis. J Clin Microbiol 1986; 23: 869-872
11. Okrend AJG, Rose BE, Lattuada CP. Use of 5-Bromo-4-Chloro-3-Indoxyl-β-D-Glucuronide in MacConkey Sorbitol Agar to aid in the isolation of Escherichia Coli 0157:H7 From Ground Beef. J Food Prot 1990; 53:941-943.
12. ISO 16654:2001. Microbiology of food and animal feeding stuffs- Horizontal method for detection of E. coli O157
13. Public Health England. Investigation of Faecal Specimens for Enteric Pathogens. B38. Issue 8.1. 2014
14. Public Health England. Identification of Vero cytotoxin-producing Escherichia coli including Escherichia coli O157. ID22. Issue 4. 2015.
15. Díaz S, Vidal D, Herrera-León S, Sánchez S. Sorbitol-fermenting, β-Glucuronidase-positive, shiga toxin-negative Escherichia coli O157:H7 in free-ranging red deer in south-central Spain. Foodborne Pathog Dis 2018; 8:1313-1315
16. Health Protection Agency (HPA). CDR Weekly. Sorbitol-fermenting Vero cytotoxin-producing E. coli (VTEC 0157). CDR 16(21) 2006b.

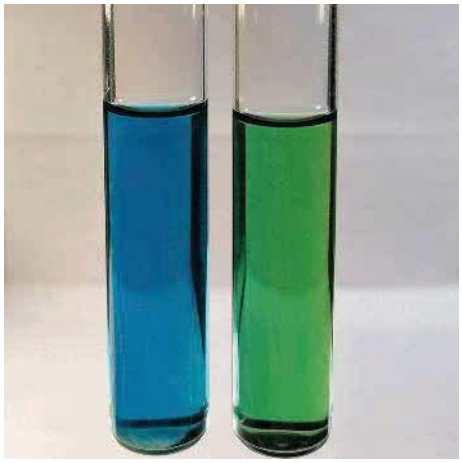
PACKAGING

Product	Type	REF	Pack
Mac Conkey Sorbitol MUG Agar	Dehydrated medium	4016692	500 g (9,8 L)
Cefixime Tellurite O157 Supplement	Freeze-dried supplement	42ISEC	10 vials, each for 500 mL of medium

IFU rev 2, 2022/05

MALONATE BROTH

Dehydrated culture medium



Malonate Broth
From left: *E. aerogenes* malonate +, *E. coli*, malonate -

INTENDED USE

In vitro diagnostic. For the differentiation of *Enterobacter* from *Escherichia* based on malonate utilisation.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Ammonium sulphate	2.000 g
Dipotassium phosphate	0.600 g
Monopotassium phosphate	0.400 g
Sodium chloride	2.000 g
Sodium malonate	3.000 g
Bromothymol blue	0.025 g
Glucose	0.250 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The malonate test was introduced by Leifson (1933)[1] to help distinguish *Escherichia coli* from *'Klebsiella aerogenes*', and with these organisms he found a perfect correlation with the VP test. An organism that simultaneously can utilize sodium malonate as a carbon source and ammonium sulphate as a nitrogen source produces an alkalinity due to the formation of sodium hydroxide. This results in an alkaline reaction which in a medium containing malonate, changes the indicator (bromothymol blue) from its original green colour to light blue or Prussian blue. Organisms which cannot utilize malonate and ammonium sulphate produce no colour change.
Malonate Broth is prepared according to a modification of the original formulation proposed by Leifson and does not include yeast extract as in Ewing's modified version.[2]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 8.3 g in 1000 mL of cold purified water, heat to dissolve, distribute into tubes and sterilise by autoclaving at 121 °C for 15 minutes. All glassware must be chemically cleaned and alkali-free.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-green, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	green, limpid
Final pH at 20-25 °C	6.7 ± 0.1

SPECIMENS

The sample consists of bacterial cultures isolated from clinical samples or other materials, purified on Tryptic Soy Agar or blood agar or other suitable medium.

TEST PROCEDURE

Inoculate the medium lightly with a loopful of a pure culture.
Incubate aerobically at 35-37°C for 24-48 hours. Observe the growth at the end of each period.

READING AND INTERPRETATION

Examine the tubes for colour change.
Positive test: alkaline reaction, light blue to Prussian blue colour throughout the medium.
Negative test: no colour change of the medium
Bacterial genera in which the majority of species yields a positive alkaline reaction include: *Enterobacter, Klebsiella, Citrobacter.*
Bacterial genera in which the majority of species yields a negative reaction include: *Escherichia, Serratia, Salmonella, Morganella, Shigella Proteus, Edwardsiella, Providencia, Yersinia.*
Consult the suitable references for the expected reactions for specific microbial species.[3,4]
Consult the suitable references for the expected reactions for specific microbial species.[3,4]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.
Malonate positive strain: *E. aerogenes* ATCC 13048
Malonate negative strain: *E. coli* ATCC 25923

Limitations of the method

- Some malonate negative organisms produce only a slight alkalinity that causes the results to be difficult to interpret. In case of doubt, compare with an uninoculated malonate tube. Any trace of a blue colour denotes a positive test at the end of 48 h incubation period. A final negative interpretation should be made until the tubes have been incubated for 48 h.[5]
- *E. coli* and *Klebsiella/Enterobacter* groups do not absolutely require yeast extract and glucose enrichments; however, when attempting to differentiate the *Salmonella* species from *S. arizonae* it is recommended that 1 g/L of yeast extract and 0,25 g/L of glucose be incorporated into the malonate medium, before autoclaving.[5]
- Even if the microbial colonies are differentiated on the basis malonate test, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

Storage conditions

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 2 weeks.[5]

References

1. Leifson E. The Fermentation of Sodium Malonate as a Means of Differentiating Aerobacter and Escherichia. J Bacteriol. 1933 Sep; 26(3): 329–330.
2. Ewing WH, Davis BR, Reavis RW. Phenylalanine and Malonate Media and Their Use in Enteric Bacteriology. Public Health Lab. 1957; 15: 153-167.
3. Buchan BW et al Escherichia, Shigella and Salmonella. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. Forsythe SJ et al Klebisella and selected Enterobacterales. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

Packaging

Product	Type	REF	Pack
Malonate Broth	Dehydrated medium	4016852	500 g (60.2 L)

IFU rev 1, 2022/04

MALT AGAR

Dehydrated and ready-to-use culture medium

Intended use

For the enumeration of yeasts and moulds in cosmetic and food samples and for cultivating yeast and mould stock cultures.

Composition - typical formula *
(after reconstitution with 1 L of water)

Dehydrated medium and ready-to-use flasks

Malt extract	30 g
Agar	17 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Traditionally acidified media based on malt extract have been used to enumerate yeasts and moulds in different commodities. A medium with 30 g/L of malt extract and agar is known as malt extract agar and is recommended by APHA[1] for the detection and enumeration of heat-resistant moulds in foods. The same medium supplemented with chlortetracycline HCl, is recommended by FDA-BAM[1] (Medium M93) for the enumeration of yeasts and moulds in cosmetics. Malt Agar is included in Official Methods of Analysis of AOAC International.[3] The medium is used for cultivation and maintenance of yeast and mould stock cultures.
Malt Agar contains malt extract, which provides carbon, protein and nutrient sources required for the growth of yeasts and moulds and agar as the solidifying agent. The acidic pH restricts the bacterial growth.

Directions for medium preparation (dehydrated medium)

Suspend 47 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes. If required, add 40 mg/L of chlortetracycline HCl (REF 4240024) after autoclaving.
To prepare slants dispense 5-6 mL of boiled medium into 16 x 125 mm screw-cap, loosely cap tubes and sterilize as above. After autoclaving lay tubes in a slanting position and let them solidify.
When the user's procedures require an acidified medium, melt the sterilised medium in boiling water and acidify to pH 4.5 with sterile tartaric acid. To preserve the solidifying properties of the agar do not heat after the addition of tartaric acid.

Directions for medium preparation (ready-to-use flasks)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

Physical characteristics

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, clear or slightly opalescent
Final pH at 20-25 °C	5.5 ± 0.2

Specimens

Foods, animal feeding stuffs and other samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Prepare suitable decimal dilutions of the samples.
Add 1 mL to empty Petri dishes using two dishes for each dilution. Pour into each dish approximately 15 mL of melted medium, cooled to 44-47°C. Mix gently, allowing the medium to solidify.
Alternatively, directly inoculate the agar plates using surface spread technique with 0.1 or 0.2 mL of decimal dilutions.
Invert the plates and incubate at 22°C for 5-7 days.

READING AND INTERPRETATION
After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies
Count colonies on plates that contain an estimated 50-100 colonies. Report as number of yeasts or moulds per gram of food by multiplying the number of colonies by the dilution factor.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25°C/ 72h/A	good growth
Aspergillus brasiliensis ATCC 16404	25°C/ 72h/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[4]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[4]
- Non-linearity of counts from dilution plating often occurs, i.e.10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[4]
- It is recommended that identification testing be performed on isolates, from pure culture.

STORAGE CONDITIONS
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the plated medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks while the tubed medium at +2°C/+8°C for 6 months.[5]
Ready-to-use flasks
Store flasks in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 23 Microbiological Methods for Cosmetics. Rev December 2021
3. Official methods of analysis of AOAC International, 18th ed. 2007. AOAC International, Gaithersburg, Md.
4. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
Malt Agar	Dehydrated medium	4016452	500 g (10.6 L)
Malt Agar	Ready-to-use flasks	5116452	6 x 100 mL

IFU rev 2, 2022/10

MALT EXTRACT AGAR
Dehydrated culture medium

INTENDED USE
For the enumeration of yeasts and moulds in water, food and other samples and for cultivating yeast and mould stock cultures.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Maltose	12.75 g
Dextrin	2. 50 g
Glycerol	1.00 g
Peptocomplex	2.60 g
Agar	17.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Traditionally acidified media based on malt and malt extracts have been used to enumerate yeasts and moulds in different commodities. Malt Extract Agar is recommended by APHA[1] for the enumeration of yeasts and moulds in water samples and for purifying yeast isolates and studying yeast species in various tests; it is also useful for maintaining stock cultures.
Malt Extract Agar contains maltose as an energy source. Dextrin, derived from starch, and glycerol are carbon sources. Peptocomplex provides nitrogen and minerals for microbial growth. The acidic pH restricts the bacterial growth.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 35.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes. To prepare slants dispense 5-6 mL of boiled medium into 16 x 125 mm screw-cap, loosely cap tubes and sterilize as above. After autoclaving lay tubes in a slanting position and let them solidify.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	white, opalescent
Final pH at 20-25 °C	4.7 ± 0.2

SPECIMENS
Waters, foods and other samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Prepare suitable decimal dilutions of the samples.
Add 1 mL to empty Petri dishes using two dishes for each dilution. Pour into each dish approximately 15 mL of melted medium, cooled to 44-47°C. Mix gently, allowing the medium to solidify.
Alternatively, directly inoculate the agar plates using surface spread technique with 0.1 or 0.2 mL of decimal dilutions.
Invert the plates and incubate at 22°C for 5-7 days.

READING AND INTERPRETATION
After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies
Count colonies on plates that contain an estimated 50-100 colonies. Report as number of yeasts or moulds per gram of food by multiplying the number of colonies by the dilution factor.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25°C/72h/A	good growth
Aspergillus brasiliensis ATCC 16404	25°C/72h/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[2]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[2]
- Non-linearity of counts from dilution plating often occurs, i.e.10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[2]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that identification testing be performed on isolates, from pure culture.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the plated medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks while the tubed medium at +2°C/+8°C for 6 months.[3]

REFERENCES

1. APHA Standards Methods for the Microbiological of Water and Wastewater. American Public Health Association, Washington D.C. 23rd, 2017.
2. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
Malt Extract Agar	Dehydrated medium	4016552	500 g (14 L)

IFU rev 2, 2022/10

MALT EXTRACT BROTH
Dehydrated culture medium

INTENDED USE
For the detection, isolation and enumeration of fungi in various materials and for cultivating yeast and mould stock cultures

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)
Malt extract	17 g
Mycological peptone	3 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Traditionally, acidified media based on malt extract have been used to enumerate yeasts and moulds in different commodities. Malt Extract Broth is used for the detection, isolation and enumeration of fungi in various materials and for cultivating yeast and mould stock cultures for microbiological assays and for biochemical and molecular studies.
Mycological peptone provides nitrogen, whereas malt extract is a source of carbon and nutrients for the growth of yeasts and moulds. The acidic pH restricts the bacterial growth. Selectivity may be enhanced by decreasing the pH with lactic acid or tartaric acid and by addition of antibiotics.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 20 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute into final containers and sterilise by autoclaving at 115ºC for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear
Final pH at 20-25 °C	5.4 ± 0.2

SPECIMENS
Foods, animal feeding stuffs and other samples; pure stock cultures. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Inoculate each test strain or specimen onto tubes. Incubate at 22-25°C for 2-7 days.
The incubation conditions may vary according to the type of expected microorganisms and can be extended.
The user is responsible for choosing the appropriate incubation time, and temperature depending on the processed sample or inoculated strain, the requirements of organisms to be recovered or cultivated and the local applicable protocols.

READING AND INTERPRETATION
After incubation, the presence of microbial growth is evidenced by the presence of turbidity compared to an un-inoculated control. The characteristic of the growth is closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25°C/ 72h/A	good growth
Aspergillus brasiliensis ATCC 16404	25°C/ 72h/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Since Malt Extract Broth is a general-purpose medium with poor selective properties, bacterial strains will also grow.

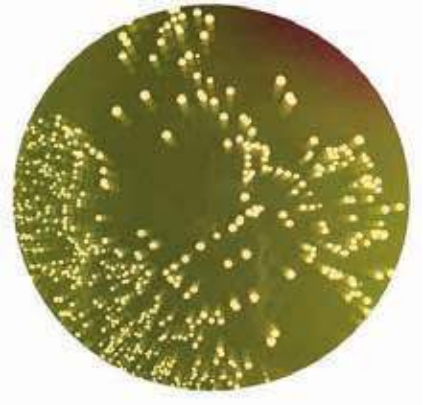
STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months.[1]

REFERENCES
1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
Malt Extract Broth	Dehydrated medium	4016602	500 g (25 L)

IFU rev 2, 2022/10

. MANNITOL SALT AGAR
Dehydrated and ready-to-use culture medium



S. aureus on Mannitol Salt Agar

INTENDED USE
In vitro diagnostics. Selective medium for the isolation and differentiation of staphylococci from clinical specimens. For the detection of *Staphylococcus aureus* in non-sterile pharmaceutical products according to the harmonized method EP, USP, JP and in cosmetics.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Pancreatic digest of casein	5.000 g
Pancreatic digest of animal tissue	5.000 g
Beef extract	1.000 g
Sodium chloride	75.000 g
Mannitol	10.000 g
Phenol red	0.025 g
Agar	15.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Staphylococcus species are Gram positive, non-motile, non-sporeforming cocci of varying size occurring singly, in pairs and in irregular clusters. The optimum growth temperature is 30-37°C. They are facultative anaerobes and have a fermentative metabolism. *S. aureus* is a ubiquitous commensal bacterium on human skins and anterior naris, but frequently causes severe infections in humans. Mannitol Salt Agar is based on early work by Gordon[1] indicating that the fermentation of mannitol could be used as a mean of differentiating pathogenic from non-pathogenic staphylococci, and by Koch[2] discovery that the presence of 7.5% sodium chloride in media inhibited the growth of most organisms except staphylococci. Chapman[3] utilized this information to formulate phenol-red mannitol agar with 7,5% of sodium chloride.

Mannitol Salt Agar is a selective and differential medium intended for the isolation of staphylococci from clinical specimens[4,5] and for the differentiation of mannitol fermenting from non-fermenting staphylococci. In clinical samples, mannitol positive isolates are suggestive of *S. aureus* and should be tested further.[6]

Mannitol Salt Agar meets harmonised EP, USP, JP requirements [7] for the detection of *S. aureus* in non-sterile pharmaceutical products and is recommended for the detection of *S. aureus* in cosmetics[8,9].

Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride at the concentration of 75 g/L creates a high osmotic pressure: staphylococci can withstand the pressure, while this concentration will inhibit the growth of most other gram-positive and gram-negative bacteria[2]. Additionally, the medium contains mannitol as fermentable carbohydrate and phenol red as a pH indicator. When mannitol is fermented acid is produced, which lowers the pH and results in the formation of a yellow area surrounding an isolated colony. Mannitol non-fermenters that withstand the high salt concentration, would display a red to pink area due to peptone breakdown[6].

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 111 g in 1000 mL of cold purified water. Heat to boiling with agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Mannitol Salt Agar is intended for the bacteriological examination of human clinical specimens from contaminated sources, such as faeces, materials from respiratory tract, purulent exudates, wounds and abscesses.[4,5,10] and non-clinical specimens, such as non-sterile pharmaceutical products and cosmetics[8,9] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[11] Consult appropriate standard methods for details on sample collection and preparation of non-sterile pharmaceutical products and cosmetics [7,8,9]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic conditions at 35-37°C and record the results after 24 and 48 hours.

For the detection of *S. aureus* in non-sterile pharmaceutical products follow the test method described by EP and summarized below.

Use 10 mL or the quantity corresponding to 1 g or 1 mL of sample to inoculate a suitable amount of Tryptic Soy Broth. Mix and incubate at 30–35 °C for 18–24 h. Subculture by streaking on a plate of Mannitol Salt Agar, and incubate at 30–35°C for 18–72 h.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Suspected *S. aureus* colonies are yellow/white surrounded by a yellow zone.

Mannitol non-fermenting staphylococci produce small colonies with no colour change of the medium or with red or purple zone.

It is mandatory to confirm putative *S. aureus* isolates recovered on Mannitol Salt Agar.[10]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[12]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538 or 25923	35°C / 18-24 H / A	growth with yellow colonies and yellow halo
S. epidermidis ATCC12228	35°C / 18-24 H /A	growth with small colourless colonies with violet halo
P. mirabilis ATCC 12453	35°C / 18-24 H / A	partially inhibited
E. coli ATCC 8739	35°C / 68-72 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Enterococci may exhibit growth and slight mannitol fermentation; however, catalase test and Gram morphology should separate the two genera.[13]
- Few strains of *S. aureus* may exhibit a delayed mannitol fermentation; negative plates should be re-incubated for additional 24 hours before being discarded.[13]
- A putative *S. aureus* strain must be confirmed by the coagulase test. Subculture the colonies on a non-inhibitory agar for performing the test.
- Mannitol Salt Agar is a selective medium for pathogenic *S. aureus* isolation when distinct colonies are observed after 24 hours of incubation; however, if incubated 48 hours even *Micrococcus* and *Bacillus* and certain *Serratia* strains may grow.[13]
- Media colour change demonstrates mannitol fermentation, not the colony colour. This is particularly important as many micrococci are pigmented.[6]
- There are reports that some coagulase negative staphylococci can produce yellow colonies with yellow zone on Mannitol Salt Agar.[14]
- Some target organisms (potentially pathogen *Staphylococcus* strains) may be inhibited on this medium. The sensitivity of the described procedure varies depending of the clinical specimens, the amount of competitive non-target organisms and the number of target organisms. If it is necessary to detect all potential pathogens it is advisable to use also a non-selective and non-differential medium together with Mannitol Salt Agar.
- For the detection of Methicillin Resistant Staphylococcus Aureus (MRSA) strains more accurate detection methods, such as molecular techniques, should be applied.

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 6-8 weeks.

Ready-to-use plates

Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Gordon, M. H. 1903-04. Reports of some characters by which various streptococci and staphylococci may be differentiated and identified. Local British Government Board, Rept. Med. Officer. 33:388–430.
2. Koch, F. E. 1942. Electivnährboden für Staphylokokken. Zentr. Bakt. Parasitenk. I Orig.149:122–124.
3. Chapman, G. H. 1945. The significance of sodium chloride in studies of staphylococci. J.Bacteriol. 50:201–203.
4. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
5. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
6. Shields P, Tsang AY. Mannitol salt agar plates protocol. American Society for Microbiology (ASM), October 9, 2006.
7. European Pharmacopoeia, current edition
8. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 23 Microbiological Methods for Cosmetics. July 2017
9. ISO 22718:2015 Cosmetics –Microbiology- Detection of Staphylococcus aureus
10. Becker K, Skow R, Von Eiff C. Staphylococcus, Micrococcus, and Other Catalase Poistive Cocci. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.354
11. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
12. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
13. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
14. Thakur P, Nayyar C, Tak V, Karnika Saigal K. Mannitol-fermenting and tube coagulase-negative staphylococcal isolates: unraveling the diagnostic dilemma. J Lab Physicians 2017; 9(1):65–66.

PACKAGING

Product	Type	REF	Pack
Mannitol Salt Agar	Dehydrated medium	4016652 4016654	500 g (4.5L) 5 kg (45 L)
Mannitol Salt Agar	Ready-to-use plates	541665	2 x 10 plates ø 90 mm

IFU rev 3, 2022/01

. MANNITOL SALT BROTH

Dehydrated culture medium

INTENDED USE

Selective liquid medium for the detection and differentiation of staphylococci.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10.000 g
Beef extract	1.000 g
Sodium chloride	75.000 g
Mannitol	10.000 g
Phenol red	0.025 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Mannitol Salt Broth is based on early work by Gordon[1] indicating that the fermentation of mannitol could be used as a mean of differentiating pathogenic from non-pathogenic staphylococci, and by Koch[2] discovery that the presence of 7.5% sodium chloride in media inhibited the growth of most organisms except staphylococci. Chapman[3] utilized this information to formulate phenol-red mannitol media with 7.5% of sodium chloride. Mannitol Salt Broth is a selective and differential medium intended for enrichment of staphylococci and for their enumeration by MPN or MF techniques. Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride at the concentration of 75 g/L creates a high osmotic pressure: staphylococci can withstand the pressure, while this concentration will inhibit the growth of most other gram-positive and gram-negative bacteria[2]. Additionally, the medium contains mannitol as fermentable carbohydrate and phenol red as a pH indicator. When mannitol is fermented acid is produced, which lowers the pH and results in the formation of a yellow colour. Mannitol non-fermenters that withstand the high salt concentration, would display a red to pink colour due to peptone breakdown[4].

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 96 g in 1000 mL of cold purified water. Mix thoroughly and heat if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	orange, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-orange, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Foodstuffs and other materials of sanitary importance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Place 1 mL of the sample in a 9 mL tube, or 10 mL in a 90 mL bottle. Alternatively pour 2 mL onto absorbent pad placed in a 55 mm Ø Petri dish and then place the membrane used for the filtration of the sample.
Incubate at 37 °C for 24-48 hours.

READING AND INTERPRETATION
After incubation, observe the bacterial growth: yellow tubes presumably contain *S. aureus*, turbid, non-yellow tubes presumably contain other staphylococci.
With membrane filtration, yellow colonies are presumed to be *S. aureus* and red colonies are presumed to be other staphylococci.
Confirm the isolates by coagulase test or latex agglutination test.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538 or 25923	37°C / 18-24 H / A	growth the medium turns yellow
S. epidermidis ATCC12228	37°C / 18-24 H /A	growth, no colour changes of the medium
E. coli ATCC 8739	37°C / 24-48 H / A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Enterococci may exhibit growth and slight mannitol fermentation; however, catalase test and Gram morphology should separate the two genera.[5]
- Few strains of *S. aureus* may exhibit a delayed mannitol fermentation; negative tubes should be re-incubated for additional 24 hours before being discarded.[5]
- Mannitol Salt Broth is a selective medium however, if incubated 48 hours, *Micrococcus* and *Bacillus* and certain *Serratia* strains may grow.[5]
- There are reports that some coagulase negative staphylococci can acidify the medium.[6]
- Some target organisms (potentially pathogen *Staphylococcus* strains) may be inhibited on this medium. The sensitivity of the described procedure varies depending of the specimens, the amount of competitive non-target organisms and the number of target organisms.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Gordon, M. H. 1903-04. Reports of some characters by which various streptococci and staphylococci may be differentiated and identified. Local British Government Board, Rept. Med. Officer. 33:388–430.
2. Koch, F. E. 1942. Electivnährboden für Staphylokokken. Zentr. Bakt. Parasitenk. I Orig.149:122–124.
3. Chapman, G. H. 1945. The significance of sodium chloride in studies of staphylococci. J.Bacteriol. 50:201–203.
4. Shields P, Tsang AY. Mannitol salt agar plates protocol. American Society for Microbiology (ASM), October 9, 2006.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Thakur P, Nayyar C, Tak V, Karnika Saigal K. Mannitol-fermenting and tube coagulase-negative staphylococcal isolates: unraveling the diagnostic dilemma. J Lab Physicians 2017; 9(1):65–66.

PACKAGING
Product	Type	REF	Pack
Mannitol Salt Broth	Dehydrated medium	4016662	500 g (5.2 L)

IFU rev 1, 2022/09

MAXIMUM RECOVERY DILUENT
(PEPTONE SALT SOLUTION)
Dehydrated and ready-to-use culture medium

INTENDED USE
Isotonic diluent for the preparation of the initial suspension and dilutions of foods and animal feed stuffs for microbiological examination.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED AND READY-TO-USE TUBES AND FLASKS

Enzymatic digest of casein	1.0 g
Sodium chloride	8.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Maximum Recovery Diluent, also known as peptone salt solution, is formulated as recommended by ISO 6887[1]. It is an isotonic diluent for maximal recovery of microorganisms from samples of the food chain.
The presence of low levels of enzymatic digest of casein in the diluent at a pH 7.0 protects the bacteria and does not allow them to multiply for at least 1-2 hours during the dilution phase. Sodium chloride at physiological strength maintains the osmotic equilibrium contributing to the recovery of microorganisms.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 9.5 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 9 mL in tubes or 90 mL or 225 mL in flasks and sterilise in the autoclave at 121°C for 15 minutes. Cool to room temperature before the use.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared medium appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Waters, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations. [1]

TEST PROCEDURE

The working procedure described here is a summary taken from ISO 6887[1]. For operational details please refer to the cited Standard.

1. Weigh a mass m g or measure a volume V mL (minimum 10 g or 10 mL, unless otherwise stated by specific method), to a tolerance of ± 5 %, representative of the test sample.
2. Add a quantity of Maximum Recovery Diluent equal to 9 × m g or 9 × V mL, to a tolerance of ± 2 %, to prepare the initial suspension (primary decimal dilution).
3. Homogenize the mixture.
4. Allow large particles to settle, if necessary, for up to 15 min. Filtration systems giving equivalent results, such as plastic bags with integral filter liners, may also be used.
5. For a decimal dilution series for use in enumeration tests, transfer, using a pipette, 1 mL ± 0.02 mL of the initial suspension into a tube containing 9 mL of Maximum Recovery Diluent and mix thoroughly (10^{-2} dilution).
6. If necessary, repeat these operations using the 10^{-2} and further dilutions to obtain 10^{-3}, 10^{-4} etc. dilutions, until the appropriate number of microorganisms has been obtained.
7. The time between the end of the preparation of the initial suspension and the moment when the inoculum comes into contact with the final culture medium shall not exceed 45 min. Additionally, the time between the preparation of the initial suspension and the beginning of preparation of any subsequent dilutions shall not exceed 30 min.
8. Use initial suspension and decimal dilutions for the purposes of specific microbiological analyses

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of strains depends on the intended use. Here below are listed some test strains useful for the quality control of medium.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	60 min at room temperature	± 30% original count (subculture in Tryptic Soy Agar)
E. coli ATCC 25922	60 min at room temperature	± 30% original count (subculture in Tryptic Soy Agar)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The test sample may increase the turbidity of the medium although bacterial growth is not present. Subculture to appropriate media is necessary to verify growth of organisms.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Ready-to-use medium in tubes and flasks

Store tubes and flasks in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. ISO 6887-1:2017 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

PACKAGING

Product	Type	REF	Pack
Maximum Recovery Diluent	Dehydrated medium	4016912 4016914	500 g (52.6 L) 5 kg (526 L)
Maximum Recovery Diluent	Ready-to-use medium in tubes	551691	20 x 9 mL
Maximum Recovery Diluent	Ready-to-use medium in flasks	5116912 5116913	6 x 90 mL 6 x 225 mL

IFU rev 2, 2022/08

mCCDA: see CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON

m-CP AGAR BASE
m-CP ANTIMICROBIC SUPPLEMENT
m-CP SUPPLEMENTS A / B / C
m-CP AGAR

Dehydrated and ready-to-use culture medium, selective and differential supplements

INTENDED USE

For the isolation, enumeration and presumptive identification of *Clostridium perfringens* from water samples.

COMPOSITION*

M-CP AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	30.0 g
Yeast extract	20.0 g
Sucrose	5.0 g
L-cysteine HCl	1.0 g
Magnesium sulphate. $7H_2O$	0.1 g
Agar	13.2 g
Bromocresol purple	0.04 g

M-CP ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 ML OF MEDIUM BASE)

Polymyxin B sulphate	12.5 mg (105,000 IU)
D-cycloserine	200 mg

M-CP SUPPLEMENT A
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Phenolphthalein diphosphate	750 mg

M-CP AGAR
READY-TO-USE PLATES, TYPICAL FORMULA

M-CP Agar Base	69.7 g
Polymyxin B sulphate	25.0 mg (210,000 IU)
D-cycloserine	400.0 mg
Phenolphthalein diphosphate	100.0 mg
Ferric chloride	90.0 mg
Indoxyl β-D glucoside	60.0 mg
Purified water	1000 mL

M-CP SUPPLEMENT B
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Ferric chloride	700 mg

M-CP SUPPLEMENT C
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Indoxyl β-D glucoside	450 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

m-CP Agar is a selective and chromogenic medium for the isolation, enumeration and presumptive identification of *Clostridium perfringens* from water samples. m-CP Agar was devised by Bisson and Cabelli[1] who included a number of compatible, differential tests in a selective medium to be used in a membrane filter procedure. The medium has been recommended by the European Council Directive 98/83/EC[2] in its original drafting for the examination of water intended for human consumption. The amendment of 6 October 2015[3] of the Directive refers to the EN ISO 14189[4] for the enumeration of *C. perfringens* including spores.
Tryptose provides nitrogen, carbon, minerals and amino acids for the microbial growth. The yeast extract is a source of vitamins particularly of the B-group. L-cysteine hydrochloride is a reducing agent. Magnesium and iron salts enhance the microbial growth. Sucrose is the fermentable carbohydrate and bromo cresol purple is a pH indicator: sucrose fermenting bacteria exhibit yellow colonies due to the colour change of the pH indicator. Indoxyl-β-D-glucoside is a chromogenic substrate for β-D-gucosidase which cleaves the substrate with the formation of a blue chromophore (*C. perfringens* does not cleave the chromogen). The presence of phosphatase results in the cleavage of phenolphthalein diphosphate, evidenced by the development of a pink-red colour by exposing the colonies to ammonium hydroxide vapour; no colour change will be seen with colonies of organisms that do not possess acid phosphatase. *C. perfringens* does not cleave indoxyl-β-D-glucoside, ferments sucrose and is positive to phosphatase. D-cycloserine and polymyxin B help in the selective isolation of *C. perfringens* by inhibiting accompanying flora.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 34.7 g in 500 mL of cold purified water, heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add and the contents of one vial of m-CP Antimicrobic Supplement (REF 4240070), reconstituted with 5 mL of sterile purified water. Aseptically add the following fresh filter sterilised solutions, prepared with the powdered compounds supplied with the vials of m-CP Supplements A, B and C dissolved in purified water:

Phenolphthalein diphosphate (REF 4240070A)	0.5% solution	10 mL in 500 mL of medium base
Ferric chloride hexahydrate (REF 4240070B)	4.5% solution	1 mL in 500 mL of medium base
Indoxyl β -D-glucoside (REF 4240070C)	0.75% solution	4 mL in 500 mL of medium base

Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

m-CP Agar Base	
Dehydrated medium appearance	pale blue, fine, homogeneous, free-flowing powder
Solution appearance	violet, limpid
Prepared plates appearance	violet, limpid
Final pH at 20-25 °C	7.6 ± 0.2
m-CP Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid
m-CP Supplement A	
Powder appearance	white to faint yellow or tan powder
m-CP Supplement B	
Powder appearance	faint yellow to yellow-brown powder
m-CP Supplement C	
Powder appearance	almost white powder

SPECIMENS

Water samples. Refer to applicable International Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

1. Filter a suitable volume of sample through a 0.45 µm cellulose acetate or cellulose nitrate membrane.
2. Using aseptic technique, roll the membrane filter onto the surface of the m-CP Agar prepared as described above, so as to avoid the formation of air bubbles between the filter and the agar surface.
3. Incubate in anaerobic conditions for 21 ± 3 hours at 44 ± 1° C.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
The following typical colonies can be observed on m-CP Agar:

Microorganism	Colony characteristics	Sucrose	β -D-glucosidase	Phosphatase
C. perfringens	Opaque yellow; pink/red after exposure to ammonium hydroxide vapours for 20-30 seconds	+	-	+
Other clostridia (*C. baratii, C.paraputrificum, C.tertium*)	Blue-green	+	+	
Other clostridia (*C.bifermentans, C.difficile, C.sporogenes*)	Purple	-	+ or -	
Other clostridia	Opaque yellow, negative to exposure to ammonium hydroxide vapours	+	-	-

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	44°C/ 18-24 H / AN	good growth, opaque yellow colonies, pink/red after exposure to ammonium
C. bifermentas NCTC 506	44°C/ 18-24 H / AN	growth, blue colonies
E. coli ATCC 25922	44°C/ 18-24 H / AN	totally inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection Type Cultures.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at 2-8°C away from direct light.
Powdered supplements
m-CP Supplement A: Store the product in the original package at -20°C away from direct light
m-CP Supplement B: Store the product in the original package at +10/+30°C away from direct light
m-CP Supplement C: Store the product in the original package at -20°C away from direct light
Ready-to-use plates
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Bisson JW, Cabelli VJ. Membrane filter enumeration method for *Clostridium perfringens*. Appl Environ Microbiol 1979; 37: 55-88.
2. E.U. (1998) 98/83/EC of Council of 3rd of November 1998 on the quality of water intended for human consumption. Off J Eur Commun L330: 32-54
3. Commission Directive (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83/EC on the quality of water intended for human consumption. Off J Eur Commun L260: 6-17
4. ISO 14189:2013 Water quality - Enumeration of *Clostridium perfringens* - Method using membrane filtration

PACKAGING

Product	Type	REF	Pack
m-CP Agar Base	Dehydrated medium	4013202	500 g (7.2 L)
m-CP Antimicrobic Supplement	Freeze-dried supplement	4240070	10 vials, each for 500 mL of medium
m-CP Supplement A (Phenolphthalein diphosphate	Powdered supplement	4240070A	1 vial (750 mg for 500 g of medium)
m-CP Supplement B (Ferric chloride)	Powdered supplement	4240070B	1 vial (700 mg for 500 g of medium)
m-CP Supplement C (Indoxyl β -D-glucoside)	Powdered supplement	4240070C	1 vial (450 mg for 500 g of medium)
m-CP Agar	Ready-to-use plates	491320	3 x 10 plates, ø 55 mm

IFU rev 1, 2022/06

MEAT LIVER SR AGAR

Dehydrated culture medium

INTENDED USE

For the enumeration of spores of sulphite reducing anaerobic bacteria.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Meat-liver peptone	30.0 g
Glucose	1.0 g
Sodium sulphite	0.5 g
Ferric ammonium citrate	0.5 g
Starch	1.0 g
Agar	8.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Sulphite reductase activity is a common property among clostridia, however some species such as *Clostridium butyricum*, *Clostridium saccharobutyricum*, *Clostridium septicum, Clostridium tertium*, are particularly sensitive to sulphite.[1] For this reason most enumeration media, including Meat Liver SR Agar, contain no more than 0.5 g/L sulphite.
Meat-liver peptone provides a rich supply of nutrients for the microbial growth, particularly that of anaerobic bacteria. Glucose is a source of carbon and energy. Starch promotes spore germination. Reduction of sodium sulphite and precipitation of the resultant sulphide involves ferric ammonium citrate that yields iron sulphide.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and dispense 20 mL portions into test tubes 20x200 mm. Sterilise by autoclaving at 115°C for 20 minutes. Cool and maintain the medium in a molten state at 44-47 °C.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	dark yellow with a flocculent precipitate
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Water, food, environmental specimens. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

- Heat the sample 10 minutes at 80 ± 2 °C in order to destroy vegetative cells and activate spores.
- Inoculate the tubes containing 20 mL of medium cooled to 44-47°C, with 1mL of sample and/or with 1 mL of its decimal dilutions.
- Homogenise thoroughly by inversion, avoiding the incorporation of air.
- Cool in an ice water bath.
- Incubate at 37°C for up to 48 hours.

Alternatively, inoculation can be performed by the pour plate method or by surface spreading. Inoculated plates must be incubated in an anaerobic atmosphere.

READING AND INTERPRETATION

Examine the tubes for growth and blackening of the medium (iron sulphide precipitate).

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C/ 18-24 H / AN	growth with presence of a black precipitate

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The identification of isolated strains must be confirmed by suitable tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Mead GC. Principles involved in the detection and enumeration of clostridia in foods. Int J Food Microbiol 1992; 17:113

PACKAGING

Product	Type	REF	Pack
Meat Liver SR Agar	Dehydrated medium	4016892	500 g (12.2 L)

IFU rev 1, 2022/08

m-ENDO BROTH

Dehydrated culture medium

INTENDED USE

For the enumeration of coliforms in water samples by membrane filtration.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	1.500 g
Tryptone	5.000 g
Peptone	5.000 g
Tryptose	10.000 g
Lactose	12.500 g
Dipotassium hydrogen phosphate	4.375 g
Potassium dihydrogen phosphate	1.375 g
Sodium chloride	5.000 g
Sodium deoxycholate	0.100 g
Sodium lauryl sulphate	0.050 g
Sodium sulphite	2.100 g
Basic fuchsin	1.050 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Endo media were originally developed by Endo[1] for the isolation of the typhoid bacillus. McCarthy, Delaney, and Grasso[2] modified Endo's formulation and proposed the LES (Lawrence Experimental Station) Endo media, for the recovery of coliforms with a membrane filter two steps technique.
Both one and two steps membrane filter procedures have been included in the APHA Standard Methods for the detection of coliforms in drinking, non-potable, and other waters.[3]
Endo Broth Membrane Filter (m-Endo Broth) has a formulation with the same components as LES Endo Agar, at slightly different concentrations and without agar.
In m-Endo Broth, essential growth factors are provided by peptones which are sources of nitrogen, carbon and minerals. Yeast extract is a source of vitamins, particularly of the B-group. Phosphates are used as buffering agents to control the pH in the medium. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. The slight inhibition of Gram-positive bacteria achieved with the sodium sulphite/acid fuchsin combination in classical Endo formulation, has been improved in "Endo Broth" formulation by inclusion of sodium deoxycholate and sodium lauryl sulphite. The sodium sulphite in the medium also has the function of decolourising acid fuchsin as it does in Schiff's reagent. Lactose-fermenting bacteria produce acetaldehyde from lactose which releases the fuchsin from the colourless fuchsin-sulphite compound and colours the colonies red; when the reaction is rapid and very intense (e.g in the case of *E. coli*), the fuchsin crystallises and produces a metallic sheen on the colonies. In areas of the plate with intense growth, the metal sheen is suppressed. Non-lactose-fermenting organisms produce colourless colonies against the pink background of the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 48 g in 1000 mL of cold purified water. Add 20 mL of 95% ethanol, heat to boiling with frequent agitation to dissolve completely. Do not autoclave, do not overboil. Cool to 47-50°C, mix well for resuspending the precipitate.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	purple, fine, homogeneous, free-flowing powder with small dark particles
Solution appearance	pink-orange, slightly opalescent to opalescent with small dark particles
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Water samples. Consult the appropriate references for sample collection, storage and preparation.[3]

TEST PROCEDURE

One step technique[3]

- Place an absorbent pad in a 55 mm Petri dish and pipette at least 2-3 mL of m-Endo Broth to saturate pad.
- Using an appropriate sterile filtration unit, filter the water sample.
- Aseptically, place the membrane filter on the pad avoiding the formation of air bubbles between the filter and the pad surface.
- Incubate at 35°C for 22 to 24 hours.

Two steps technique[3]

- Place an absorbent pad in a 55 mm Petri dish and pipette at least 2 mL of Lauryl Pepto Bios Broth (REF 401580), to saturate pad.
- Using an appropriate sterile filtration unit, filter the water sample aseptically, place the membrane filter on the pad and incubate for 1.5 - 2 hours at 35°C in a moist atmosphere.
- Transfer the membrane from the pad to 55 mm Petri dish containing a pad saturated with at least 2-3 mL of m-Endo Broth, avoiding the formation of air bubbles between the filter and the agar surface. Incubate at 35°C for 22 to 24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical coliforms colonies are pink to red with metallic sheen. The sheen may cover the entire colony or may only appear in the centre or on the periphery.
Some colonies will appear pink or red but lack the characteristic metallic sheen. These colonies are classified as atypical coliforms and need to be verified through further testing.
Typical non-lactose fermenters colonies are colourless against the pink-red background of the medium

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C/24H-A	good growth, pink-red colonies with metallic sheen
S. Enteritidis ATCC 13076	37°C/24H-A	good growth, colourless colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- Occasionally, non-coliform organisms may produce typical shining colonies.
- Occasionally, some colonies will appear pink or red but lack the characteristic metallic sheen. These colonies are classified as atypical coliforms and need to be verified with further tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to APHA, the bottled medium may be stored at +2-+8°C in the dark, for up to 96 hours.[3]

REFERENCES

1. Endo S. Über ein Verfahren zum Nachweis der Typhus bacillen. Centr f Bakt 1904; 35:109-110.
2. McCarthy JA, Delaney JE, Grasso RJ. Measuring coliforms in water. Water Sewage Works 1961; 108:238
3. APHA. Standard methods for the examination of water and wastewater, 23st ed., 2017. American Public Health Association, Washington, D.C.

PACKAGING

Product	Type	REF	Pack
m- Endo Broth	Dehydrated medium	4014612	500 g (10.41 L)

IFU rev 1, 2022/08

m-FAECAL COLIFORM AGAR (m-FC AGAR)

Dehydrated and ready-to-use culture medium

INTENDED USE

m-Faecal Coliform Agar is used with rosolic acid for the cultivation and enumeration of faecal coliforms by the membrane filter technique.

COMPOSITION*

DEHYDRATED M-FAECAL COLIFORM AGAR
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	10.0 g
Peptocomplex	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts N° 3	1.5 g
Aniline blue	0.1 g
Agar	13.0 g

M-FC AGAR, READY-TO-USE PLATES
TYPICAL FORMULA

Tryptose	10.0 g
Peptocomplex	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts N. 3	1.5 g
Aniline blue	0.1 g
Agar	13.0 g
Rosolic acid 1%	10 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Faecal coliforms are found in the gastro intestinal tracts and faeces of warm-blooded animals and may be differentiated from coliforms from environmental sources by their ability to grow at 44.5 °C.
m-Faecal Coliform Agar is prepared according to the formulation reported by Geldrich et al. in 1965[1] for the enumeration of faecal coliforms using the membrane filter technique without prior enrichment. The medium is recommended by many International Authorities for the enumeration of faecal coliforms using the membrane filter technique.[2-4] Faecal coliforms (or thermotolerant coliform bacteria) are frequently used as indicators of faecal pollution although they are a less specific indicator of faecal contamination than *Escherichia coli*, since they may sometimes arise from nonfecal sources, especially in tropical climates.[5]
Tryptose and peptocomplex provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is a fermentable carbohydrate and a source of carbon and energy, sodium chloride maintains the osmotic balance. Bile salts N° 3 and aniline blue inhibit the growth of Gram-positive bacteria. The high incubation temperature makes the medium more selective. Aniline blue and rosolic acid form the indicator system of the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 50 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, and add 10 mL of Rosolic Acid (REF 4211901) 1% solution in NaOH 0.2 N and continue to boil for 1 minute. Do not sterilize in the autoclave. Cool to 47-50 °C, mix well and pour into sterile dishes for MF technique (55 mm diameter).

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	grey-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

1. Filter an appropriate volume of water onto the membrane depending on the expected faecal coliforms number. When the sample's bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate (20-60 CFU/dish).

2. Using aseptic technique, roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
3. Within 30 minutes place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record any specific morphological and colour characteristic of the colonies.
Count and record colonies with various shades of blue as faecal coliforms.
Colonies of non-faecal coliforms are grey or cream or pink.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 25922	44.5°/ 24 H-A	growth with blue colonies
E. faecalis ATCC 19433	44.5°/ 24 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since the incubation temperature is critical, the use of submerged waterproofed MF culture is recommended or the use of an incubator that is documented to hold the temperature at 44.5°C± 0.2°C throughout the chamber over a 24 hours period.[2]
- There are limitations to the interpretation of a thermotolerant coliform result from thermal waters and pulp and paper mill effluent samples where thermotolerant *Klebsiella* have predominated and not been indicative of a sewerage source. Approximately 60% to 80% of all *Klebisella* from faeces and clinical samples are positive in the thermotolerant coliform test and are *K. pneumoniae*.[2]

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use plates
Store plates in their original pack at +2°C / +8°C away from direct light.

REFERENCES

1. Geldreich EE, Clark HF, Huff CB, Best LC. Faecal coliform organisms medium for membrane filtration technique. J Am Water Works Assoc 1965; 57:208.
2. APHA Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C., 2017.
3. AOAC Official methods of analysis, 18th ed., AOAC International. Gaithersburg, Md. 2007
4. U.S. Environmental Protection Agency. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, USEPA, Cincinnati, Ohio.
5. Cisneros BJ, in Treatise on Water Science, 4.06.4.1.5 Biological indicators, 2011.

PACKAGING

Product	Type	REF	Pack
m-Faecal Coliform Agar (m-FC Agar)	Dehydrated medium	4014872	500 g (10 L)
m-FC Agar	Ready-to-use plates	491487	3 x 10 plates ø 55 mm

IFU rev 1, 2023/01

m-FAECAL COLIFORM BROTH (m-FC BROTH)

Dehydrated culture medium

INTENDED USE

m-Faecal Coliform Broth is used with rosolic acid for the cultivation and enumeration of faecal coliforms by the membrane filter technique.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	10.0 g
Peptocomplex	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts N° 3	1.5 g
Aniline blue	0.1 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Faecal (thermotolerant) coliforms are found in the gastro intestinal tracts and faeces of warm-blooded animals and may be differentiated from coliforms from environmental sources by their ability to grow at 44.5 °C.
m-Faecal Coliform Broth is prepared according to the formulation reported by Geldrich et al. in 1965[1] for the enumeration of faecal coliforms using the membrane filter technique without prior enrichment. Faecal Coliform Media are recommended by many International Authorities for the enumeration of faecal coliforms using the membrane filter technique.[2-4] Faecal coliforms (or thermotolerant coliform bacteria) are frequently used as indicators of faecal pollution although they are less specific indicators of faecal contamination than *Escherichia coli*, since they may sometimes arise from nonfecal sources, especially in tropical climates.[5] Tryptose and peptocomplex provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is a fermentable carbohydrate and a source of carbon and energy, sodium chloride maintains the osmotic balance. Bile salts N° 3 and aniline blue inhibit growth of Gram-positive bacteria. The high incubation temperature makes the medium more selective. Aniline blue and rosolic acid form the indicator system of the medium.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 37 g in 1000 mL of cold purified water. Add 10 mL of Rosolic Acid (REF 4211901) 1% solution in NaOH 0.2 N, heat to boiling with frequent agitation and continue to boil for 1 minute. Do not sterilize in the autoclave. Cool and use for absorbing the suitable sterile absorbent pads.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	grey, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	grey-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.1

SPECIMENS
Water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
1. Filter an appropriate volume of water onto the membrane depending on the expected faecal coliforms number. When the sample's bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate (20-60 CFU/dish).
2. Using aseptic technique, place a sterile absorbent pad in each culture dish and pipet at least 2 mL of broth. Carefully remove any excess of liquid from culture dish by decanting plate.
3. Roll the membrane filter used to collect the water sample onto the surface of the pad, so as to avoid the formation of air bubbles between the filter and the pad.
4. Within 30 minutes place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

READING AND INTERPRETATION
After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Count and record colonies with various shades of blue as faecal coliforms. Colonies of non-faecal coliforms are grey or cream or pink.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 25922	44.5°/ 24 H-A	growth with blue colonies
E. faecalis ATCC 19433	44.5°/ 24 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Since the incubation temperature is critical, the use of submerged waterproofed MF culture is recommended or the use of an incubator that is documented to hold the temperature at 44.5°C± 0.2°C throughout the chamber over a 24 hours period.[2]
- There are limitations to the interpretation of a thermotolerant coliform result from thermal waters and pulp and paper mill effluent samples where thermotolerant *Klebsiella* have predominated and not been indicative of a sewerage source. Approximately 60% to 80% of all *Klebsiella* from faeces and clinical samples are positive in the thermotolerant coliform test and are *K. pneumoniae*.[2]

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Geldreich EE, Clark HF, Huff CB, Best LC. Faecal coliform organisms medium for membrane filtration technique. J Am Water Works Assoc 1965; 57:208.
2. APHA Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, Washington, D.C., 2017
3. AOAC Official methods of analysis, 18th ed., AOAC International. Gaithersburg, Md. 2007
4. U.S. Environmental Protection Agency. Manual for the certification of laboratories analysing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, USEPA, Cincinnati, Ohio.
5. Cisneros BJ, in Treatise on Water Science, 4.06.4.1.5 Biological indicators, 2011.

PACKAGING
Product	Type	REF	Pack
m-Faecal Coliform Broth (m-FC Broth)	Dehydrated medium	4014862	500 g (13.5 L)

IFU rev 1, 2023/01

m-GREEN AGAR
Dehydrated culture medium

INTENDED USE
For the enumeration of yeasts and moulds in beverages, with membrane filter method.

COMPOSITION - TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *
Yeast extract	9.0 g
Dextrose	50.00 g
Peptone	10.00 g
Magnesium sulphate	2.10 g
Potassium phosphate	2.0 g
Diastase	0.05 g
Thiamine hydrochloride	0.05 g
Bromocresol green	0.026 g
Agar	23.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

m-Green Agar is a complex composition medium used by the beverages industry of for the detection and enumeration of yeast and moulds by the membrane filter method. It is prepared by adding agar to the formulation reported by ISO 10718[1] for counting yeasts and moulds, capable of both being extracted and growing in alcoholic medium, in cork stoppers.
Peptone and yeast extract provide the nitrogen, and amino acids, vitamins and minerals for microbial growth. Dextrose is a source of carbon and energy. Potassium phosphate buffers the medium. Magnesium sulphate, thiamine, and diastase (a mixture containing amylolytic enzymes) provide important ions, minerals, and nutrients for mycological growth. The composition includes bromocresol green which facilitates the visualization and counting of fungal colonies that are green due to diffusion of the stain into the colonies. Acidic end products from colonies diffuse into the medium, reducing the pH and causing the indicator to turn yellow (acid reaction) around the colonies. The growth of non-acidophilic bacteria is inhibited by the acidic pH of the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 96.7 g in 1000 mL of cold purified water; heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes. Mix well and pour into sterile Petri dishes. Prolonged or excessive heating will diminish the gel strength of the agar.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	greenish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	green, clear
Final pH at 20-25 °C	4.6 ± 0.2

SPECIMENS

Non-alcoholic and alcoholic beverages. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Filter an appropriate volume of sample onto the membrane, depending on the expected yeast and mould charge.
Roll the membrane onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
Incubate at 30 °C ± 2 °C for 72 hours.

READING AND INTERPRETATION

Enumerate the number of colonies of yeasts and moulds per plate and calculate the microbial count.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	28-32°/ 72 H-A	good growth, pale green colonies
A. brasiliensis ATCC 16404	28-32°/ 72 H-A	good growth
B. subtilis ATCC 6633	28-32°/ 72 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Avoid over-heating and remelting of medium.
- The isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. ISO 10718:2015 Cork stoppers – Characterization of a low-in-germs stopper, through the enumeration of colony-forming units of yeast, moulds and bacteria, capable of both being extracted and growing in alcoholic medium.

PACKAGING

Product	Type	REF	Pack
m-Green Agar	Dehydrated medium	4015272	500 g (5.1 L)

IFU rev 1, 2022/08

m-Green Agar: membrane filter
with colonies of *Saccharomyces cerevisiae*

MICROBIAL CONTENT TEST AGAR
(TSA+LECITHIN+TWEEN® 80)
Dehydrated culture medium

INTENDED USE
General purpose medium for the detection and enumeration of microorganisms on treated sanitary areas, containers, equipment.

COMPOSITION -TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	15.0 g
Soy peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Lecithin	0.7 g
Polysorbate 80	5.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Detection and enumeration of microorganisms on environmental surfaces treated with antiseptics have been the subject of many investigations by microbiologists in the public health field. Quisno *et al*[1] reported that lecithin would effectively neutralizes quaternary ammonium compounds and Brummer[2] combined lecithin and polysorbate 80 for the neutralisation of disinfectants by replicate organism detection and counting (RODAC) technique.
Microbial Content Test Agar, prepared with Tryptic Soy Agar supplemented with lecithin and polysorbate 80, is used for the detection and enumeration of microorganisms surviving after treatment of surfaces and materials with antiseptics and in environmental air sampling procedures. Tryptic Soy Agar is the medium specified as “casein soya bean digest agar” in the harmonised EP, USP JP method[3] for microbial enumeration of non-sterile pharmaceutical products. Lecithin is incorporated to neutralize quaternary ammonium compounds and polysorbate 80 is used to neutralize substituted phenolic disinfectants.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 45.7 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C mix well and pour into sterile Petri dishes or RODAC plates.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, opalescent
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS
Sanitary areas, containers, equipment treated with disinfectants.

TEST PROCEDURE
RODAC plates filled with Microbial Content Test Agar are employed for the quantitative method to enumerate microbes on open flat work surfaces.[4]
- Gently but firmly touch the RODAC agar surface against the area being sampled, exert moderate, even, vertical pressure and then carefully replace lid. Avoid using rubbing motions of the plate at the sample site as this may break the agar.
- Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

Qualitative methods utilizing sponges/ swabs are used for hard-to-reach areas.[4]
- Apply swab (or sponge with handle) to surface (or equipment) being monitored with firm application pressure.
- When sampling (monitoring) flat surfaces allow the swab (or sponge with handle) to firmly rub an area of approximately 24 to 30 cm^2.
- Apply the swab (or sponge) within this contact area in both a horizontal and vertical direction for approximately 10 seconds.
- Roll the swab directly on the surface of Microbial Content Test Agar plates.
- Incubate inoculated plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

READING AND INTERPRETATION
After incubation, the presence of microorganisms is indicated by the appearance of colonies of various morphology and size on medium surface. The characteristics of the growth are closely related to the type or types of cultivated microorganisms.
Count all developed colonies.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS		INCUBATION T°/ t / ATM	EXPECTED RESULTS
S. aureus	ATCC 6538	35-37°C / 18-24H / A	good growth
E. coli	ATCC 25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place
According to MacFaddin the self-prepared plates may be stored at 2-8°C for 6-8 weeks.[5]

REFERENCES
1. Quisno R, Gibby IW, Foter MJ. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am J Pharm 1946; 118:320.

2. Brummer B. Influence of Possible Disinfectant Transfer on Staphylococcus aureus Plate Counts After Agar Contact Sampling. App Environ Microbiol 1976; 32:80-84.
3. European Pharmacopoeia 10.3, 2021.
4. Food and Drug Administration. Office of Regulatory Affairs. Office of Regulatory Science. Pharmaceutical Microbiology Manual. Document n° ORA.007. Rev 2. 25 Aug 2020.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Microbial Content Test Agar	Dehydrated medium	4016992	500 g (10.9 L)

IFU rev 1, 2022/09

MINERALS MODIFIED GLUTAMATE MEDIUM (MMGM) BASE
MINERALS MODIFIED GLUTAMATE MEDIUM (MMGM)
SODIUM GLUTAMATE

Dehydrated culture medium and ready-to-use tubes

INTENDED USE

Chemical defined enrichment broth for detection of *Escherichia coli* according to ISO 16649 and other coliform organisms in food, water and wastewater samples.

COMPOSITION - TYPICAL FORMULA *

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

	DOUBLE STRENGTH	SINGLE STRENGTH
401737 MINERALS MODIFIED GLUTAMATE MEDIUM BASE (MMGM)		
Lactose	20.000 g	10.000 g
Sodium formate	0.500 g	0.250 g
L-cystine	0.04 g	0.020 g
L (-) aspartic acid	0.048 g	0.024 g
L (+) arginine	0.040 g	0.020 g
Thiamine	0.002 g	0.001 g
Nicotinic acid	0.002 g	0.001 g
Pantothenic acid	0.002 g	0.001 g
Magnesium sulphate heptahydrate	0.200 g	0.100 g
Ammonium iron (III) citrate	0.02 g	0.010 g
Calcium chloride dihydrate	0.020 g	0.010 g
Di-potassium hydrogen phosphate	1.800 g	0.900 g
Bromocresol purple	0.020 g	0.010 g
4123642 Sodium Glutamate		
Sodium glutamate**	12.70 g	6.35 g
Ammonium chloride**	5.00 g	2.50 g
Water	1000 mL	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.
** Not included in the dehydrated medium; it must be added to the basal medium.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

A chemically defined medium based on glutamic acid was first advocated by Folpmers[1] in 1948 for the enumeration of the coliform group of bacteria in water. This basic formulation was modified by Burman and Oliver in 1952[2] and by the UK Public Health Laboratory Service[3] substituting glucose with lactose. The lactose medium was then further improved by Gray in 1959[4], who increased the pH and added sodium formate to increase gas production. Further modifications were made by Windle Taylor[5]: the concentration of lactose was increased and the phosphate decreased, and the modified medium was adopted in UK in place of MacConkey broth for all routine samples examined by the multiple tube method. Simultaneously and independently Gray[6] also made further modifications in 1964 resulting in the publication of an improved formate lactose glutamate medium.
Comparative trials with MacConkey Broth[7] demonstrated that Gray's modification of the medium gave significantly higher numbers of positive results for coliform organisms and *E. coli*, after only 24 hours incubation. In a comparative evaluation with Lauryl Tryptose Broth (LTB)[8], at 48 h of incubation, Minerals Modified Glutamate Medium (MMGM) gave better results for total coliform organisms including *E. coli* in chlorinate waters, especially if the numbers were small. MMGM was evaluated by Abbis et al[9] with reference to Lauryl Sulfate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth for the enumeration of coliforms in different food samples and provided the best sensitivity results.
MMGM is recommended by ISO 16649-3[10] and, supplemented with agar, by ISO 16649-1[11]. Both the techniques include a resuscitation step and are used for the enumeration of β-glucuronidase positive *E. coli* from foodstuffs likely to contain sub-lethally injured cells.
Essential growth factors are provided by sodium glutamate and sodium formate; lactose is a fermentable carbohydrate. The presence of B-complex vitamins, amino acids and magnesium ions allows an increased rate of growth and fermentation. The addition of ammonium chloride (not included in the dehydrated medium) increases gas production by target strains, while di-potassium hydrogen phosphate acts as buffer system during lactose fermentation. Bromocresol purple serves as an acid-base indicator giving a yellow colour to the broth with lactose fermenting bacteria while non-lactose-fermenting bacteria develop a blue colour. To improve the stability of the dehydrated medium on storage, the sodium glutamate is supplied separately (REF 4123642) and must be added to the basal medium REF 401737.

Directions for medium preparation

Single strength medium

Dissolve 2.5 g of ammonium chloride in 1000 mL of cold purified water. Add 11.4 g of Minerals Modified Glutamate Medium Base, and 6.35 g of Sodium Glutamate (REF 4123642).

Double strength broth

Dissolve 5 g of ammonium chloride in 1000 mL of cold purified water. Add 22.7 g of Minerals Modified Glutamate Medium Base, and 12.7 g of Sodium Glutamate (REF 4123642).

Mix and heat if necessary to dissolve the medium completely.
Dispense the single strength medium in 10 mL volumes into tubes or bottle of dimensions at least 16 mm x 160 mm.
Dispense the double strength medium in 10 mL volumes into tubes or bottles of dimensions at least 18 mm x180 mm or 20 mm x 200 mm. Place an inverted fermentation tube in each container if required. Sterilise by autoclaving for 10 minutes at 116°C.
Minerals Modified Glutamate Medium Base may be supplemented with 13 g/L of Agar Bios LL (REF 401030) before sterilisation for the preparation of Minerals Modified Glutamate Agar.[11]

Physical characteristics

Dehydrated medium appearance	whitish to mauve, fine, homogeneous, free-flowing powder
Prepared tubes appearance	purple, limpid
Final pH at 20-25 °C	6.7 ± 0.1

Specimens

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling; water and wastewater. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

Test procedure

Enumeration of *E. coli* by MPN technique in foodstuffs (ISO 16649-3)[10]

- Inoculate 3 or 5 double-strength MMGM tubes with 10 mL aliquots of the test sample, if liquid, or with 10 mL aliquots of the initial suspension in the case of other products.
- Inoculate 3 or 5 tubes of single-strength MMGM with 1 mL aliquots of the test sample, if liquid or with 1 mL aliquots of the initial suspension in the case of other products.
- Repeat the inoculation of the single strength liquid medium for each of the further decimal dilutions, using a fresh pipette for each dilution.
- Incubate the tubes at 37°C for 24 ± 2 hours
- From each of the incubated tubes showing yellow colour subculture with a loop on a plate of TBX Agar (402156) by streaking to obtain isolated colonies and incubate at 44°C for 24 ± 2 hours
- Examine the TBX Agar plates for the presence of green-blue colonies (*E. coli* beta-glucuronidase positive).
- Express the results as the Most Probable Number of *E. coli* on the basis of the presence of green-blue colonies on TBX plates.

Enumeration of coliforms/*E. coli* in water

Inoculate the water sample into the medium in the following volumes:

- 50 mL of sample into 50 mL of double-strength medium or 5 x 10 mL of sample into 5 x 10 mL of double-strength medium (for suspected low number of target organisms).
- 5 x 1 mL of sample into 5 x 5 mL of single-strength medium or 5 x 1 mL of a 1:10 dilution of the sample into 5 x 5 mL of single-strength medium (for suspected high number of target organisms).
- Incubate the tubes at 37°C. Examine after 18-24 hours incubation and again at 48 hours.

Enumeration of *E. coli* in foodstuffs with MMG Agar (ISO 16649-1)[11]

This technique requires the use of Minerals Modified Glutamate Medium (MMGM) Base supplemented as described above and with 13 g/L of Agar Bios LL (REF 411030): MMG Agar.

- Transfer in the centre of the membrane 1 mL of the sample or 1 mL of the initial suspension and spread the inoculum on the surface of the membrane. Repeat the procedure with further decimal dilutions if necessary.
- Using a sterile spreader, spread the inoculum evenly over the whole membrane surface, avoiding any spillage from the membrane.
- Leave the plates at room temperature for 15 minutes in order the medium adsorbs the liquid sample.
- Incubate the plates for 4 h ± 0.25 h at 37 °C, with the membrane/agar surface uppermost.
- After this resuscitation step transfer the membranes onto TBX Agar plates and incubate at 44°C for 18-24 hours.

Reading and interpretation

MMGM becomes turbid when bacteria are growing; lactose fermentation can be detected by gas formation and by yellow colour development. Each presumptive positive tube should be confirmed with appropriate sub-cultures and with additional biochemical tests.
Examine the TBX Agar plates for the presence of typical, blue or blue-green colonies indicating the presence of β-glucuronidase-positive *E. coli.*

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 8739	37°C/24 H/A	growth, with gas production; the medium turns yellow
E. faecalis ATCC 29212	37°C/24 H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- The pH of the medium significantly influences its performance. Avoid overheating and check the pH of each preparation batch.
- Some strains of *E. coli* may grow poorly or not at all in media incubated at 44 °C. Consequently, some strains of *E. coli*, including pathogenic ones, will not be detected by the methods reported above taken from ISO Standards. β-glucuronidase activity may also be exhibited at 44 °C by certain other members of the *Enterobacteriaceae*, notably *Shigella* and *Salmonella*.[10]
- Some organisms other than coliform bacteria can grow in the medium with acid and gas production. Each presumptive positive tube should be confirmed with appropriate sub-cultures and with additional biochemical tests.

STORAGE CONDITIONS

Dehydrated medium and Sodium Glutamate
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use medium in tubes
Store tubes in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Folpmers T. Is it justified to use lactose broth for the detection of Bact. coli in the presumptive test of routine water analysis? Antonie van Leeuwenhoek. 1948; 14: 58-64.
2. Burman NP, Oliver CW. A comparative study of Folpmer's glutamic acid medium for the detection of Bact. coti in water. Proc Soc Appl Bact. 1952; 15:1-7.
3. Public Health Laboratory Service, Water Sub-Committee. A comparison between MacConkey broth and glutamic acid media for the detection of coliform organisms in water. J Hyg Camb 1958; 56:377-88.
4. Gray RD. Formate lactose glutamate: a chemically defined medium as a possible substitute for MacConkey broth in the presumptive coliform examination of water. J Hyg Camb 1959;57: 249-65.
5. Windle Taylor E. (1961--62). Glutamic acid media. Rep Results Bact Chem Biol Exam Lond Wat. 1961-1962; 40, 18-22.
6. Gray RD. An improved formate lactose glutamate medium for the detection of Escherichia coli and other coliform organisms in water. J Hyg Camb 1964; 62:495–508
7. P. H. L. S. Standing Committee on the Bacteriological Examination of Water Supplies. Comparison of MacConkey broth, Teepol broth and glutamic acid media for the enumeration of coliform organisms in water. J Hyg Camb 1968; 65:67-82.
8. Joint Committee of the P. H. L. S. and the Standing Committee of Analysts. A comparison between minerals-modified glutamate medium and lauryl tryptose lactose broth for the enumeration of Escherichia coli and coliform organisms in water by the multiple tube method. J. Hyg., Camb.1980;85:35-49.
9. Abbiss JS, Wilson JM, Blood RM, Jarvis B. A comparison of minerals modified glutamate medium with other media for the enumeration of coliforms in delicatessen foods. J Appl Bacteriol 1981; 51:121-127.
10. ISO 16649-3:2016. Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide
11. ISO 16649-1:2018. Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

PACKAGING

Product	Type	REF	Pack
Minerals Modified Glutamate Medium (MMGM) Base	Dehydrated medium	4017372	500 g (44 or 22 L)
Sodium Glutamate	Raw material/supplement	4123642	300 g (46.9 or 23.4 L)
Minerals Modified Glutamate Medium (MMGM)	Ready-to-use tubes	551737	20 x 10 mL
Minerals Modified Glutamate Medium (MMGM) 2x	Ready-to-use tubes	551737D	20 x 10 mL

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m-LES ENDO AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

For the enumeration of coliforms in water samples by membrane filtration.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

M-LES ENDO AGAR, DEHYDRATED MEDIUM

Yeast extract	1.20 g
Tryptone	3.70 g
Peptone	3.70 g
Tryptose	7.50 g
Lactose	9.40 g
Dipotassium hydrogen phosphate	3.30 g
Potassium dihydrogen phosphate	1.00 g
Sodium chloride	3.70 g
Sodium deoxycholate	0.10 g
Sodium lauryl sulphate	0.05 g
Sodium sulphite	1.60 g
Basic fuchsin	0.80 g
Agar	15.00 g

M-LES ENDO AGAR, READY-TO-USE PLATES
TYPICAL FORMULA

m-LES Endo Agar	51 g
Ethanol 95%	20 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Endo Agar was originally developed by Endo[1] for the isolation of the typhoid bacillus. McCarthy, Delaney, and Grasso[2] modified Endo's formulation and proposed the LES (Lawrence Experimental Station) Endo Agar, for the recovery of coliforms with a membrane filter 2 steps technique: 1- pre-enrichment of the filter in Lauryl Sulphate Broth, 2- incubation of the filter on a LES Endo Agar plate. McCarthey *et al.* recovered higher numbers of coliforms with the 2 steps method compared to the one-step technique with m-Endo medium.[2]
Both one and two steps Membrane Filter procedures have been included in the APHA Standard Methods for the detection of coliforms in drinking, non-potable, and other waters.[3]
In m-LES Endo Agar, essential growth factors are provided by peptones which are sources of nitrogen, carbon and minerals. Yeast extract is a source of vitamins, particularly of the B-group. Phosphates are used as buffering agents to control the pH in the medium. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. The slight inhibition of Gram-positive bacteria achieved with the sodium sulphite/acid fuchsin combination in classical Endo formulation, has been improved in "LES" formulation by inclusion of sodium deoxycholate and sodium lauryl sulphate. The sodium sulphite in the medium also has the function of decolourising acid fuchsin as it does in Schiff's reagent. Lactose-fermenting bacteria produce acetaldehyde from lactose which releases the fuchsin from the colourless fuchsin-sulphite compound and colours the colonies red; when the reaction is rapid and very intense (e.g in the case of *E. coli*), the fuchsin crystallises and produces a metallic sheen on the colonies. In areas of the plate with intense growth, the metal sheen is suppressed. Non-lactose-fermenting organisms produce colourless colonies against the pink background of the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 51 g in 980 mL of cold purified water and add 20 mL of 95% ethanol. Heat to boiling with frequent agitation to dissolve completely. Cool to 47-50°C, mix well for resuspending the precipitate and distribute into sterile Petri dishes. Do not autoclave, avoid direct sunlight.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	purple, fine, homogeneous, free-flowing powder with small dark particles
Solution appearance	pink-red, slightly opalescent to opalescent with precipitate
Prepared medium appearance	pink-red, slightly opalescent
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

Water samples. Consult the appropriate references for sample collection, storage and preparation.[3,4]

TEST PROCEDURE

One step technique.[3]

- Using an appropriate sterile filtration unit, filter the water sample.
- Aseptically, place the membrane filter on the m-LES Endo Agar plate, invert dish and incubate for 22 to 24 hours at 35°C

Two steps technique.[3]

- Place an absorbent pad in a 55 mm Petri dish and pipette at least 2 mL of Lauryl Pepto Bios Broth (REF 401580), to saturate pad.
- Using an appropriate sterile filtration unit, filter the water sample.
- Aseptically, place the membrane filter on the pad and incubate for 1.5 - 2 hours at 35°C in a moist atmosphere.
- Transfer the membrane from the pad to 55 mm Petri dish containing m-LES Endo Agar, avoiding the formation of air bubbles between the filter and the agar surface.
- Incubate at 35°C for 20 to 24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical coliforms colonies are pink to red with metallic sheen. The sheen may cover the entire colony or may only appear in the centre or on the periphery.
Some colonies will appear pink or red but lack the characteristic metallic sheen. These colonies are classified as atypical coliforms and need to be verified through further testing.
Typical non-lactose fermenters colonies are colourless against the pink-red background of the medium

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	37°C/24H-A	good growth, pink-red colonies with metallic sheen
S. Enteritidis ATCC 13076	37°C/24H-A	good growth, colourless colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- Occasionally, non-coliform organisms may produce typical sheen colonies.
- Occasionally, some colonies will appear pink or red but lack the characteristic metallic sheen. These colonies are classified as atypical coliforms and need to be verified with further tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.
According to APHA, the self-prepared plates may be stored at 2-8°C in the dark, preferably in sealed plastic bag, for up to 2 weeks.[3] Discard the medium sooner than 2 weeks if there is evidence of moisture loss, medium contamination, medium darkening, or surface sheening formation.[3]

Ready-to-use plates

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Endo S. Über ein Verfahren zum Nachweis der Typhus bacillen. Centr f Bakt 1904; 35:109-110.
2. McCarthy JA, Delaney JE, Grasso RJ. Measuring coliforms in water. Water Sewage Works 1961; 108:238
3. APHA. Standard methods for the examination of water and wastewater, 23th ed., 2017. American Public Health Association, Washington, D.C.

PACKAGING

Product	Type	REF	Pack
m- LES Endo Agar	Dehydrated medium	4015932	500 g (9.8 L)
LES Endo Agar	Ready-to-use plates	541593	2 x 10 plates ø 90 mm
LES Endo Agar	Ready-to-use plates	491543	3 x 10 plates ø 55 mm

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m-LES Endo Agar:
E. coli colonies

MODIFIED LAURYL SULFATE TRYPTOSE (mLST) BROTH BASE
VANCOMYCIN ANTIMICROBIC SUPPLEMENT
MODIFIED LAURYL SULFATE TRYPTOSE (mLST) BROTH

Dehydrated culture medium, selective supplement and ready-to-use tubes

INTENDED USE

Enrichment selective broth for the detection of *Cronobacter sakazakii* in milk and milk products.

COMPOSITIONS*

MODIFIED LAURYL SULFATE TRYPTOSE (MLST) BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Enzymatic digest of animal and plant tissue	20.00 g
Lactose	5.00 g
Sodium chloride	34.00 g
Sodium lauryl sulphate	0.10 g
Potassium dihydrogen phosphate	2.75 g
Potassium hydrogen phosphate	2.75 g

VANCOMYCIN ANTIMICROBIC SUPPLEMENT (VIAL CONTENT)

Vancomycin	25 mg

MODIFIED LAURYL SULFATE TRYPTOSE (MLST) BROTH, READY-TO-USE TUBES
TYPICAL FORMULA

Modified Lauryl Sulfate Tryptose (mLST) Broth Base	64.6 g
Vancomycin	10.0 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Cronobacter species (formerly known as *Enterobacter sakazakii*) are Gram-negative rod-shaped, motile pathogenic bacteria of the family *Enterobacteriaceae*. These organisms are regarded as opportunistic pathogens linked with life-threatening infections predominantly in neonates.[1] Clinical syndromes of *Cronobacter* infection include necrotizing enterocolitis (NEC), bacteraemia and meningitis, with case fatality rates ranging from 40-80%.[1,2] The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, water, rice and others.[1,3,4]
Modified Lauryl Sulfate Tryptose (mLST) Broth is a selective broth for the enrichment step of the isolation procedure of *Cronobacter*.
The use of Buffered Peptone Water as a non-selective enrichment, mLST Broth as a selective enrichment and Enterobacter Sakasakii Isolation Agar (ESIA REF 401478) allow the specific detection of *C. sakazakii* in food samples especially in milk powder and powdered infant formula. The above culture media and the work procedure described below are in accordance with the withdrawn standard ISO/TS 22964:2006[4], replaced by ISO Standard 22964:2017.[5]
Essential growth factors are provided by enzymatic digest of animal and plant tissue which is a source of nitrogen, carbon, amino acids and minerals. Lactose is a fermentable carbohydrate and a source of carbon and energy. Phosphates act as buffer system. The high concentration of sodium chloride and the surface-active agent sodium lauryl sulphate act as selective agents in restricting the growth of bacteria other than coliforms, while vancomycin is inhibitory for Gram positive bacteria.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 32.3 g in 500 mL of cold purified water. Heat to dissolve and sterilize by autoclaving at 121°C for 15 minutes. Cool to room temperature and add 1 mL of Vancomycin Antimicrobic Supplement (REF 4240057), reconstituted with 5 mL of sterile purified water. Final vancomycin concentration in the medium: 10 mg/L. Mix well and distribute into sterile tubes (10 mL/tube) under aseptic conditions. The vancomycin solution may be kept at 0 °C to 5 °C for 15 days.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear
Freeze-dried selective supplement	high, soft white, pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	6.8 ± 0.2

SPECIMENS

Milk powder, powdered infant formula and environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

TEST PROCEDURE

- Prepare the initial sample suspension (primary dilution) by adding *x* g of the test sample to 9 times *x* mL of Buffered Peptone Water Casein (REF 401278C): e.g., 25 g + 225 mL of Buffered Peptone Water Casein or 10 g + 90 mL of Buffered Peptone Water Casein.
- Incubate at 37 ± 1°C for 18 ± 2 hours.
- After incubation of the inoculated pre-enrichment medium, transfer 0.1 mL of the obtained culture into 10 mL mLST Broth.
- Incubate at 44 ± 0.5°C for 24 ± 2 hours.
- After incubation, streak a 10 µ loopful from the mLST broth onto the surface of the ESIA plate (REF 401478) and incubate at 44 ± 1°C for 24 ± 2 hours

READING AND INTERPRETATION

The presence of microorganisms in mLST Broth is indicated by a varying degree of turbidity, specks and flocculation.
Subculture onto ESIA plates:
Presumptive positive result for *C. sakazakii*: presence of blue to green colonies, 1 to 3 mm in diameter.
Negative result for *C. sakazakii*: absence of typical blue-green colonies or presence of mauve-violet colonies.
Confirm colonies with biochemical tests recommended by ISO 22964.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
C. sakazakii ATCC 29544	44°C / 24 h / A	growth
S. aureus ATCC 25923	44°C / 24 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- *Cronobacter* may be present in low numbers in the samples, along with other *Enterobacteriaceae*, such as *E. cloacae*, which may interfere in the determination of the target microorganism.[5]
- Some coliforms grow on ESIA with violet colonies, easily distinguishable from the blue colonies of *C. sakazakii*.

Storage conditions

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Freeze-dried supplement

Store the product in the original package at +2/ +8°C away from direct light.

According to ISO/TS 22964:2006 the self-prepared tubes of mLST/vancomycin medium may be kept at 0 °C to 5 °C for 1 day and the unused vancomycin solution may be may be kept at 0 °C to 5 °C for 15 days.

Ready-to-use medium in tubes

Store tubes in their original pack at 2-8°C away from direct light.

References

1. Carol Iversen *et al*., The taxonomy of Enterobacter sakazakii: proposal of a new genus Cronobacter gen. nov. and descriptions of Cronobacter sakazakii comb. nov. Cronobacter sakazakii subsp. sakazakii, comb. nov., Cronobacter sakazakii subsp. malonaticus subsp. nov., Cronobacter turicensis sp. nov., Cronobacter muytjensii sp. nov., Cronobacter dublinensis sp. nov. and Cronobacter genomospecies BMC Evol Biol. 2007; 7: 64.
2. Simmons BP et al. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of powdered infant formula. Infect Control Hosp Epidemiol 1989; 10: 398.
3. Van Acker J et al. Outbreack of necrotizing enterocolitis associated with *E.sakazakii* in powdered milk formula. J Clin Microbiol 2001; 39:293-297.
4. ISO/TS 22964:2006. Milk and milk products – Detection of *Enterobacter sakazakii*
5. ISO 22964:2017. Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

Packaging

Product	Type	REF	Pack
Modified Lauryl Sulfate Tryptose (mLST) Broth Base	Dehydrated medium	4014762	500 g (7.7 L)
Vancomycin Antimicrobic Supplement	Freeze-dried supplement	4240057	10 vials (25 mg/vial)
Modified Lauryl Sulfate Tryptose (mLST) Broth	Ready-to-use tubes	551476	20 x 10 mL

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MODIFIED THAYER MARTIN (MTM) MEDIUM

Ready-to-use plates

MTM Medium:
Neisseria gonorrhoeae

Intended use

In vitro diagnostic device. Selective and enriched medium for the isolation and cultivation of *Neisseria gonorrhoeae* from clinical specimens.

Composition - typical formula *

Peptocomplex	15 g
Corn starch	1 g
Dipotassium hydrogen phosphate	4 g
Potassium dihydrogen phosphate	1 g
Sodium chloride	5 g
Agar	12 g
80°C heated defibrinated sheep blood	50 mL
Purified water	1000 mL
VCNT Supplement	
Vancomycin	3 mg
Colistin	7.5 mg
Nystatin	12,500 IU
Trimethoprim	5 mg
Biovitex Enrichment Supplement	
Nicotinamide adenine dinucleotide (NAD)	2.5 mg
Cocarboxylase	1 mg
p-aminobenzoic acid	0.13 mg
Thiamine	0.03 mg
Vitamin B12	0.1 mg
L-glutamine	100 mg
L-cystine	11 mg
L-cysteine HCl	259 mg
Adenine	10 mg
Guanine HCl	0.3 mg
Ferric nitrate.6H_20	0.2 mg
Glucose	1 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In 1964 Thayer and Martin[1] formulated a selective medium for the cultivation of *Neisseria gonorrhoeae* and *Neisseria meningitidis*, incorporating haemoglobin, yeast supplement B, polymyxin B and ristocetin into GC Agar. Thayer and Martin improved in 1966[2] the formulation substituting the original antibiotics with vancomycin, colistin and nystatin (VCN). In 1970 Martin and Lester[3] modified the new Thayer Martin Medium by increasing agar and glucose content and by incorporating an additional antibiotic, trimethoprim lactate; this improved medium is called Modified Thayer Martin (MTM) medium.
Biolife Modified Thayer Martin (MTM) Medium is prepared with GC Medium Base, supplemented with defibrinated sheep blood chocolatized at 80°C for 15 minutes, Biovitex and VCNT supplements.
Heat-lysed sheep blood is a good source of both hemin (X factor) and NAD (V factor), that enhance the growth of *Neisseria*[4]. V factor and various other factors such as glutamine, cocarboxylase, cystine etc., enhancing the growth of *N. gonorrhoeae* [5], are supplied by the chemically defined enrichment supplement Biovitex.
Vancomycin inhibits Gram-positive bacterial contamination, nystatin is an anti-fungal agent, colistin inhibits Gram-negative microbial flora and almost all saprophytic *Neisseria* spp., trimethoprim suppresses *Proteus* swarming.[5] Peptocomplex provides carbon, nitrogen and trace elements for bacterial growth, sodium chloride maintains the osmotic balance, dibasic and monobasic potassium phosphates buffer prevents pH changes due to amine production, corn starch is included to absorb toxic by-products contained in the specimen and is an energy source for bacterial growth.[5]
Modified Thayer Martin (MTM) Medium is intended for the isolation and cultivation of *N. gonorrhoeae* from clinical specimens, containing a mixed flora of bacteria and/or fungi.[5-8]

PHYSICAL CHARACTERISTICS

Medium appearance	brown, opaque
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Modified Thayer Martin (MTM) Medium can be directly inoculated with specimens from non-sterile human sites contaminated by mixed flora of bacteria and/or fungi (urogenital tract, upper respiratory tract, pus and exudates).[8-11] This medium is not useful for the isolation of *Neisseria* spp. from supposedly sterile sites as cerebrospinal fluid, conjunctival swab, skin biopsy, joint fluid.[5] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information because *Neisseria* spp. are very sensitive to collection and storage procedures.[9]

TEST PROCEDURE

Allow plates to come to room temperature. The agar surface should be smooth and moist, but without excessive water.
Process the specimen as soon as possible after it is received in the laboratory to avoid loss of gonococci viability and overgrowth of contaminants.
Roll the swab over one quadrant of the surface then streak the specimen over the other quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap.
Alternatively, since swabs for gonococcal culture may contain only small numbers of organisms, roll the swabs directly on the medium in a large "Z" pattern to sufficiently transfer the specimen; cross-streak the "Z" pattern with a sterile loop.
Incubate at 35-36.5°C in a moist atmosphere supplemented with 3-7% CO_2; cultures should be examined daily for growth and held for a maximum of 72 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
N. gonorrhoeae colonies are variable in size, usually small (0,5-2 mm), moderately convex, raised, granular, glistening, moist, with entire to lobate margins, usually greyish-white to translucent; almost all strains become mucoid after 48 hours.
A Gram staining must be performed on suspected *Neisseria* colonies to confirm the presence of uniform Gram-negative diplococci. Performance of oxidase test is mandatory for colonies suspected to belong to *Neisseria* that shall be positive for *N. gonorrhoeae*.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[12]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
N. gonorrhoeae	ATCC	43069	35-36.5°C / 24-48H / CO_2	good growth
S. epidermidis	ATCC	12228	35-36.5°C / 24-48H / CO_2	inhibited
P. mirabilis	ATCC	43071	35-36.5°C / 24-48H / CO_2	inhibited
C. albicans	ATCC	60193	35-36.5°C / 24-48H / CO_2	growth partially inhibited

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The presence of small particles may sometimes be observed in the agar. However, this phenomenon does not affect the performance of the medium.
- Vancomycin sensitive strains of some auxotypes of *N. gonorrhoeae* (e.g. strains that require arginine, uracil and hypoxanthine for growth) which fail to grow on MTM, have been reported from 3% to 10% of the total isolates.[13,14] Some gonococci are susceptible to trimethoprim too.[15]
- It is recommended that both a selective and a non-selective medium be used when isolating pathogenic *Neisseria* in order to avoid the loss of vancomycin and/or trimethoprim sensitive strains.[5]
- This medium is not useful for the isolation of *Neisseria* spp. from supposedly sterile sites as cerebrospinal fluid, conjunctival swab, skin biopsy, joint fluid for which non-selective media are recommended.[5]
- For the growth of *N. gonorrhoeae,* it is necessary that the surface of the plates is moist; if it appears dry, humidify with a few drops of sterile purified water. Place damp gauze or paper towels in the CO_2 container before incubation or use an incubator with humidifier.[5]
- On this medium *N. gonorrhoeae* grows with smaller and more granular colonies than with non-selective chocolate agar.
- Some saprophytic non-target microorganisms, resistant to antimicrobials present in the medium may grow. *N. lactamica* may grow on this medium with colonies smaller and less moist than gonococci, occasionally with a yellowish tint [5].
- Use dacron or calcium alginate swabs for specimen collection, avoid cotton swabs since they contain fatty acids which are inhibitory for *N. gonorrhoeae*.
- The gonococci are one of the most fragile Gram-negative bacteria. It is recommended that any suspected *Neisseria* containing specimen should be inoculated onto primary isolation medium immediately on collection to avoid any loss in viability and/or overgrowth of contaminants; if this is not possible *N. gonorrhoeae* swabs are better held at 4-6° C for not more than 3 hours.[5]

- The incubator temperature should be set at 35-36.5°C [6] because many strains of *N. gonorrhoea*e will not grow well at 37°C [5].
- Examine plates after 24 hours incubation. At 48 hours the Gram morphology may exhibit atypical forms.
- Many standard protocols[4,7,8,10] describe the use of MTM medium for the detection of meningococcal carriage in oropharyngeal and nasopharyngeal swabs. This application is out of Biolife MTM Medium intended use. The end-user should validate this application before routinely using MTM Medium for *N. meningitidis* detection in clinical specimens.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Thayer JD, E. Martin Jr E. A selective medium for cultivation of N. gonorrhoeae and N. meningitidis. Pub. Health Rep. 1964; 79:49.
2. Thayer JD, E. Martin Jr E. Improved medium selective for cultivation of N. gonorrhoeae and N. meningitidis. Pub. Health Rep. 1966; 81:559-562.
3. Martin JE Jr, Lester A. Transgrow, a maedium for transport and growth of N. gonorrhoeae and N. Meningitides. HSMHA Helth Service Rep. 1971: 86:30
4. CDC Lab Manual, meningitides; Annex: Preparation of Media and Reagents, 2016
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. CDC: Morbidity and Mortality Weekly Report (MMWR). Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae. Infections Recommendations and Reports. October 18, 2002 / Vol. 51 / No. RR-15
7. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
8. Elias J, Frosh M, Vogel U. Neisseria. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.635.
9. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
10. Public Health England: Standards for microbiology investigations (UK SMI)- Bacteriology: UK SMI B2:2017, UK SMI B9:2015, UK SMI B14:2016; UK SMI B28:2017; B51:2014
11. Vandepitte J, Verhaegen J, P. Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd edition Geneve: World Health Organization Geneva; 2003.
12. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
13. Talbot V. et al. Vancomycin sensitive penicillinase producing Neisseria gonorrhoea. Br. J Ven Dis. 1983; 59:277
14. Mirret S, Reller B, Knapp JS. Neisseria gonorrhoeae Strains inhibited by vancomycin in selective media and correlation with auxotype. J Clin Microbiol 1981; 14: 94
15. Lai-King Ng, Martin IE. The laboratory diagnosis of Neisseria gonorrhoeae Can J Infect Dis Med Microbiol. 2005; 16(1): 15–25.

PACKAGING
Product	Type	REF	Pack
Modified Thayer Martin (MTM) Medium	Ready-to-use plates	541522	2 x 10 plates ø 90 mm

IFU rev 2, 2021/03

MOPS-BUFFERED LISTERIA ENRICHEMENT BROTH (MOPS-BLEB)

Dehydrated and ready-to-use culture medium

MOPS-BLEB- From left: uninoculated tube, growth of *L. monocytogenes*

INTENDED USE
Secondary enrichment broth for the isolation and identification procedure of *Listeria monocytogenes* in foods according to USDA-FSIS.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Pancreatic digest of casein	17.000 g
Soy peptone	3.000 g
Yeast extract	6.000 g
Sodium chloride	5.000 g
Glucose	2.500 g
Dipotassium hydrogen phosphate	2.500 g
MOPS free acid	6.700 g
MOPS sodium salt	10.500 g
Cycloheximide	0.05 g
Acriflavine HCl	0.015 g
Nalidixic Acid	0.040 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
MOPS Buffered Listeria Enrichment Broth (MOPS-BLEB) is prepared according to a modification described by USDA-FSIS[1] of the original formulation of J. Lovett[2], with the introduction of a strong buffer system that improves the enrichment properties. The medium is recommended by USDA-FSIS[3,4] as the second enrichment broth to be used for subculture of the primary enrichment in UVM 1 medium.
Casein peptone, yeast extract and soy peptone provide essential nitrogen and carbon-based nutrients and trace elements for microbial growth; glucose is a carbohydrate that increases the growth rate of *Listeria*; MOPS compounds and dipotassium hydrogen phosphate act as a buffer system; sodium chloride maintains osmotic balance. Selectivity is provided by the presence of the antifungal compound cycloheximide, nalidixic acid with a marked antibacterial activity against primarily Gram-negative bacteria and acriflavine, an acridine derivative with bacteriostatic properties towards many Gram-positive bacteria and weak antifungal activity.

DIRECTIONS FOR MEDIA PREPARATION

Suspend 53.3 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder. Distribute 10 mL in tubes and autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, limpid
Final pH of complete medium (at 20-25°C)	7.3 ± 0.2

SPECIMENS

Red meat, poultry, ready-to-eat siluriformes (fish) and egg products, and environmental samples. When collecting, storing, transporting and preparing food samples, follow the rules of good laboratory practice and refer to USDA-FSIS document MLG 8.11.[3]

TEST PROCEDURE

- Perform the primary enrichment by adding 225 ml of Listeria UVM1 Enrichment Broth (REF 4015982) to 25 g or 25 ml of sample.
- Homogenise for 2 minutes and incubate at 30° ± 2°C for 20-26 hours.
- Transfer 0.1 mL from the UVM broth into 10 mL of MOPS-BLEB and incubate at 35° ± 2°C for 18-24 hours
- At the same time, from the primary enrichment broth, streak 0.1 mL onto a MOX medium plate (401601 Listeria Oxford Agar Base + 4240039 MOX COL Selective Supplement) and incubate at 35° ± 2°C for 24-28 hours.
- Use the culture in MOPS-BLEB for inoculating a second MOX medium plate and incubate at 35° ± 2°C for 24-28 hours (culture procedure only) or for molecular detection of *Listeria monocytogenes.*

READING AND INTERPRETATION

After incubation, typically *Listeria* spp. produce a turbidity into the enrichment broth.
After subculture on the plating media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Follow the procedure described by USDA-FSIS MLG method 8.11[3] for the identification of colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. monocytogenes ATCC 19117	35°C / 24h / A	good growth
E. coli ATCC 25922	35°C / 24h / A	inhibited
E. faecalis ATCC 29212	35°C / 24h / A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since *Listeria* species other than *L. monocytogenes* can grow, an identification of *Listeria monocytogenes* must be confirmed by suitable tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C / +30°C away from direct light in a dry place.
Ready-to-use medium in tubes
Store tubes in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Laboratory Guidebook, Notice of Change: Media and Reagents. USDA-FSIS, Chapter MLG Appendix 1.09, 12/29/2017.
2. Lovett, J., Francis D.W. and Hunt J.M. (1987) Listeria monocytogenes in raw milk: detection, incidence and pathogenicity. J. Food Prot. 50:188-192
3. Laboratory Guidebook, Notice of Change: Isolation and Identification of Listeria monocytogenes from Meat, Poultry, Ready to Eat Siluriformes (Fish) and Egg Products, and Environmental Sponges. USDA-FSIS, Chapter MLG 8.11, 1/02/2019.
4. Laboratory Guidebook, Notice of Change: Flow Chart Specific for FISI Isolation and Identification of Listeria monocytogenes - Isolation and Identification of Listeria monocytogenes (Culture Method only). USDA-FSIS, Chapter MLG 8 Appendix 1.4, 1/02/2019.

PACKAGING

Product	Type	REF	Pack
MOPS Buffered Listeria Enrichment Broth (MOPS-BLEB)	Dehydrated medium	401601M2 401601M4	500 g (9.4 L) 5 kg (94 L)
MOPS Buffered Listeria Enrichment Broth (MOPS-BLEB)	Ready-to-use tubes	551601M	20 x 10 mL

IFU rev 0, 2022/07

MOTILITY NITRATE CP MEDIUM

Dehydrated culture medium

INTENDED USE

For the confirmation of *Clostridium perfringens* colonies isolated from foods and other materials.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	5.0 g
Beef extract	3.0 g
Galactose	5.0 g
Potassium nitrate	1.0 g
Disodium hydrogen phosphate	2.5 g
Agar	3.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Motility Nitrate CP Medium is prepared according to the formulation recommended by ISO 7937[1] and by FDA-BAM[2,3] for the confirmation of *C. perfringens* colonies isolated from foods and other materials, based on motility test and nitrate reduction reaction. Motility Nitrate CP Medium should be used in combination with Lactose Gelatin Medium for confirmatory purposes.[1,3]

Essential growth factors are provided by enzymatic digest of casein and beef extract which are sources of nitrogen, carbon and minerals. Galactose is the fermentable carbohydrate and a source of energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium. The agar at a concentration of 0.3% is used to demonstrate the motility of organisms. Potassium nitrate serves as a base for evaluation of nitrate reduction to nitrite; galactose and glycerol improve the consistency of the reaction. *C. perfringens* is nonmotile and reduces nitrates to nitrites.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 19.5 g in 1000 mL of cold purified water and add 5 g of Glycerol (REF 421015). Heat to boiling with frequent agitation to dissolve completely the powder. Dispense 10 mL portions into test tubes and sterilise by autoclaving at 121°C for 15 minutes. If not used the same day, just before to use, heat in boiling water or steam for 15 minutes, then cool rapidly to the incubation temperature.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Colonies isolated on plating media such as TSC Agar.

TEST PROCEDURE

For the confirmation of *C. perfringens* one of the following two techniques may be followed:

- confirmation technique using Nitrate Motility CP Medium (REF 401726) and Lactose Gelatin Medium (REF 4015762).[1,3]
- confirmation technique using Lactose Sulfite Medium (REF 401579).[1]

Confirmation using Nitrate Motility CP Medium

- Stab-inoculate each well-isolated characteristic colony selected from TSC Agar into the freshly deaerated Nitrate Motility CP Medium.
- Incubate under anaerobic conditions at 37 °C for 24 h.
- Examine the tube of medium for the type of growth along the stab line.
- Test for the presence of nitrite by adding, with a graduated pipette and the rubber bulb, 0.2 mL to 0.5 mL of the nitrite detection reagent^ to each tube of medium. WARNING: carry out this test under a fume hood.

^Nitrite detection reagent

Dissolve 0.1 g of 5-Amino-2-naphthalenesulfonic acid in 100 mL of 15 % acetic acid solution. Filter through a filter paper. Store in a well-stoppered brown bottle (preferably with a bulb type dropper) at 3 °C ± 2 °C.

Sulfanilic acid solution: Dissolve 0.4 g of sulfanilic acid in 100 mL of 15 % acetic acid solution. Filter through a filter paper. Store in a well-stoppered brown bottle (preferably with a bulb type dropper) at 3 °C ± 2 °C.

Preparation of complete reagent: mix equal amounts of the two solutions just before use. Discard unused reagent immediately.

READING AND INTERPRETATION

Nonmotile organisms produce growth only in and along stab line, while motile organisms usually produce diffuse growth out into the medium, away from the stab.

The formation of a red colour after the addition of nitrite detection reagent confirms the reduction of nitrate to nitrite. If no red colour is formed within 15 mm, add a small amount of zinc dust and allow to stand for 10 min. If a red colour is formed after the addition of zinc dust, no reduction of nitrate has taken place. Bacteria that produce black colonies in TSC Agar, are non-motile, reduce nitrate to nitrite, produce acid and gas from lactose, and liquefy gelatin in 48 h are considered to be *C. perfringens*. Cultures that show a faint reaction for nitrite (i.e. a pink colour) shall be eliminated, since *C. perfringens* consistently gives an intense and immediate reaction.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C/ 24-48 H / AN	non-motile, positive to nitrate reduction test
C. bifermentas NCTC 506	37°C/ 24-48 H / AN	negative to nitrate reduction test

A: anaerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Type Cultures Collection.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

According to ISO 7937 the complete medium Motility Nitrate CP Medium in tubes can be stored at 3 °C ± 2 °C for 4 weeks but, just prior to use, heat in boiling water or flowing steam for 15 min, then cool rapidly to the incubation temperature.[1]

References

1. ISO 7937:2004. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of Clostridium perfringens -- Colony-count technique
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). M102: Motility-Nitrate Medium, Buffered (for C. perfringens).
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens

Packaging

Product	Type	REF	Pack
Motility Nitrate CP Medium	Dehydrated medium	4017262	500 g (25.6 L)

IFU rev 1, 2022/08

MOTILITY MEDIUM

Dehydrated culture medium

Intended use

Semi-solid medium for motility test.

Composition - typical formula * (after reconstitution with 1 L of water)

Pancreatic digest of casein	20.0 g
Enzymatic digest of meat	6.1 g
Agar	3.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Since the early days of microbiology, the motility of bacteria has been used as a means of differentiation and classification.[1] In the early, motility was detected by microscopic observation, later in the late 1800s, with culture media containing agar, gelatin and various infusions. In 1935, Tittsler and Sandholzer[2] developed a semi-solid agar to determine motility by observing the spread of growth beyond the inoculation line.
Motility in bacteria develops through a variety of mechanisms, but the most common involve flagella which are mainly present in bacilli, but there are some flagellated cocci, so motility is a very important means of identification in the *Enterobacteriaceae* family.[1]
Motility Medium is prepared according to the formula described by ISO 11290 and is recommended in the procedure for confirmation of *Listeria* spp. including *Listeria monocytogenes*. [3,4]
The medium contains casein and meat peptones which provide the essential growth factors for microbial growth. Small amount of agar helps to create a semisolid medium for motility test.

Directions for medium preparation

Suspend 29.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, dispense into tubes in quantities of about 5 mL and sterilise by autoclaving at 121°C for 15 minutes.

Physical characteristics

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

Specimens

The specimens consist of colonies grown on plated media.

Test procedure

Using an inoculating needle, stab the medium with a pure culture obtained in Tryptic Soy Yeast Extract Agar (REF 402167).
Incubate at 25 °C for 48 h ± 2 h.

Reading and interpretation

Examine for growth around the stab line.
A positive motility test is indicated by a diffuse growth outward away from stab line or turbidity of the medium; a negative motility test is indicated by growth confined to the stab line.
Listeria spp. are motile, giving a typical umbrella-like growth pattern. If growth is not sufficient, incubate for up to an additional five days and observe the stab again.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t - ATM	Expected results
L. monocytogenes NCTC 7973	25°/ 48 H-A	good growth spreading beyond the inoculation line
L. innocua ATCC 33080	25°/ 48 H-A	good growth spreading beyond the inoculation line
E. coli ATCC 25922	25°/ 48 H-A	good growth spreading beyond the inoculation line
S. aureus ATCC 6538	25°/ 48 H-A	good growth, not motile

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Some new *Listeria* species have been recently isolated. Most of them are not motile in the motility agar.[3,4]
- Motile bacteria but with damaged flagella can give false negative results.
- The presence of excess water in the tubes may lead to false positives.[1]

- The success of this test depends upon proper stab technique and the quality of the inoculating needle. It is recommended that a straight inoculating needle be used. If this is not available, consider using a disposable inoculating needle.[1]
- It is necessary to inoculate the medium taking care to remove the needle along the same stabbing line.
- Do not take inoculums from liquid or broth suspension.
- The isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Shields P, Cathcart L. Motility Test Medium Protocol. 01 November 2011. American Society for Microbiology © 2016
2. Tittsler RP, Sandholzer LA. The use of semi-solid agar for the detection of bacterial motility. J Bacteriol 1936; 31:575–580.
3. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
4. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.

PACKAGING
Product	Type	REF	Pack
Motility Medium	Dehydrated medium	4017142	500 g (16.9 L)

IFU rev 1, 2022/08

MRS AGAR ISO FORMULATION
Dehydrated culture medium

INTENDED USE
For the enumeration of viable mesophilic lactic acid bacteria in food and animal feeding stuffs.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Enzymatic digest of casein	10.00 g
Beef extract	10.00 g
Yeast extract	4.000 g
Glucose	20.00 g
Di-potassium hydrogen phosphate	2.00 g
Sodium acetate	5.00 g
Tri-ammonium citrate	2.00 g
Magnesium sulphate heptahydrate	0.20 g
Manganous sulphate tetrahydrate	0.05 g
Agar	13.00 g
Tween® 80	1.08 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
The so-called “lactic acid bacteria” (LAB) group produces lactic acid as the major metabolic end product of carbohydrate fermentation. Production of lactic acid has linked LAB with food fermentations, as acidification inhibits the growth of spoilage agents. The genera that comprise the LAB are at its core *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus*, and *Streptococcus*, as well as other more peripheral genera.
MRS Agar is a medium devised by DeMan, Rogosa and Sharpe[1] in 1960 primarily for the cultivation of lactobacilli from oral, faecal, dairy and other sources, with the intention of producing a defined medium as a substitute of tomato juice agar. The medium results in good productivity for nearly all LAB but is not selective in the original version.[2] It becomes selective for LAB if the pH is lowered to 5.7 and sorbic acid is added in a concentration of 0.14%.[2]
MRS Agar ISO Formulation is prepared according to the formulation specified by ISO 15124[3] at pH 5.7 for the enumeration of viable mesophilic lactic acid bacteria. If there is a risk of extensive yeast contamination (e.g., in dried sausage), the ISO Standard recommends the addition of sorbic acid.
Peptones provide nitrogen and minerals for microbial growth; yeast extract is a source of B-vitamins complex for growth stimulation. Tween® 80 provides the fatty acids necessary for the metabolism of lactobacilli while magnesium sulphate and manganese sulphate provide essential ions for the multiplication of lactobacilli. Glucose is the fermentable carbohydrate and a source of carbon and energy for microbial growth. Dipotassium phosphate buffers the medium. Selectivity is provided by the presence of ammonium citrate and sodium acetate which, at low pH, allows the growth of LAB while inhibiting a number of other groups of microorganisms.
MRS Agar with Tween 80 differs from MRS Agar with Tween 80 (REF 401728) in that it contains tri-ammonium citrate instead of diammonium citrate and in the final pH.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 67.3 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute into bottles of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.
If needed add sorbic acid: dissolve 1.4 g of sorbic acid in about 10 mL of 1 mol/L solution of sodium hydroxide and sterilize by filtration. Add this solution to 1000 mL of MRS Agar ISO Formulation, previously cooled to approximately 47°C. The final pH of the medium shall be maintained at 5.7 ± 0.1 at 25 °C.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid or slightly opalescent
Final pH at 20-25 °C	5.7 ± 0.1

SPECIMENS
Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

For the enumeration of mesophilic lactic acid bacteria in foodstuffs, ISO 15214 recommends the following technique[3]:

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerned.
2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of MRS Agar ISO Formulation, cooled to approximately 47°C into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.
5. Invert the prepared dishes and incubate at 30°C for 72 h ± 3 h.

Surface plating in combination with incubation under anaerobic or microaerobic conditions can be applied instead of the pour-plating procedure described. Candle jars may be used to obtain appropriate conditions. It is also possible to use a double-layer MRS medium.
According to ISO 20128 (IDF 102)[4], *L. acidophilus* in yogurt must be enumerated with MRS Agar ISO Formulation with the pH adjusted to 6.2 and supplemented with clindamycin and ciprofloxacin. Transfer 0.1 mL of the appropriate dilution(s) onto the surface of two Petri dishes containing the medium. Spread the sample over the entire surface of the medium and incubate anaerobically at 37 °C for 72 h ± 3 h.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Lactobacilli grow with lenticular, often sharp-shaped, colonies of diameter 1 mm to 3 mmm, embedded in or on MRS Agar.
Count the colonies on plates containing between 15 and 300 colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
L. sakei ATCC 15521	30°/ 72 H-A	growth
E. coli ATCC 25922	30°/ 72 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Some *Leuconostoc* spp. may form large slimy colonies, which may hinder the development of other colonies, thus causing an underestimation of the number of lactic acid bacteria.[3]
- Due to the possible development of microorganisms other than lactic acid bacteria, it may be necessary in some cases and for some products to confirm the colonies by simple techniques (such as Gram staining, or the test for catalase).[3]
- If there is a risk of extensive yeast contamination (e.g., in dried sausage), add sorbic acid to the medium.[3]
- Do not permit plates to dry out; on drying, acetate concentration increases at surface which inhibits growth of lactobacilli.[4]

STORAGE CONDITIONS

Store at +2°C /+8°C away from direct light in a dry place.
According to Baird RM et al. the prepared medium without sorbic acid may be stored at 2-8°C for 14 days, while with sorbic acid the shelf life is 7 days at 2-8°C.[2] According to ISO 20128, MRS Agar without supplementations prepared in flasks may be stored in the dark at 1 °C to 5 °C for 6 months.[4]

REFERENCES

1. DeMan JC, Rogosa M, Sharpe ME. (1960). An improved medium for the cultivation of Lactobacilli. 1960; J Appl Bact 23,130-135.
2. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:228-232.
3. ISO 15214:1998. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony-count technique at 30°C.
4. ISO 20128 (IDF 192: 2006. Milk products — Enumeration of presumptive Lactobacillus acidophilus on a selective medium — Colony-count technique at 37 °C.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
MRS Agar ISO Formulation	Dehydrated medium	401728S2	500 g (7.4 L)

IFU rev 1, 2023/01

MRS AGAR WITH TWEEN® 80

Dehydrated and ready-to-use culture medium

INTENDED USE

For the detection and enumeration of *Lactobacillus* and other lactic acid bacteria in dairy products and other food products as well as in products intended for animal feed.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Peptone	10.00 g
Beef extract	10.00 g
Yeast extract	5.00 g
Glucose	20.00 g
Dipotassium hydrogen phosphate	2.00 g
Sodium acetate	5.00 g
Diammonium citrate	2.00 g
Magnesium sulphate	0.20 g
Manganous sulphate	0.05 g
Agar	15.00 g
Tween® 80	1.00 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactobacilli are lactic acid bacteria, a group that also includes, among others, *Leuconostoc, Pediococcus, Lactococcus*, and *Streptococcus*. All these species can produce lactic acid in considerable amounts. They are Gram-positive, catalase and oxidase negative and are fastidious in their nutritional requirements. Growth is enhanced considerably by microaerobic conditions.

MRS Agar with Tween 80 is based on the formulation devised by Johannes Cornelis de Man, Morrison Rogosa and Margaret Elisabeth Sharpe[1] in 1960 primarily for the cultivation of lactobacilli from oral, faecal, dairy and other sources, with the intention of producing a defined medium as a substitute of tomato juice agar. The medium allows a luxuriant growth of all strains of lactobacilli, and more particularly strains with slow and difficult development such as *L. brevis* and *L. fermenti*.

MRS Agar with Tween 80 is slightly selective for lactobacilli and some growth of leuconostocs and pediococci may occur. Selectivity can be improved by the addition of selective compounds such as sorbic acid or antibiotics, adapting the incubation temperature and decreasing the pH: lactobacilli will tolerate lower pH levels than streptococci (pH 5.0-6.5) with pediococci and leuconostocs growing best within this range.[2,3]

Peptones provide nitrogen and minerals for microbial growth; yeast extract is a source of B-vitamins complex for growth stimulation. Tween® 80 provides the fatty acids necessary for the metabolism of lactobacilli while magnesium sulphate and manganese sulphate provide essential ions for the multiplication of lactobacilli. Glucose is the fermentable carbohydrate and a source of carbon and energy for microbial growth. Dipotassium phosphate buffers the medium. Selectivity is provided by the presence of ammonium citrate and sodium acetate which, at low pH, allows the growth of lactic acid bacteria while inhibiting a number of other groups of microorganisms.

MRS Agar with Tween 80 differs from MRS Agar ISO Formulation (REF 401728S) in that it contains ammonium citrate bibasic instead of tri-ammonium citrate and in the final pH.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 70.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise in the autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid or slightly opalescent
Final pH at 20-25 °C	6.4 ± 0.2

SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerned.
2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of MRS Agar with Tween 80, cooled to approximately 47°C into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.

Choose incubation time, temperature and atmosphere based on the research to be performed (e.g 35°C for 3 days, or 30°C for 5 days, in an aerobic atmosphere supplemented with carbon dioxide). Consult the cited references for further information.[5,6]

MRS Agar with Tween 80, acidified at pH 5.4 ± 0.1 with acetic acid, may be used for the enumeration of *Lactobacillus delbrueckii* subsp. *bulgaricus* in yogurt according to the method described by ISO 7889:[4] transfer with a sterile pipette 1 ml of each dilution into Petri dishes and pour 15 ml of acidified MRS Agar. Mix the inoculum with the medium, allow the mixture to solidify and incubate in anaerobic conditions at 37 °C for 72 h.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Lactobacilli grow with lenticular, often sharp-shaped, colonies of diameter 1 mm to 3 mmm, embedded in or on MRS Agar.
Count the colonies on plates having between 15 and 300 colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
L. sakei ATCC 15521	30°/ 72 H-A	growth
E. coli ATCC 25922	30°/ 72 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Some *Leuconostoc* spp. may form large slimy colonies, which may hinder the development of other colonies, thus causing an underestimation of the number of lactic acid bacteria.
- *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* are frequently found in the same habitat as lactobacilli, especially *Lactobacillus brevis*, and can grow on MRS Agar. These two microorganisms however, can be distinguished by their ability to ferment trehalose, and their inability to hydrolyse arginine.
- Due to the possible development of microorganisms other than lactic acid bacteria, it may be necessary in some cases and for some products to confirm the colonies by simple techniques (such as Gram staining, or the test for catalase).[2]
- If there is a risk of extensive yeast contamination (e.g., in dried sausage), add sorbic acid to the medium.[2]
- Do not permit plates to dry out; on drying, acetate concentration increases at surface which inhibits growth of lactobacilli.[7]

STORAGE CONDITIONS

Dehydrated medium

Store at +2°C /+8°C away from direct light in a dry place.
According to Baird RM *et al.* the prepared plates may be stored at 2-8°C for 14 days.[8]

Ready-to-use plates

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. DeMan JC, Rogosa M, Sharpe ME. (1960). An improved medium for the cultivation of Lactobacilli. 1960; J Appl Bact 23,130-135.
2. ISO 15214:1998. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony-count technique at 30°C.
3. Reuter G. Elective and selective media for lactic acid bacteria. Int J Food Microbiol 1985; 2:55-68
4. ISO 7889 (IDF 117): 2003. Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 °C.
5. Njongmenta NA et al. APHA Compendium of Methods for the Microbiological Examination of Foods. Chapter 19 Acid-producing microorganism. American Public Health Association, Washington D.C. 5th Ed, 2015
6. Schoeni JL. APHA Compendium of Methods for the Microbiological Examination of Foods. Chapter 20 Probiotics. American Public Health Association, Washington D.C. 5th Ed, 2015
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
8. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:228-232.

PACKAGING

Product	Type	REF	Pack
MRS Agar with Tween® 80	Dehydrated medium	4017282	500 g (7.1 L)
MRS Agar with Tween® 80	Ready-to-use plates	541728	2 x 10 plates ø 90 mm

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IFU rev 2, 2023/001

MRS BROTH WITH TWEEN 80

Dehydrated culture medium

INTENDED USE

For the cultivation of *Lactobacillus* spp. and other lactic acid bacteria in dairy products and other foods as well as in products intended for animal feed.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.00 g
Beef extract	10.00 g
Yeast extract	5.00 g
Glucose	20.00 g
Dipotassium hydrogen phosphate	2.00 g
Sodium acetate	5.00 g
Ammonium citrate	2.00 g
Magnesium sulphate	0.20 g
Manganous sulphate	0.05 g
Tween® 80	1.00 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactobacilli are lactic acid bacteria, a group that also includes, among others, *Leuconostoc, Pediococcus, Lactococcus*, and *Streptococcus*. All these species can produce lactic acid in considerable amounts. They are Gram-positive, catalase and oxidase negative and are fastidious in their nutritional requirements. Growth is enhanced considerably by microaerobic conditions.
MRS Broth with Tween 80 is based on the formulation devised by de Man, Rogosa and Sharpe[1] in 1960, primarily for the cultivation of lactobacilli from oral, faecal, dairy and other sources, with the intention of producing a defined medium as a substitute of tomato juice.
The medium allows a luxuriant growth of all strains of lactobacilli, and more particularly strains with slow and difficult development such as *L. brevis* and *L. fermenti*.
MRS Agar with Tween 80 is slightly selective for lactobacilli and some growth of leuconostocs and pediococci may occur. Selectivity can be improved by the addition of selective compounds such as sorbic acid, adapting the incubation temperature and decreasing the pH: lactobacilli will tolerate lower pH levels than streptococci (pH 5.0-6.5) with pediococci and leuconostocs growing best within this range.[2,3]
MRS Broth is recommended by APHA[4] to screen heterofermentative lactic acid producers with MPN procedure.

Essential growth factors are provided by peptones and yeast extract which are sources of nitrogen, carbon, vitamins and minerals. Polysorbate 80 provides the fatty acids necessary for the metabolism of lactobacilli while magnesium sulphate and manganese sulphate provide essential ions for the multiplication of lactobacilli. Glucose is the fermentable carbohydrate and a source of carbon and energy for microbial growth. Dipotassium phosphate buffers the medium. Selectivity is provided by the presence of ammonium citrate and sodium acetate which, at low pH, allows the growth of lactic acid bacteria while inhibiting a number of other groups of microorganisms.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 55.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute into tubes or bottles of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	brown, limpid
Final pH at 20-25 °C	6.4 ± 0.2

SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

The sample and its dilutions can be inoculated directly into MRS Broth with Tween 80. Choose incubation time, temperature and atmosphere based on the research to be performed (e.g at 35°C for 4 days, or at 30°C for 5 days, in an aerobic atmosphere).
APHA[4] describes a MPN procedure for detecting lactic acid producers:
MRS Broth tubes containing inverted Durham tubes are inoculated in a three-tube MPN method; after incubation at 35°C for 4 days, tubes showing gas formation are counted as presumptive for heterofermentative organisms.

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
L. sakei ATCC 15521	30-37°/ 72 H-A	growth
P. damnosus ATCC 29358	30-37°/ 72 H-A	growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- *L. mesenteroides* and *L. dextranicum* are frequently found in the same habitat as lactobacilli, especially *Lactobacillus brevis*, and can grow on MRS Broth. These two microorganisms however, can be distinguished by their ability to ferment trehalose, and their inability to hydrolyse arginine.[2]
- If there is a risk of extensive yeast contamination (e.g., in dried sausage), add sorbic acid to the medium.[2]

STORAGE CONDITIONS

Store at +2°C /+8°C away from direct light in a dry place.

REFERENCES

1. DeMan JC, Rogosa M, Sharpe ME. (1960). An improved medium for the cultivation of Lactobacilli. 1960; J Appl Bact 23,130-135.
2. ISO 15214:1998. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony-count technique at 30°C.
3. Reuter G. Elective and selective media for lactic acid bacteria. Int J Food Microbiol 1985; 2:55-68.
4. Njongmenta NA et al. APHA Compendium of Methods for the Microbiological Examination of Foods. Chapter 19 Acid-producing microorganism. American Public Health Association, Washington D.C. 5th Ed, 2015

PACKAGING

Product	Type	REF	Pack
MRS Broth with Tween® 80	Dehydrated medium	4017282	500 g (9.1 L)

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IFU rev 2, 2022/08

MRVP MEDIUM

Dehydrated culture medium

INTENDED USE

In vitro diagnostic. For the differentiation of bacteria by means of the methyl red and Voges Proskauer reactions.

COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	7
Glucose	5
Phosphate buffer	5

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Voges and Proskauer[1] in 1898 and Clark and Lubs[2] in 1915 were the first bacteriologists to observe that the gas produced during fermentation was a mix of CO_2 and H_2 and the red colour reaction produced in appropriate culture medium after treatment with potassium hydroxide. Clark and Lubs optimized the culture medium proposing a formulation with 0.5% peptone, 0.5% glucose and, 0.5% K_2HPO_4, that is the base of the current MRVP medium.

Both methyl red and Voges-Proskauer tests are commonly used in conjunction with the indole and citrate tests, to form a group of tests known as IMViC which aid in the differentiation of *Enterobacteriaceae*, but are now also used to characterize other groups of bacteria including *Actinobacteria*.[3] The medium is recommended by FDA BAM for MR and VP tests in the identification procedures of *Salmonella*.[4]

Escherichia coli and other members of the low-ratio organisms described by Clark and Lubs, those which produce a low ratio of CO_2 to H_2 from the fermentation of glucose, ferment glucose by the mixed acid pathway resulting in a large quantity of acids produced (lactic, succinic, acetic, formic acids) exceeding the buffer system of the medium and lowering the pH to values below 4.4.[3] *Enterobacter aerogenes* and other members of the high-ratio of CO_2 to H_2 organisms, ferment sugars via the butanediol pathway, producing only 1 mol of acid per mol of glucose. This pathway results in a lower degree of acidification of the culture medium.[3] The pH indicator methyl red has been found to be suitable to measure the concentration of hydrogen ions between pH 4.4 (red) and 6.0 (yellow).[5]

The Voges-Proskauer test determines the ability of bacteria to ferment glucose via the butanediol pathway. The pyruvate that is formed by glycolysis in the Embden-Meyerhof pathway, is transformed into α-acetolactate, then into acetoin and finally into butanediol. In ~~the~~ presence of oxygen and KOH, the intermediate acetoin is oxidized to diacetyl, a reaction which is catalysed by α-naphthol. Diacetyl reacts with the guanidine groups of the arginine contained in the peptones, giving rise to a pinkish-red-coloured product. α-naphthol and creatine are catalysts and intensifiers of the chromatic reaction.

For lactose fermenting enteric bacteria there is almost always a negative correlation between MR test and VP test: *Escherichia coli* is MR positive and VP negative, *Enterobacter aerogenes* is MR negative and VP positive.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 17 g in 1000 mL of cold purified water and heat slightly to dissolve, with frequent agitation. Dispense 5 mL in screwcap tubes and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	beige, limpid
Final pH at 20-25 °C	6.9 ± 0.2

SPECIMENS

MRVP Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

TEST PROCEDURE

Transfer a light inoculum from an isolated colony and re-suspend it in the tube. Note that the use of a heavy inoculum may result in aberrant results.

Incubate in aerobic atmosphere for a minimum of 48 hours and up to 5 days at 35 ± 2°C.

For a rapid VP test, with heavy inoculation, it is possible to incubate at 35°C in a water bath for 4 hours.[6]

READING AND INTERPRETATION

One medium is used for two test procedures, MR and VP; the MR test is performed after 48 hours or longer of incubation, the VP test usually after 24 and 48 hours. Therefore, it is necessary to test aliquots for the VP test. In case it is necessary to prolong incubation for MR test, it is recommended that an aliquot also be used for the MR test.[6]

1- Methyl Red test (MR)

Aseptically, remove a 2.5 mL aliquot and add 5 drops of Methyl Red Reagent. Interpret colour result immediately.

Positive test: red colour at the surface of the medium. Negative test: yellow colour at the surface of the medium.

In case of delayed reaction, indicated by the development of an orange colour, continue the incubation to 5 days and repeat the test.

2- Voges-Proskauer Test (VP)

Aseptically, remove a 2.5 mL aliquot for the Barrit test[7], or 1 mL for the O'Meara test[8], or 0.2 mL for the rapid Barry and Feeney test[9].

2A - Barrit test: to 2.5 mL of MRVP Medium add 0.6 mL of Barrit Reagent A and 0.2 mL of Barrit Reagent B; shake gently after each addition to aerate the culture. Read after 10-15 minutes.

Positive test: pinkish red colour at the surface of the medium.

Negative test: yellow colour at the surface of the medium; a coppery colour is a sign of negative test.

2B - O'Meara test: to 1 mL of MRVP medium incubated at 35°C for 4 hours in a water-batch, add 1 mL of O'Meara reagent. Shake the tube gently 30 sec to 1 min to expose the medium to oxygen in order to oxidize the acetoin.

Positive test: pinkish red colour at the surface of the medium.

Negative test: yellow colour at the surface of the medium; a coppery colour is a sign of negative test.

2C - Barry and Feeney rapid test: to 0.2 mL of MRVP Medium inoculated with a single colony from selective medium, incubated at 35°C for 4-6 hours, add 2 drops of Barry and Feeney Reagent, 2-3 drops of Barrit Reagent A and 2-3 drops of Barrit Reagent B. Shake the tubes after each addition of reagent. Read after 15 minutes.

Positive test: cherry red colour within 15 minutes.
Negative test: yellow colour at the surface of the medium.

3-Reagents

Methyl Red Reagent: completely dissolve 0.1 g of methyl red in 300 mL of ethanol (95%). Add 200 mL of purified water to make 500 mL.
Barrit Reagent A: 5 g of α-naphthol in 100 mL of absolute ethyl alcohol.
Barrit Reagent B: 40% KOH in purified water;
O'Meara reagent: 40% KOH + 0.3% creatine in purified water.
Barry and Feeney Reagent: 0.3% creatine in purified water

In the table below, adapted from Edwards and Ewing[10] and MacFaddin[6], the MR/VP results for *Enterobacteriaceae*

Microorganism	MR	VP
Escherichia coli	+	-
Shigella	+	-
Edwardsiella tarda	+	-
Salmonella spp.	+	-
Salmonella arizonae	+	-
Citrobacter spp.	+	-
Klebsiella ozoaenae	+	-
K. rhinoscleromatis	+	-
Morganella morganii	+	-
Proteus vulgaris	+	-
Providencia spp.	+	-
Klebsiella pneumoniae	-	+
Enterobacter aerogenes	-	+
Enterobacter cloacae	-	+
Serratia marcescens	V	+
Hafnia alvei (35°C)	V+	V
Yersinia enterocolitica	+	- (35°C)
Proteus mirabilis	+	V-
Klebsiella oxytoca	V+	V-

V+: variable, usually positive; V-: variable, usually negative; V: variable

USER QUALITY CONTROL

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CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	33-37°C / 48H / A	MR + / VP -
K. pneumoniae ATCC 27736	33-37°C / 48H / A	MR - / VP +

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The results of the MR and VP tests must be used together with other biochemical tests to differentiate the genera and species within the *Enterobacteriaceae*.
- For the MR test, in order to obtain reproducible results, each laboratory should standardize the inoculum density, the total volume of the broth and the size of the test tubes. Sometimes an orange reaction occurs when using too large volume of broth.[6]
- The reaction of methyl red cannot be accelerated by increasing the glucose concentration of the medium.[6]
- Read the MR test not earlier than 48 hours of incubation. If the MR test is performed too early the results are often equivocal or falsely positive.[6] Incubate MR-negative tests for more than 48 hours and test again.
- Read the VP test after 48 hours of incubation. Longer incubations can produce acidic conditions in the broth that will interfere with reading the results. Only in the case of a negative test continue the incubation.[6]
- Do not automatically consider a VP positive strain as MR negative or vice versa; although it is true in most cases, there are certain organisms such as *Hafnia alvei* and *Proteus mirabilis*, which can be positive in both tests, even if the VP reaction is often delayed.[6]
- It is important to follow the order indicated for the addition of Barrit's A and B reagents: first α-naphthol, then KOH. A reversal of order of reagents addition may give weak positive or false negative reactions.
- Do not exceed the volume of 0.2 mL of 40% KOH, since the excess of the reagent can mask a weak VP positive reaction by exhibiting a copper-like colour due to its reaction with α-naphthol.[6]
- The maximum colour for the VP positive test occurs 1 hour after adding the reagents. By exceeding the time of 1 hour, the negative strains may show a copper-liker colour, leading to false positive results. According to Vaughn[11], these false positives are more frequent with incubation at 30°C rather than at 35°C.
- After each addition of the α-naphthol and KOH reagents, the test tubes must be gently shaken to expose the broth to atmospheric oxygen to promote the oxidation of acetoin, if present, to diacetyl.[6]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks.[6]

REFERENCES

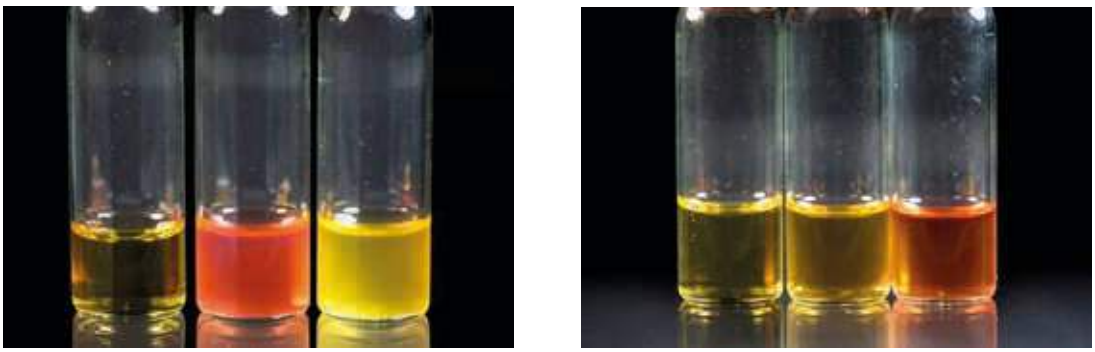
1. Voges O, B. Proskauer B. Beitraege zur Ernaehrungsphysiologie und zur Differential Diagnose der Bakterien der hemmorrhagischen Septicamie. Z. Hyg. 1898; 28:20–32.
2. Clark WM, Lubs HA. The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J Infect Dis 1915; 17:169–173.
3. McDevitt S. Methyl Red and Voges-Proskauer Test Protocols. American Society for Microbiology. 08 December 2009
4. Food and Drug Administration (FDA), Bacteriological Analytical Manual (BAM). Chapter 5: Salmonella; 11/13/2019.
5. Palitzsch, S. Application of methyl-red to the colorimetric estimation of hydrogen ion concentrations. Biochem. Z. 1911; 37:131–138.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Barritt MM. The intensification of the Voges-Proskauer reaction by the addition of a-naphthol. J Pathol Bacteriol 1936; 42:441–454.

8. O'Meara RAQ. A simple delicate and rapid method of detecting the formation of acetyl-methylcarbinol by bacteria fermenting carbohydrate. J Pathol Bacteriol 1931; 34:401–406.
9. Barry L, Feeney KL. Two quick methods for Voges-Proskauer test. App Microbiol 1967; 15:1138
10. Edwards R. & Ewing V.H. (1955) - Identification of Enterobacteriaceae. Minneapolis: Burgess Publishing Company.
11. Vaughn R, Mitchell NB, Levine M. J. The Voges-Proskauer and methyl red reactions in the coli-aerogenes group. J Amer Water Works Assoc 1939; 31: 993

PACKAGING

Product	Type	REF	Pack
MRVP Medium	Dehydrated culture medium	4017352	500 g (29.4L)

IFU rev 2, 2022/03



MRVP Medium:
on top: MR test (uninoculated tube, *E. coli* +, *E. aerogens* -)
at left: Voges-Proskauer test (uninoculated tube, *E. coli* -, *E. aerogenes* +)

MSE AGAR

Dehydrated culture medium

INTENDED USE

For the detection and enumeration of *Leuconostoc* in dairy products and other materials.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.0 g
Gelatin	2.5 g
Yeast extract	5.0 g
Sucrose	100.0 g
Glucose	5.0 g
Sodium citrate	1.0 g
Agar	13.0 g
Sodium azide	75.0 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Leuconostoc spp. are mesophilic Gram-positive cocci belonging to the Lactic Acid Bacteria group, characterised by heterolactic fermentation. They are found commonly on plants, especially sugar cane and leafy vegetables, in dairy products and wine. They are used in the food industry as starter cultures in food production.
MSE agar is a selective medium for the detection and enumeration of *Leuconostoc* in milk, milk products, sweet foods, butter, fermented vegetables, sour pastes. MSE Agar is suitable for counting *Leuconostoc* in starter cultures.[1]
The acronym MSE comes from the initials of the microbiologists who devised the medium: Mayeux, Sandine, and Elliker.[2]
From the sucrose in the medium, *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* synthesise polysaccharides (dextrans) and grow with mucous, gelatinous colonies. *Leuconostoc* species that do not synthesise dextrans grow with small, colourless colonies. Sodium azide inhibits Gram-negative bacteria and lactococci coexisting with *Leuconostoc* in dairy products.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 136.5 g in 1000 ml of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 110°C for 15 minutes. Cool to 47-50°C and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	beige, limpid
Final pH at 20-25 °C	6.9 ± 0.2

SPECIMENS
Milk, milk products, sweet foods, butter, fermented vegetables, sour pastes, starter cultures. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
- Inoculate with 0.1 mL of the sample suspension and/or its decimal dilutions, and spread the inoculum on the surface of the plates.
- Incubate at 21-22°C for 4 days in aerobic atmosphere.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
L. mesenteroides and *L. dextranicum* grow with large mucous, gelatinous colonies.
Other *Leuconostoc* spp. grow with small, colourless colonies

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
L. mesenteroides ATCC 14935	21°/ 72 H-A	growth with large mucous, gelatinous colonies
E. coli ATCC 25922	21°/ 72 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- MSE Agar is a moderately selective medium and microorganism other than *Leuconostoc* can develop colonies.
- After 4 days of incubation, lactic streptococci may develop on the medium as small, opaque, white or yellowish-white colonies

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. FIL-IDF 149A. 1997. Levains lactiques de cultures de bactéries lactiques. Norme de composition.
2. Mayeux JV, Sandine WE, Elliker PR. A selective medium for detecting Leuconostoc in mixed-strain starter cultures. J Dairy Sci 1962;45, 655.

PACKAGING
Product	Type	REF	Pack
MSE Agar	Dehydrated medium	4015292	500 g (3.7 L)

IFU rev 1, 2022/08

MUELLER HINTON AGAR BLOOD SHEEP
Ready-to-use plates

INTENDED USE
In vitro diagnostic device. Culture medium for Antimicrobial Susceptibility Testing (AST) by disk diffusion method of streptococci and *Neisseria meningitidis*, isolated from clinical specimens, according to CLSI.

COMPOSITION - TYPICAL FORMULA *
Beef extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Defibrinated sheep blood	50.0 mL
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
The development of bacterial resistance to antimicrobials in the first half of the twentieth century, resulted in the need for physicians to request the microbiology lab to test a patient's pathogen against various concentrations of a given antimicrobial to determine susceptibility or resistance to that drug.[1] The culture medium proposed for Kirby-Bauer method was Mueller Hinton Agar, originally developed by Howard Mueller and Jane Hilton in 1941 for the isolation of gonococcus and meningoccus.[2] Currently, the Clinical Laboratory Standards Institute (CLSI) for USA and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Europe are responsible for updating and modifying the original procedure through a global consensus process.[3,4] Interpretative guidelines for inhibition zone sizes are included in their publications.[3,5]
Mueller Hinton Agar supplemented with defibrinated sheep blood is recommended and standardized by CLSI[3] for testing the following fastidious organisms: *Streptococcus pneumoniae, Streptococcus* spp. β-haemolytic group, *Streptococcus* spp. *viridans* group, *Neisseria meningitidis*. Defibrinated sheep blood enables the growth of fastidious bacteria with minimal interference in the results of the antimicrobial susceptibility test.

PHYSICAL CHARACTERISTICS
Medium appearance	brilliant red
Final pH at 20-25°C	7.3 ± 0.1
Agar depth	4.0 ± 0.5 mm

SPECIMENS
AST by disk diffusion method is designed to be used with pure culture of strains isolated from clinical specimens. Mueller Hinton Agar Blood Sheep is not intended for microbial isolation directly from clinical specimens. A Gram stain and a preliminary bacterial identification are required for choosing the appropriate antimicrobial agents to be tested.

TEST PROCEDURE

- The surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. Do not over-dry plates.
- **Streptococci**: prepare the inoculum using colonies from an overnight (18 to 20 hours) culture on a blood agar plate. Suspend the colonies in saline and mix to an even turbidity. Adjust the density of the organism suspension to 0.5 McFarland by adding saline or more bacteria.
- ***N. meningitidis***: prepare the inoculum using colonies from an overnight (18 to 20 hours) culture on a chocolate agar plate incubated at 35°C with 5% CO_2. Suspend the colonies in saline and mix to an even turbidity. Adjust the density of the organism suspension to 0.5 McFarland by adding saline or more bacteria. Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep blood agar will contain approximately 50% fewer CFU/mL.
- Dip a sterile cotton swab into the suspension. Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
- Allow disks to reach room temperature before opening cartridges or containers used for disk storage.
- Apply disks firmly to the surface of the inoculated agar plate within 15 minutes of inoculation. Disks must be in close and even contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from disks is very rapid.
- The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. With streptococci test a maximum of 9 disks on a 140 mm plates and 4 disks on a 90 mm plate. With *N. meningitidis* test a maximum of 5 disks on a 140 mm plates and 2 disks on a 90 mm plate.
- Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition.
- Incubate at 35 ± 2°C, 5% CO_2 for 20-24 hours.

READING AND INTERPRETATION

Measure the diameter of zones of complete inhibition, considering the area showing no obvious, visible growth that can be detected with the unaided eye, including the diameter of the disk. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.[3]
For haemolytic streptococci, do not measure the zone of inhibition of haemolysis.
With trimethoprim and sulphonamides, antagonists in the medium allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.[3]
For specific reading instructions consult the CLSI document.[3]
Interpret zone diameters into susceptibility categories according to the current breakpoint tables.[3]

USER QUALITY CONTROL

All manufactured lots of Mueller Hinton Agar Blood Sheep plates are released for sale after the Quality Control has been performed to check the compliance with the specifications, according to CLSI rules[3]. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Select the quality control strain specified by CLSI and reported here below, to monitor the performance of the test.
Streptococcus pneumoniae ATCC 49619
For the details about the suggested QC frequency, the choice of antibiotics and the acceptability ranges, consult the CLSI document.[3]

LIMITATIONS OF THE METHOD

- With trimethoprim and sulphonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥80% reduction in growth.[3]
- Amoxicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, ertapenem, imipenem and meropenem may be used to treat pneumococcal infections; however, reliable disk diffusion tests with these agents do not yet exists. Their *in vitro* activity is best determined using a MIC method.[3]
- For *S. pneumoniae* isolated from CSF, penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method and reported routinely. Such isolates can be tested against vancomycin using the MIC or disk diffusion method.[3]
- EUCAST has evaluated the disk potency of 16 strategically important antibiotic disks from nine manufacturers of disks for antimicrobial susceptibility testing. The study disclosed some good and some poor quality among disks and manufacturers. It is the responsibility of laboratories to perform quality control to guarantee that the material used performs to the standards of the laboratory and the health care system.[6]
- Incorrect inoculum concentration, improper storage of antimicrobial discs, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.[7] Therefore, strict adherence to protocol is required to ensure reliable results.
- CLSI specifies the applicability of interpretative breakpoints for *Streptococcus* spp. β-haemolytic group: large colony forming pyogenic strains streptococci with group A (*S. pyogenes*), C or G antigens, and strains with group B antigen (*S. agalactiae*).[3]
- CLSI specifies the applicability of interpretative breakpoints for *Streptococcus* spp. viridans group: *mutans* group, *salivarius* group, bovis group, *anginosus* group (previously "*S. milleri* group") and *mitis* group.[3]
- CLSI recommends the use of MH-F as an alternative to Mueller Hinton Blood Sheep for AST with *S. pneumoniae*.[3]
- Despite the presence of animal blood, some fastidious strains may not grow or grow lightly on the medium.
- Consult the CLSI papers for the details of disc diffusion methodology, reading and interpretations of inhibition zones, warnings, guidance documents in susceptibility testing, guidelines for detection of resistance mechanisms, clinical breakpoints.
- Mueller Hinton Agar Blood Sheep can be used for determination of MICs with strips containing antimicrobial gradients. To perform this method, it is required to follow the instructions for use of the supplier of strips and to validate the work procedure in the laboratory.
- Informational supplements to CLSI document M100, or revised versions, are periodically published, containing revised tables of antimicrobial discs and interpretative standards. The latest tables should be consulted for current recommendations.
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology (ASM), December 8, 2009.
2. Bauer AW, Perry DM, Kirby WM. Single disk antibiotic sensitivity testing of staphylococci. Analysis of technique and results. Arch Intern Med 1959; 104:208
3. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.
4. The European Committee on Antimicrobial Susceptibility Testing. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 8.0 (January 2020). http://www.eucast.org.

5. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. http://www.eucast.org.
6. Åhman J, Matuschek E, Kahlmeter G. The quality of antimicrobial discs from nine manufacturers EUCAST evaluations in 2014 and 2017. Clinical Microbiology and Infection 2019; 25:346-352
7. Matushek E. EUCAST Educational Workshop. Technical problems and controversies in antimicrobial susceptibility testing. ECCMID 2017, Vienna, Austria.

PACKAGING

Product	Type	REF	Pack
Mueller Hinton Agar Blood Sheep	Ready-to-use plates	541743	2 x 10 plates ø 90 mm
Mueller Hinton Agar Blood Sheep - 150 mm	Ready-to-use plates	501743P	5 plates ø 150 mm

IFU rev 1, 2020/10

MUELLER HINTON AGAR F (MHA-F)

Ready-to-use plates

INTENDED USE

In vitro diagnostic device. Culture medium for Antimicrobial Susceptibility Testing (AST) by disk diffusion method of fastidious organisms.

COMPOSITION - TYPICAL FORMULA *

Beef extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Defibrinated horse blood	50 ml
β-NAD	20 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The development of bacterial resistance to antimicrobials in the first half of the twentieth century, resulted in the need for physicians to request the microbiology lab to test a patient's pathogen against various concentrations of a given antimicrobial to determine susceptibility or resistance to that drug.[1] The culture medium proposed for the Kirby-Bauer method was Mueller Hinton Agar, originally developed by Howard Mueller and Jane Hilton in 1941 for the isolation of gonococcus and meningoccus.[2]
Currently, the Clinical Laboratory Standards Institute (CLSI) for USA and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Europe are responsible for updating and modifying the original procedure through a global consensus process.[3,4] Interpretative guidelines for inhibition zone sizes are included in their publications.[3,5]
Mueller Hinton Agar supplemented with defibrinated horse blood and β-NAD (MHA-F) is recommended and standardized by EUCAST[4] for testing fastidious organisms such as *Streptococcus pneumoniae*, viridans group streptococci, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Listeria monocytogenes*, *Pasteurella multocida Campylobacter jejuni* and *coli*, *Corynebacterium* spp., *Aerococcus sanguinicola* and *urinae*, *Kingella kingae, Aeromonas* spp., *Burkholderia pseudomallei, Campylobacter jejuni* and *coli*.
Defibrinated horse blood and β-NAD enable the growth of fastidious bacteria with minimal interference in the results of the antimicrobial susceptibility test.

PHYSICAL CHARACTERISTICS

Medium appearance	brilliant red
Final pH at 20-25°C	7.3 ± 0.1
Agar depth	4.0 ± 0.5 mm

SPECIMENS

AST by disk diffusion method is designed to be used with pure culture of strains isolated from clinical specimens.
Mueller Hinton Agar F is not intended for microbial isolation directly from clinical specimens.
A Gram stain and a preliminary bacterial identification are required for choosing the appropriate antimicrobial agents to be tested.
EUCAST has published a method for rapid AST (reading at 4, 6 or 8 h incubation) directly from positive blood culture bottles, validated for selected organisms; consult the EUCAST document for the test procedure, reading and interpretation of inhibition zones.[6]

TEST PROCEDURE

The test procedure and the reading and interpretation of inhibition zones here described are a summary of EUCAST documents.[4,5,7]

- The surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min. Do not over-dry plates.
- Use a sterile loop or a cotton swab to pick colonies from an overnight culture on non-selective media. Use several morphologically similar colonies (when possible) to avoid selecting an atypical variant. Suspend the colonies in saline and mix to an even turbidity. Adjust the density of the organism suspension to 0.5 McFarland by adding saline or more bacteria. The suspension must always be used within 60 min of preparation. When *Streptococcus pneumoniae* is suspended from a chocolate agar plate, the inoculum must be equivalent to a 1.0 McFarland standard.
- Dip a sterile cotton swab into the suspension. To avoid over-inoculation of Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube. For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.
- Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
- Allow disks to reach room temperature before opening cartridges or containers used for disk storage.
- Apply disks firmly to the surface of the inoculated agar plate within 15 minutes of inoculation. Disks must be in close and even contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from disks is very rapid.
- The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. The maximum number of disks depends on the organism and the selection of disks. Normally 6 and 12 disks are the maximum possible number on a 90- and 150-mm circular plate, respectively.
- Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition.

- Incubate at 35 ± 1°C in 4-6% CO_2 in air for 18 ± 2 h. Incubate *Aeromonas* spp. and *Burkholderia pseudomallei* at 35 ± 1°C in air for 18 ± 2 h. For *C. jejuni* and *coli*, incubate in microaerobic environment at 41±1°C for 24 hours.
- Isolates of *Corynebacterium, Aerococcus, Kingella* with insufficient growth after 16-20 h are re-incubated immediately and inhibition zones read after a total of 40-44 h incubation. Isolates of *Campylobacter* with insufficient growth are re-incubated for a total of 40-48 hours.

READING AND INTERPRETATION

After incubation, read plates from the front with the lid removed and with reflected light.
A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light and the test must be repeated.
The growth should be evenly distributed over the agar surface to achieve uniformly circular (non-jagged) inhibition zones.
Check that inhibition zones for quality control strains are within acceptable ranges.
For all agents, the zone edge should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye. Holding the plate at a 45-degree angle to the work bench may facilitate reading when zone edges are difficult to define.
Measure the inhibition zone diameters to the nearest millimetre with a ruler or a calliper.
For specific reading instructions consult the EUCAST document.[4] Interpret zone diameters into susceptibility categories according to the current breakpoint tables.[5]

USER QUALITY CONTROL

All manufactured lots of Mueller Hinton Agar F plates are released for sale after the Quality Control has been performed to check the compliance with the specifications, according to EUCAST rules[4,6]. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Select the quality control strains specified by EUCAST and summarized here below, to monitor the performance of the test. Principal recommended control strains are typical susceptible strains, but resistant strains can also be used to confirm that the method will detect resistance mediated by known resistance mechanisms. Check that results for control strains are within acceptable ranges in EUCAST QC tables.[6] EUCAST recommended strains[4]:

- *Streptococcus pneumoniae* ATCC 49619 - Reduced susceptibility to benzylpenicillin
- *Haemophilus influenzae* ATCC 49766 - Susceptible, wild type
- *Campylobacter jejuni* ATCC 33560 – Susceptible, wild type
- *H. influenzae* ATCC 49247 – Reduced susceptibility to β-lactam agents due to PBP mutations.

For details about the choice of antibiotics, the control strains, the frequency of the controls and the tables of the acceptability ranges, consult the EUCAST documents.[4,7]

LIMITATIONS OF THE METHOD

- EUCAST has evaluated the disk potency of 16 strategically important antibiotic disks from nine manufacturers of disks for antimicrobial susceptibility testing. The study disclosed some good and some poor quality among disks and manufacturers. It is the responsibility of laboratories to perform quality control to guarantee that the material used performs to the standards of the laboratory and the health care system.[8]
- Incorrect inoculum concentration, improper storage of antimicrobial disks, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.[9] Therefore, strict adherence to protocol is required to ensure reliable results.
- Despite the presence of animal blood and NAD, some fastidious strains may not grow or grow lightly on the medium.
- Consult the EUCAST papers for the details of disk diffusion methodology, reading and interpretations of inhibition zones, warnings, guidance documents in susceptibility testing, guidelines for detection of resistance mechanisms, clinical breakpoints.
- Mueller Hinton Agar F can be used for determination of Minimum Inhibiting Concentrations (MICs) with strips containing antimicrobial gradients. To perform this method, it is required to follow the instructions for use of the supplier of strips and to validate the work procedure in the laboratory.
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology (ASM), December 8, 2009.
2. Bauer AW, Perry DM, Kirby WM. Single disk antibiotic sensitivity testing of staphylococci. Analysis of technique and results. Arch Intern Med 1959; 104:208
3. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.
4. The European Committee on Antimicrobial Susceptibility Testing. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 8.0 (January 2020). http://www.eucast.org.
5. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. http://www.eucast.org.
6. Jonasson E, Matuschek E, Kahlmeter G. The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles. J Antimicrob Chemother 2020; 75: 968–978
7. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 10.0, 2020. http://www.eucast.org."
8. Åhman J, Matuschek E, Kahlmeter G. The quality of antimicrobial discs from nine manufacturers EUCAST evaluations in 2014 and 2017. Clinical Microbiology and Infection 2019; 25:346-352
9. Matushek E. EUCAST Educational Workshop. Technical problems and controversies in antimicrobial susceptibility testing. ECCMID 2017, Vienna, Austria.

PACKAGING

Product	Type	REF	Pack
Mueller Hinton Agar F (MHA-F)	Ready-to-use plates	541740F	2 x 10 plates ø 90 mm

IFU rev 0, 2020/08

MUELLER HINTON AGAR II

Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. Culture medium for Antimicrobial Susceptibility Testing (AST) by disk diffusion method of common, aerobic, rapidly growing bacteria.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Beef extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g
Agar	17.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The development of bacterial resistance to antimicrobials in the first half of the twentieth century, resulted in the need for physicians to request the microbiology lab to test a patient's pathogen against various concentrations of a given antimicrobial to determine susceptibility or resistance to that drug.[1] William M.M. Kirby and his colleagues proposed a single disk method for antimicrobial susceptibility testing, and thereafter Kirby and Bauer, extensively reviewed the susceptibility testing literature, consolidated and updated all the previous descriptions of the disk diffusion method and published their findings.[2]

This publication led the World Health Organization to form a committee in 1961 to lay the groundwork for the development of a defined procedure for single antimicrobial disk susceptibility testing. The result was a standardized procedure for the disk diffusion susceptibility test, henceforth called at first the Anderson and later the Kirby-Bauer disk diffusion test.[3]

The culture medium proposed for Kirby-Bauer method was Mueller Hinton Agar, originally developed by Howard Mueller and Jane Hilton in 1941 for the isolation of gonococcus and meningoccus.[4]

Currently, the Clinical Laboratory Standards Institute (CLSI) for USA and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Europe are responsible for updating and modifying the original procedure through a global consensus process.[5,6] Interpretative guidelines for inhibition zone sizes are included in their publications.[5,7]

Mueller Hinton agar is considered the best medium to use for Antimicrobial Susceptibility Testing and is recommended by both CLSI[5] and EUCAST[6]. It is suitable and standardized by EUCAST for testing the more common rapidly growing bacteria: *Enterobacteriaceae*, *Pseudomonas* spp., *Stenotrophomonas maltophilia*, *Acinetobacter* spp., *Staphylococcus* spp., *Enterococcus* spp., *Aeromonas*, *Burkholderia pseudomallei*.[6]

Variations in performances of Mueller-Hinton Agar between and with manufacturers' batches/lots, involving different causes, have been observed.[8,9] Concentration of divalent cations Mg^{++} and Ca^{++} influences susceptibility of *Pseudomonas* spp. to tetracycline, gentamicin, polymyxin B, and carbenicillin[10]; calcium concentration modifies daptomycin inhibition zones of Gram-positive bacteria.[11] Variation in thymine and thymidine content, affects sulphonamide and trimethoprim values.[12,13]. The concentration of $zinc_T$ influences resistance interpretations with carbapenems against *P.aeruginosa*,[14] and manganese levels affect resistance interpretations with tigecycline against *Enterobacteriaceae* and *A.baumannii*.[15]

Biolife Mueller Hinton Agar II shows good batch-to-batch reproducibility for susceptibility testing, is low in sulphonamide and trimethoprim-inhibitors (thymine and thymidine), supports satisfactory growth of Gram-positive and Gram-negative non-fastidious pathogens and contains controlled and adjusted levels of calcium and magnesium, low level of zinc and very low level of Mn, to guarantee optimal inhibition zones, within the quality control ranges.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 38 g in 1000 mL of cold purified water. Heat to boiling stirring constantly and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid or slightly opalescent
Final pH at 20-25 °C	7.3 ± 0,1

SPECIMENS

AST by disk diffusion method is designed to use with pure culture of strains isolated from clinical specimens.

Mueller Hinton Agar II is not intended for microbial isolation directly from clinical specimens.

A Gram stain and a preliminary bacterial identification are required for choosing the appropriate antimicrobial agents to be tested.

EUCAST has published a method for rapid AST (reading at 4, 6 or 8h incubation) directly from positive blood culture bottles, validated for selected organisms; consult the EUCAST document for the test procedure, reading and interpretation of inhibition zones.[16]

TEST PROCEDURE

The test procedure and the reading and interpretation of inhibition zones here described are a summary of EUCAST documents.[6,7,17]

- The surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min. Do not over-dry plates.
- Use a sterile loop or a cotton swab to pick colonies from an overnight culture on non-selective media. Use several morphologically similar colonies (when possible) to avoid selecting an atypical variant. Suspend the colonies in saline and mix to an even turbidity. Adjust the density of the organism suspension to 0.5 McFarland by adding saline or more bacteria. The suspension must always be used within 60 min of preparation.
- Dip a sterile cotton swab into the suspension. To avoid over-inoculation of Gram-negative bacteria, remove excess fluid by pressing and turning the swab against the inside of the tube. For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.
- Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
- Allow disks to reach room temperature before opening cartridges or containers used for disk storage.
- Apply disks firmly to the surface of the inoculated agar plate within 15 minutes of inoculation. Disks must be in close and even contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from disks is very rapid.
- The number of disks on a plate should be limited to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. The maximum number of disks depends on the organism and the selection of disks. Normally 6 and 12 disks are the maximum possible number on a 90- and 150-mm circular plate, respectively.

- To be able to detect inducible clindamycin resistance in staphylococci and streptococci, the erythromycin and clindamycin disks must be placed at a distance of 12-20 mm from edge to edge for staphylococci and 12-16 mm from edge to edge for streptococci.
- Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition.
- Incubate at 35 ± 1°C in air for 18 ± 2 h (24 h for glycopeptides and *Enterococcus*).

READING AND INTERPRETATION

After incubation, read plates from the back with reflected light and the plate held above a dark background.
A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light and the test must be repeated.
The growth should be evenly distributed over the agar surface to achieve uniformly circular (non-jagged) inhibition zones.
Check that inhibition zones for quality control strains are within acceptable ranges.
For all agents, the zone edge should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye. Holding the plate at a 45-degree angle to the work bench may facilitate reading when zone edges are difficult to define.
Measure the inhibition zone diameters to the nearest millimetre with a ruler or a calliper.
For specific reading instructions consult the EUCAST document.[6]
Interpret zone diameters into susceptibility categories according to the current breakpoint tables.[7]

USER QUALITY CONTROL

All manufactured lots of Mueller Hinton Agar II plates are released for sale after the Quality Control has been performed to check the compliance with the specifications, according to EUCAST rules[6,17]. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Use the quality control strains specified by EUCAST, summarized here below, to monitor the performance of the test. The principal recommended control strains are typical susceptible strains, but resistant strains can also be used to confirm that the method will detect resistance mediated by known resistance mechanisms. Check that results for control strains are within acceptable ranges in EUCAST QC tables.[17]

Escherichia coli ATCC 25922 - susceptible, wild-type
Escherichia coli ATCC 35218 TEM-1 ß-lactamase, ampicillin resistant (for the control of the inhibitor component of β-lactam-inhibitor combination disks)
Klebsiella pneumoniae ATCC 700603 ESBL-producing strain (SHV-18) (for the control of the inhibitor component of β-lactam-inhibitor combination disks)
Pseudomonas aeruginosa ATCC 27853 - susceptible, wild-type
Klebsiella pneumoniae ATCC BAA-2814 - KPC-3, SHV-11 and TEM-1
Staphylococcus aureus ATCC 29213 - weak β-lactamase producer
Enterococcus faecalis ATCC 29212 - susceptible, wild-type.
Staphylococcus aureus NCTC 12493 - mecA+, methicillin resistant (MRSA)
Enterococcus faecalis ATCC 51299 - HLAR, vanB+ High level aminoglycoside resistant (HLAR) and vancomycin resistant (vanB positive)
For details about the choice of antibiotics, the control strains, the frequency of the controls and the tables of the acceptability ranges, consult the EUCAST documents.[6,17]

PERFORMANCES CHARACTERISTICS

During 2018-2019 EUCAST evaluated the performance of 21 internationally available brands of dehydrated Mueller-Hinton agar from 17 manufacturers.[9] Testing included 4 test strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212) and 18 antimicrobial disks, chosen to represent different agent classes and to include agents that could reveal effects of varying pH and contents of cations and thymidine. All brands were tested blindly and in parallel. The agar depth, pH and concentration of five cations (Mg, Ca, Zn, Mn, Fe) were measured for all brands. Each brand was given a total rating based on how mean values (30 per agar) from triplicate tests related to the respective QC criteria in the EUCAST QC Tables. Biolife Mueller Hinton Agar II demonstrated excellent performance, with 99% of zone diameter readings within QC ranges and 81% on target ±1 mm.

LIMITATIONS OF THE METHOD

- EUCAST has evaluated the disk potency of 16 strategically important antibiotic disks from nine manufacturers of disks for antimicrobial susceptibility testing. The study disclosed some good and some poor quality among disks and manufacturers. It is the responsibility of laboratories to perform quality control to guarantee that the material used performs to the standards of the laboratory and the health care system.[18]
- Incorrect inoculum concentration, improper storage of antimicrobial disks, improper storage of the plates resulting in an agar depth and pH out of specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.[19,20] Therefore, strict adherence to protocol is required to ensure reliable results.
- Antimicrobial susceptibility testing of colistin has been fraught with difficulties. A joint EUCAST and CLSI subcommittee issued recommendations confirming that broth microdilution is so far the only valid method and that disk diffusion does not work because of the poor diffusion of the large colistin molecule.[21]
- Bacteria requiring thymine or thymidine may not grow satisfactorily on Mueller Hinton Agar II because of low levels of thymine or thymidine.[22]
- Mueller Hinton Agar II is not appropriate for assay by disk-diffusion method with slow growing organisms, anaerobes and capnophiles.[20]
- Consult the EUCAST and/or CLSI papers for the details of disk diffusion methodology, reading and interpretations of inhibition zones, warnings, guidance documents in susceptibility testing, guidelines for detection of resistance mechanisms, clinical breakpoints.
- Mueller Hinton Agar II can be used for the determination of Minimum Inhibiting Concentrations (MICs) with strips containing antimicrobial gradients. To perform this method, it is required to follow the instructions for use of the supplier of strips and to validate the work procedure in the laboratory.
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use plates
Store plates in their original pack at +2°C/+8°C away from direct light.

References

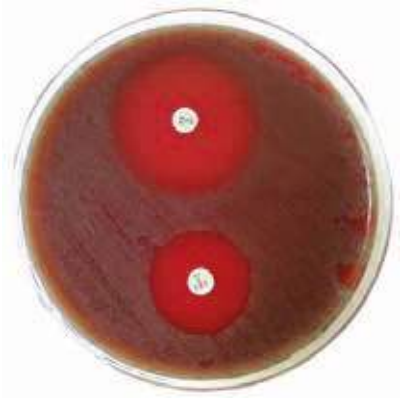
1. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology (ASM), December 8, 2009.
2. Bauer AW, Perry DM, Kirby WM. Single disk antibiotic sensitivity testing of staphylococci. Analysis of technique and results. Arch Intern Med 1959; 104:208
3. Mueller JH, Hinton J. A protein-free medium for primary isolation of the gonococcus and meningococcus. Proc Soc Exp Biol Med 1941; 48:330-333.
4. Standardisation of Methods for conducting microbic sensitivity tests. WHO Technical Report Series 210. Geneve, Word Health Organisation, 1961.
5. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.
6. The European Committee on Antimicrobial Susceptibility Testing. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 8.0 (January 2020). http://www.eucast.org.
7. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0, 2020. http://www.eucast.org.
8. Pollock HM, Minshew BH, Kenny MA, Schoenkecht FD. Effect of Different Lots of Mueller-Hinton Agar on the Interpretation of the Gentamicin Susceptibility of Pseudomonas aeruginosa Ant Microb Ag Chemother 1978; 14:360
9. The European Committee on Antimicrobial Susceptibility Testing. Warning issued 28 February, 2020 Variation in performance of Mueller Hinton dehydrated media for antimicrobial susceptibility testing. http://www.eucast.org
10. D'Amato RF, Thornsberry C, Baker CN, Kirven LA. Effect of calcium and magnesium ions on the susceptibility of Pseudomonas species to tetracycline, gentamicin, polymyxin B, and carbenicillin. Antimicrob Agents Chemother 1976; 7:596-600
11. Fuchs p. et al. Evaluation of susceptibility testing by Etest and the effect of different bacthes of media. J Antimicrob. Chemother 2001; 48:557-561
12. Koch AE, Burchall JJ. Reversal of the antimicrobial activity of trimethoprim by thymidine in commercially prepared media. Appl Microbiol 1971;22:812-817.
13. Salter AJ. Overview. Trimethoprim-Sulfamethoxazole: an assessment of more than 12 years of use. Reviews of Infectious Diseases, Vol. 4, No. 2, Symposium on Trimethoprim-Sulfamethoxazole (TMP-SMZ) (Mar. - Apr., 1982), pp. 196-236. Oxford University Press (uniformare font)
14. Daly JS, Dodge RA, Glew RH et al. Effect of zinc concentration in Mueller-Hinton Agar on susceptibility of Pseudomonas aeruginosa to imipenem. J Clin Microbiol 1997; 35:1027–1029
15. Veenemans J, Mouton JW, Kluytmans JA, Donnerly R , Verhulst C, van Keulen, PHJ. Effect of manganese in test media on in vitro susceptibility of Enterobacteriaceae and Acinetobacter baumannii to tigecycline. J.Clin.Microbiol 2012; 50: 3077-3079.
16. Jonasson E, Matuschek E, Kahlmeter G. The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles. J Antimicrob Chemother 2020; 75: 968–978
17. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 10.0, 2020. http://www.eucast.org."
18. Åhman J, Matuschek E, Kahlmeter G. The quality of antimicrobial discs from nine manufacturers , EUCAST evaluations in 2014 and 2017. Clinical Microbiology and Infection 2019; 25:346-352
19. Matushek E. EUCAST Educational Workshop. Technical problems and controversies in antimicrobial susceptibility testing. ECCMID 2017, Vienna,Austria.
20. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
21. The European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute. Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. 2016. http://www.eucast.org/guidance_documents/.
22. Haltiner RC, Migneault PC, Robertson RG. Incidence of thymidine-dependent enterococci detected on Mueller-Hinton Agar with low thymidine content Antimicrob Agents Chemother 1980; 18: 365-368

Packaging

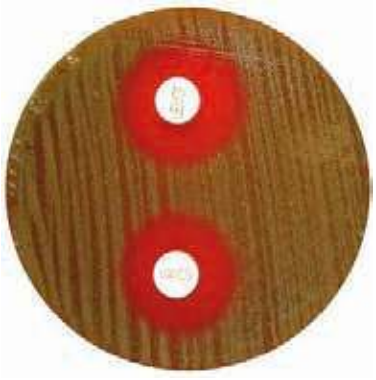
Product	Type	REF	Pack
Mueller Hinton Agar II	Dehydrated medium	4017402 4017404	500 g (13.1 L) 5 kg (131 L)
Mueller Hinton Agar II	Ready-to-use plates	541740	2 x 10 plates ø 90 mm
Mueller Hinton Agar II – 150 mm	Ready-to-use plates	501740P	5 plates ø 150 mm

IFU rev 2, 2022/01

Mueller Hinton Agar II:
P. aeruginosa ATCC 27853



Mueller Hinton Agar F:
S. pneumoniae ATCC 49619



MHA Blood Sheep:
S. pneumoniae / levofloxacin and vancomycin

MUELLER HINTON BROTH

(CATION-ADJUSTED)

Dehydrated culture medium

INTENDED USE

In vitro diagnostic. Cation adjusted liquid medium for broth dilution antimicrobial susceptibility tests (AST).

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

At the end of the twenties of the last century, Alexander Fleming contributed to the development of a broth dilution technique using broth turbidity as an end-point determination.[1] By the end of the 1950s it was apparent that there was a need to standardize AST and several organizations and investigators began addressing this critical issue. The World Health Organization (WHO) published a report on standardization of AST methodology.[3] This has been described as a forerunner of contemporary minimum inhibitory concentration (MIC) methodology. Ericsson and Sherris first used the term 'breakpoint' and published a review of several techniques for susceptibility testing and the relationship between dilution and diffusion methods.[3] Later, in the 1980s, the Clinical and Laboratory Standards Institute consolidated methods and standards for MIC determination and clinical use. The protocols using the Mueller Hinton Broth and microdilution technique and the parameters for defining susceptibility and resistance breakpoints established by CLSI[4] and EUCAST[5-7] are now considered the gold standard in the US and Europe.
With few exceptions, broth microdilution with un-supplemented cation-adjusted Mueller Hinton Broth is the reference method for antimicrobial susceptibility testing of rapidly growing aerobic bacteria.[7] For fastidious organisms, EUCAST recommends testing according to ISO 20776-1,[8] but with the use of Mueller Hinton Broth supplemented with 5% lysed horse blood and 20 mg/L β-NAD (MH-F). The lowest concentration, expressed in mg/L or µg/mL, of antimicrobial agent that inhibits visible growth of a microorganism is defined as the Minimum Inhibitory Concentration (MIC).
In Mueller Hinton Broth, acid digest of casein and beef extract provide nitrogen, carbon, and minerals for bacterial growth. These raw materials are selected with low content of thymine and thymidine as determined by MIC values with *Enterococcus faecalis* and sulfamethoxazole-trimethoprim (SXT). In Mueller Hinton Broth, calcium, magnesium and zinc ions concentrations are adjusted to provide the amounts recommended by ISO Standards[8,9]: Ca^{++}: 20-25 mg/L, Mg^{++}:10-12.5 mg/L, Zn^{++}:< 3 mg/L. Susceptibility testing of *Pseudomonas aeruginosa* with the aminoglycosides and other antibiotics is influenced by the presence of calcium and magnesium ions[10] while zinc ion has an inhibitory activity on carbapenems susceptibility of *P. aeruginosa*[11].

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 21 g in 1000 mL of cold purified water. Heat to completely dissolve the powder, distribute and sterilize by autoclaving at 121°C for 10 minutes. Do not overheat.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	light straw coloured and clear with no visible precipitate
Final pH at 20-25 °C	7.3 ± 0.1

SPECIMENS

Broth dilution antimicrobial susceptibility test is performed with pure culture of strains isolated from clinical specimens. Mueller Hinton Broth is not intended for microbial isolation directly from clinical specimens. A Gram stain and a preliminary bacterial identification are required for choosing the appropriate antimicrobial agents to be tested.

TEST PROCEDURE

Mueller Hinton Broth may be used for inoculum preparation for MIC tests and for preparation of antimicrobial dilutions for the micro-dilution or macro-dilution procedures. The micro-dilution procedure here described is a summary of ISO 20776-1 protocol.[8]

Preparation of working solutions and micro-dilution trays

The range of antibiotics concentrations selected for testing depends on the microorganisms and antimicrobial agent. The chosen range shall allow full endpoint MIC determination for appropriate reference strains. A two-fold dilution series based on 1 mg/mL stock solution is prepared in Mueller Hinton Broth. Dilutions should be prepared according to the procedure outlined in Annex C of ISO 20776-1.[8] Working solutions shall be used the same day unless information is available on stability of the solutions under specified storage conditions.
Working solutions are dispensed into micro-dilution trays at 50 µl per well with double the desired final concentrations of antimicrobial agent, or at 100 µl per well in the desired final concentrations.
At least one well, containing 50 µl or 100 µl of antimicrobial agent-free medium, should be included as a growth control for each strain tested. Likewise, a well containing 100 µl of antimicrobial agent-free medium should be included as an un-inoculated negative control well for each microorganism type tested.

Preparation of inoculum

The inoculum may be prepared by diluting an overnight broth culture or by suspending several morphologically similar colonies cultivated on non-selective agar medium in Mueller Hinton Broth.
In both cases the bacterial suspension is adjusted with saline or broth to give a turbidity equivalent to the 0.5 McFarland standard that contains approximately $1x10^8$ to $2x10^8$ CFU/mL.
The adjusted inoculum prepared as above is diluted in Mueller Hinton Broth to give a final cell number of 5×10^5 CFU/ mL

Inoculation of micro-dilution trays

The trays shall be inoculated within 30 min of standardizing the inoculum suspension
To each well containing 50 µl of diluted antimicrobial agent in broth, a volume of 50 µl of bacterial suspension is added. For tray wells that contain 100 µl of diluted antimicrobial agent in broth, up to 10 µl of diluted inoculum suspension should be added.
Viable counts shall be performed on the test suspension to ensure that test wells contain 5×10^5 CFU/ ml. This shall be done by removing 10 µl from the growth control well immediately after inoculation and diluting it in 10 ml of broth or saline. 100 µl of this dilution is spread over the surface of a suitable agar plate (e.g. Tryptic Soy Agar), which is then incubated overnight (12 h to18 h). Twenty to eighty colonies would be expected from an acceptable test suspension. If this is not achieved corrective action should be taken to ensure proper inoculum preparation.

Macro-dilution (tube) method
If the volume of antimicrobial solution in the tube is 1 mL, dilute the standardized inoculum 1:100 in Mueller Hinton Broth (0.1 mL to a 10-mL tube of broth).
Add 1.0 mL of the adjusted inoculum to each tube containing an antimicrobial agent and 2.0 mL to a sterile empty tube for a growth control.
Incubation
Incubate at 35 ± 1°C in ambient air for 18 ± 2 h for most antimicrobial agent-bacteria combinations.

READING AND INTERPRETATION

Results shall only be read when there is sufficient growth of the test organism (i.e., obvious button or definite turbidity in the positive growth control), when there is no growth in the un-inoculated or negative growth control and when purity and the appropriate cell number concentration of the inoculum has been established.
The amount of growth in each well is compared with that in the positive growth control, and the MIC recorded is the lowest concentration of the agent that completely inhibits visible growth. There are exceptions to this (e.g., trailing endpoints for linezolid, partial inhibition by sulphonamides, incomplete inhibition with some bacteriostatic agents) that will require special attention by the user.

USER QUALITY CONTROL

All manufactured lots of Mueller Hinton Broth are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. It is recommended that the user refers to pertinent ISO[9] and/or CLSI[4] guidance for appropriate Quality Control practices.

LIMITATIONS OF THE METHOD

- Incorrect inoculum concentration, improper storage of trays, pH out of specifications, improper measurement of endpoints, may produce incorrect results.[12]
- Certain resistance mechanisms may not always be expressed using the standard reference dilution method, e.g., the expression of some β-lactamases, efflux pumps or drug target site modifications. In those cases, the MIC should be interpreted with caution, or other information used instead, to guide clinical therapy.[8]
- *In vitro* susceptibility of an organism to a specific antimicrobial agent does not mean that it will be effective as a therapeutic agent *in vivo*. Consult appropriate references for details on interpretation of results.[13,14]
- Bacteria requiring thymine or thymidine may not grow satisfactorily on Mueller Hinton Broth because of low levels of thymine or thymidine.[15]
- Un-supplemented Mueller Hinton Broth is not appropriate for slow growing organisms, fastidious organisms such as *Haemophilus*, *Neisseria* and certain streptococci. For these microorganisms, refer to the methods proposed by EUCAST and CLSI.
- Tigecycline shall be tested within 12 h of preparation of the Mueller Hinton Broth.[16]
- For testing dalbavancin, televancin and oritivancin, polysorbate-80 (volume fraction 0.002%) should be added to Mueller Hinton Broth.[17]
- For testing cefiderocol, Mueller Hinton Broth is first depleted of iron with an iron chelating compound. The medium is then supplemented back with standard concentrations of calcium, magnesium and zinc.[18]
- Broth micro-dilution may not reliably detect resistance conferred by the mecA or mecC gene.[8]
- Incorporation of NaCl at a final concentration of 20 g/L in the broth is required for the detection of methicillin resistance in *Staphylococcus* spp. when testing with oxacillin.
- For testing daptomycin Mueller Hinton Broth medium shall be supplemented to a final concentration of 50 mg/L Ca^{++}.[19]
- When testing glycopeptides, the MIC should be read after 24 h incubation to give more consistent and reliable results; examine tubes or wells carefully for evidence of faint growth.[4,8]
- Studies have shown that testing of mecillinam by Mueller Hinton Broth micro-dilution, results in severe trailing endpoints and is therefore not recommended. Agar dilution or disc diffusion provide stable reproducible results.[20]
- Broth micro-dilution may not give reliable results with fosfomycin. Agar dilution should be used as the reference method.[8]
- Since colistin has affinity to plastic, the results obtained by micro-dilution trays may be prone to reproducibility issues or inaccuracy.[21]
- A distinct phenomenon commonly referred as “skipped wells”, characterized by lack of growth in wells with intermediate colistin concentrations followed by growth in wells with higher concentration, has been observed and reported by some studies.[22]
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Fleming, A. 1929. On the antimicrobial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae*. Br. J. Exp.Pathol. 10:225-236.
2. World Health Organization. (1961). Standardization of Methods for Conducting Microbic Sensitivity Tests. Second Report of the Expert Committee on Antibiotics. WHO Technical Report Series, No. 210. WHO, Geneva
3. Ericsson, H.M., and Sherris, J.H. 1971. Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sect B Suppl. 217:1-90.
4. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution Antimicrobial Susceptibility Tests for bacteria that grow aerobically 7th ed. CLSI approved standard M7 A7. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2006
5. The European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 12.0, valid from 2022-01-01
6. The European Committee on Antimicrobial Susceptibility Testing. EUCAST reading guide for broth microdilution Version 4.0, January 2022
7. The European Committee on Antimicrobial Susceptibility Testing. Media preparation for EUCAST disk diffusion testing and for determination of MIC values by the broth microdilution method. Version 6.0 January 2020
8. ISO 20776-1:2019 Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases
9. ISO/TS 16782:201 Clinical laboratory testing — Criteria for acceptable lots of dehydrated Mueller-Hinton agar and broth for antimicrobial susceptibility testing.
10. Reller, LB, Schoenknecht FD, Kenny M A, Sherris J C. Antibiotic susceptibility testing of Pseudomonas aeruginosa: selection of a control strain and criteria for magnesium and calcium control. J Infect Dis 1974; 130:454-463.
11. Daly JS, Dodge RA, Glew RH, Doja DT, Deluca BA, Herbert S. Effect of Zinc Concentration in Mueller-Hinton Agar on Susceptibility of Pseudomonas aeruginosa to Imipenem. J Clin Microbiol. 1997; 35(4): 1027-2029
12. Matushek E. EUCAST Educational Workshop. Technical problems and controversies in antimicrobial susceptibility testing. ECCMID 2017, Vienna,Austria
13. Turnidge JD, Jorgensen JH. Susceptibility Test Methods: General Considerations. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
14. Koeth LM, Miller LA. Antimicrobial Susceptibility Test Methods: Dilution and Disk Diffusion Methods. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
15. Haltiner RC, Migneault PC, Robertson RG. Incidence of thymidine-dependent enterococci detected on Mueller-Hinton Agar with low thymidine content Antimicrob Agents Chemother 1980; 18: 365-368

16. Brradford PA, Petersen PJ, Young M. et al. , Tigecycline MIC testing by broth dilution requires use of fresh medium or addition of the biocatalytic oxygen-reducing reagent oxyrase to standardize the test method. Antimicrob Agents Chemother. 2005; 49:3903-3909
17. Rennie RP, Koeth L., Jones RN et al. Factors influencing broth microdilution antimicrobial susceptibility test results for dalbavancin, a new glycopeptide agent. J Clin Microbiol. 2007; 45:3151-3154.
18. Akinobu I, Toru Nishikawa T, Matsumoto S. et al. , Siderophore cephalosporin cefiderocol utilizes ferric iron transporter systems for antibacterial activity against Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2016; 60:7396-7401.
19. Fucus PC, Barry AL, Brown SD. Daptomycin susceptibility tests: interpretive criteria, quality control and effect of calcium on in vitro tests. Diag Microbiol Infect Dis. 2000; 38:51-58
20. Skov R, Frimot-Moller N, Menday P. et al. Susceptibility testing of urinary isolates of Escherichia coli to mecillinam using NCCLS methodology. International Antimicrob Agents. 2005; 25:198-202
21. European Centre for Disease Prevention and Control Recommendations for MIC Determination of Colistin (Polymyxin E) As Recommended by the Joint CLSI-EUCAST Polymyxin Breakpoints Working Group. ECDC; Stockholm, Sweden: 2016.
22. Elias R, et al. Klebsiella pneumoniae and Colistin Susceptibility Testing: Performance Evaluation for Broth Microdilution, Agar Dilution and Minimum Inhibitory Concentration Test Strips and Impact of the “Skipped Well” Phenomenon. Diagnostics (Basel). 2021 Dec; 11(12): 2352.

PACKAGING

Product	Type	REF	Pack
Mueller Hinton Broth	Dehydrated medium	4017412	500 g (23.8 L)

IFU rev 1, 2022/04

MULLER KAUFFMANN TETRATHIONATE BROTH BASE

Dehydrated culture medium and supplements

INTENDED USE

In vitro diagnostic. Selective liquid medium for the enrichment of *Salmonella* from food and faecal specimens.

COMPOSITION *

MULLER KAUFFMANN TETRATHIONATE BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	7.00 g
Soy Peptone	2.30 g
Sodium Chloride	2.30 g
Calcium Carbonate	25.00 g
Sodium Thiosulphate	40.70 g
Bile Salts	4.75 g

MULLER KAUFFMANN TETRATHIONATE BROTH, READY-TO-USE TUBES
TYPICAL FORMULA

Muller Kauffmann Tetrathionate Broth Base	82.00 g
Iodine	3.80 g
Potassium Iodide	4.75 g
Brilliant Green	9.50 mg
Purified Water	1000 mL

BRILLIANT GREEN 0.1% SOLUTION
FLASK CONTENT (50 ML)

Brilliant green	50 mg
Purified water	50 ml

IODINE SOLUTION
FLASK CONTENT (50 ML)

Iodine	10.0 g
Potassium iodide	12.5 g
Purified water	50 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Muller Kauffmann Tetrathionate Broth has been originally described by Muller[1] and later modified by Kauffmann[2] by the inclusion of ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp.
The medium is included in the reviews ISTISAN 05/27[3] and ISTISAN 96/35[4] for the selective enrichment of *Salmonella* from samples of the food chain and from faeces prior to selective isolation.
Tryptone and soy peptone provide carbon, nitrogen, vitamins and minerals for microbial growth; the selective agents of the medium are bile salts, the added brilliant green and sodium tetrathionate which is formed from the sodium thiosulfate when the iodine / potassium iodide solution is added to the medium; calcium carbonate neutralizes the sulfuric acid that is produced by the reduction of tetrathionate during the growth of salmonellae, keeping the pH at neutral values. The complete medium allows the development of salmonellae and is inhibitory for Gram-positive bacteria and for a large part of Gram-negative bacteria of enteric origin.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 82 g in 1000 mL of cold purified water and bring with frequent agitation. Cool to 42-45°C and, immediately before use, add 19 mL of Jodine Solution (REF 421501) and 9.5 mL of Brilliant Green 0.1% Solution (REF 421505). Mix well and distribute into test sterile tubes (10 mL) or flasks (100 mL).

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale green with white precipitate
Final pH at 20-25°C	8.0 ± 0.2

SPECIMENS

Muller Kauffmann Tetrathionate Broth may be used for the enrichment of faecal specimens. Collect stool according to standard procedures, with swab preferably with transport medium or in a stool container with or without transport fluid. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied
Food samples: refer to applicable Standards and laws.

Test procedure

Allow tubes to come to room temperature. For faeces examination, inoculate test tubes with 1 g of faeces, or 1 mL of faecal suspension obtained suspending 1 g of faeces in 1 mL of saline solution. Rectal swabs received fresh or in transport medium should be rinsed thoroughly in 1 mL of saline.
Incubate the inoculated tubes in aerobic atmosphere at 35-37°C for 18-24 hours.
For milk and dairy products, the following procedure can be used:
Transfer 25 g of sample to 225 mL of Buffered Peptone Water and incubate at 35-37°C for 18-24 hours.
From the pre-enrichment broth transfer 2 aliquots of 10 mL respectively into 100 mL of Muller Kauffmann Tetrathionate Broth and into 100 mL of Selenite Cystine Broth.
Incubate Muller Kauffmann Tetrathionate Broth at 42-43°C for 24 and 48 hours and Selenite Cystine Broth at 35-37°C for 24 and 48 hours. After 24 and 48 hours of incubation subculture on appropriate selective enteric media.

Reading and interpretation

After incubation, growth of organisms is indicated by turbidity and discolouration. Subculture by streaking a loopful of broth on selective enteric plating media. The plating media should be chosen as a combination of greater and lesser inhibitory selective agars.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of medium.

Control strains	Incubation T°/ t / ATM	Expected results
S. Typhimurium ATCC 14028	35-37°C / 18-24h / A	good growth after subculture to TSA plate
E. coli ATCC 25922	35-37°C / 18-24h / A	scanty growth after subculture to TSA plate

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- Muller Kauffmann Tetrathionate Broth is not suitable for growth of S. Typhi, S. Paratyphi, S. Sendai, S. Gallinarum; it is not recommended for examination of typhoid fever.[5]
- After the enrichment in Muller Kauffmann Tetrathionate Broth, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions

Dehydrated medium: store at +10°C /+30°C away from direct light in a dry place.
Liquid supplements: store the products in the original package at +2°C/+8°C away from direct light
Ready-to-use tubes: store tubes in their original pack at +2°C/+8°C away from direct light.

References

1. Muller, L. (1923) C.R. Soc. Biol. (Paris) **89**, 434-443
2. Kauffmann, F. (1935) Z.f. Hyg. **117**, 26-32
3. Rapporto ISTISAN 05/27. ISSN 1127-3117. Infezioni da Salmonella: diagnostica, epidemiologia e sorveglianza. Raccolta a cura di C.Graziani, P.Galetta, L.Busani, AM Dionisi, E.Filetici, A.Ricci, A.Caprioli, I.Luzzi.
4. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti. Raccolta a cura di D. De Medici, L. Fenicia, L. Orefice e A.Stacchini.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

Packaging

Product	Type	REF	Pack
Muller Kauffmann Tetrathionate Broth Base	Dehydrated culture medium	4017432 4017434	500 g (6,1 L) 5 kg (61 L)
Muller Kauffmann Tetrathionate Broth	Ready-to-use tubes	551743	20 x 10 mL glass tubes, 17x125 mm
Brilliant Green 0.1% Solution	Liquid supplement	421505	50 mL
Iodine Solution	Liquid supplement	421501	50 mL

IFU rev 1, 2022/03

Muller KauffmannTetrathionate Broth – from the left:
uninoculated tube and tube with growth of *S.* Typhimurium.

MULLER KAUFFMANN TETRATHIONATE BROTH BASE ISO FORMULATION
NOVOBIOCIN MKTT ANTIMICROBIC SUPPLEMENT
IODINE SOLUTION
MK TETRATHIONATE NOVOBIOCIN BROTH

Dehydrated and ready-to-use culture medium, selective supplements

INTENDED USE

Selective liquid medium and supplements for the enrichment of *Salmonella* from food and animal feeding stuffs.

COMPOSITION *

MULLER KAUFFMANN TETRATHIONATE BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of meat	4.30 g
Enzymatic digest of casein	8.60 g
Sodium chloride	2.60 g
Calcium carbonate	38.70 g
Sodium thiosulphate anhydrous	30.30 g ^
Ox bile	4.78 g
Brilliant green	9.60 mg

NOVOBIOCIN MKTT SELECTIVE SUPPLEMENT
VIAL CONTENT (FOR 500 mL OF MEDIUM)

Novobiocin	20 mg

IODINE SOLUTION
FLASK CONTENT (50 mL)

Iodine	10.0 g
Potassium iodide	12.5 g
Purified water	50 mL

MK TETRATHIONATE NOVOBIOCIN BROTH, READY-TO-USE TUBES
TYPICAL FORMULA

Muller Kauffmann Tetrathionate Broth Base	1000 mL
Novobiocin	40 mg
Iodine Solution	10 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.
^Equivalent to 47.8 g of sodium thiosulphate pentahydrate

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Muller Kauffmann Tetrathionate (MKTT) Broth was originally described by Muller[1] for the isolation of salmonellae and later modified by Kauffmann[2] by the inclusion of ox bile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp. Jeffries[3] proposed the addition of novobiocin at 40 mg per litre of broth to improve the selective properties of the medium.
The detection of *Salmonella* in foods necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in two selective liquid media, plating out and recognition, confirmation.
MKTT Broth Base with added novobiocin and iodine solution is used for the selective enrichment of *Salmonella* from food chain samples, together with RVS Broth or MSRV Agar, and meets the requirements of ISO 6579.[4]
Peptones provide carbon, nitrogen, vitamins and minerals for microbial growth; the selective agents of the medium are ox bile, brilliant green and sodium tetrathionate which is formed from sodium thiosulfate when the iodine/potassium iodide solution is added to the medium; calcium carbonate neutralizes the sulfuric acid that is produced by the reduction of tetrathionate during the growth of salmonellae, keeping the pH at neutral values. Novobiocin is active mostly against Gram-positive bacteria but also against a few Gram-negative bacteria. The complete medium allows the development of salmonellae and it is inhibitory for Gram-positive bacteria and for a large part of Gram-negative bacteria of enteric origin.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 44.6 g in 500 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Avoid overheating, do not autoclave. Cool to below 47°C and add the contents of 1 vial of Novobiocin MKTT Antimicrobic Supplement (ref. n°4240047) reconstituted with 5 mL of sterile purified water and 10 mL of Iodine Solution (ref. n° 421501). Mix well and aseptically distribute 10 mL into sterile tubes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale green, fine, homogeneous, free-flowing powder.
Solution and prepared tubes appearance	pale green with white precipitate.
Freeze-dried novobiocin supplement appearance	short, dense white pellet; after reconstitution colourless limpid solution.
Liquid iodine solution appearance	brown, limpid.
Final pH of the complete medium at 20-25°C	8.0 ± 0.2

SPECIMENS

Food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[4]

TEST PROCEDURE

The following method is a summary taken from the ISO 6579.[4]

1. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. In general, an amount of test portion is added to a quantity of pre-warmed Buffered Peptone Water (REF 401278) to yield a tenfold dilution (e.g., 25 g test portion is mixed with 225 mL of Buffered Peptone Water).
2. Incubate between 34 °C and 38 °C for 18 h ± 2 h.
3. Transfer 0.1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of the RVS broth (REF 401981) or to the surface of a MSRV Agar plate (REF 401982).
4. Transfer 1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of Muller Kauffmann Tetrathionate Novobiocin Broth (REF 401745 - MKTTn Broth).
5. Incubate the inoculated RVS Broth (or MSRV plates) at 41.5 °C ± 1 for 24 h ± 3 h.
6. Incubate the inoculated MKTTn Broth between 34 °C and 38 °C for 24 h ± 3 h.
7. From RVS Broth or MSRV medium and MKTTn Broth transfer a loopful of growth on a plate of XLD Agar ISO Formulation and on another selective medium for *Salmonella* based on different diagnostic characteristics to those of XLD agar (e.g. Chromogenic Salmonella Agar REF 405350). With MSRV medium positive plates use a 1 µL loop, with MKTTn Broth use a 10 µL loop.

8. Incubate the XLD Agar ISO Formulation inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use.

NOTES

After incubation, it is permissible to store the pre-enriched sample and selective enrichment at 2-8 °C for a maximum of 72 h.[4]
In dried milk products and cheese, *Salmonella* may be sub lethally injured. Incubate the selective enrichment media from these products for an additional 24 h ± 3 h. When investigating outbreak samples, this additional incubation time may also be beneficial.[4]

READING AND INTERPRETATION

After incubation, growth of organisms is indicated by turbidity and discolouration.
Refer to the instructions for use of the two plated media for the description of *Salmonella* colony characteristics.
Mark suspect colonies on each plate. Select suspect colonies for subculture and confirmation.
Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of β-galactosidase (optional), indole detection (optional).[4] Serological confirmation includes the detection of the presence of *Salmonella* O- and H-antigens.
Biochemical confirmation can be substituted with the rapid MUCAP Test (REF 191500). All the colonies MUCAP Test positive must be serologically confirmed.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of medium.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028 + *P. aeruginosa* ATCC 27853 + *E. coli* ATCC 25922	34-38°C / 24 h ± 3 h A	> 10 typical colonies after subculture on XLD Agar
E. faecalis ATCC 29212	34-38°C / 24 h ± 3 h A	< 100 colonies after subculture on TSA
E. coli ATCC 25922	34-38°C / 24 h ± 3 h A	partially inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Muller Kauffmann Tetrathionate Broth is not suitable for growth of S. Typhi, S. Paratyphi, S. Sendai, S. Gallinarum. [5]
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried novobiocin supplement
Store the product in the original package at 2-8°C away from direct light.
Liquid iodine supplement
Store the product in the original package at 2-8°C away from direct light.
According to ISO 6579 the base medium may be stored in closed flasks at 2-8 °C for up to three months. If the complete medium is not used immediately, store it in the dark at 2-8°C. The pH may drop during storage due to chemical reactions. Do not use the complete medium if the pH drops below 7.0.[4]
Ready-to-use medium in tubes
Store tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Muller L. A nouveau milieu d'enrichissement pour la recherche du bacille typhique e des paratyphiques. C.R. Soc. Biol. (Paris) 1923; 89:434-443
2. Kauffmann F. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z Hyg Infektionskr. 1935; 117: 26-32
3. Jeffries L. Novobiocin-tetrathionate broth: a medium of improved selectivity for the isolation of Salmonellae from faeces. J Clin Pathol 1959; Nov;12(6):568-71.
4. ISO 6579-1:2017-2. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella —Part 1: Detection of Salmonella spp.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Muller Kauffmann Tetrathionate Broth Base ISO Formulation	Dehydrated culture medium	4017452 4017454	500 g (5.6 L) 5 kg (56 L)
Novobiocin MKTT Selective Supplement	Freeze-dried supplement	4240047	10 vials, each for 500 mL of medium
Iodine Solution	Liquid supplement	421501	50 mL
MK Tetrathionate Novobiocin Broth	Ready-to-use tubes	551745	20 x 10 mL

IFU rev 2, 2022/08

NEOMYCIN ASSAY AGAR 11: see ANTIBIOTIC MEDIA

NUTRIENT AGAR

Dehydrated and ready-to-use culture medium

Salmonella Typhimurium on Nutrient Agar

INTENDED USE

In vitro diagnostics General purpose medium for the cultivation, sub-culture and purification of colonies of non-fastidious microorganisms isolated from clinical and non-clinical specimens.

COMPOSITION*

DEHYDRATED MEDIUM, READY-TO-USE PLATES, TUBES AND FLASKS
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	3 g
Peptone	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Nutrient Agar is a culture medium based on meat peptones, used for the cultivation of non-fastidious microorganisms. The peptones provide carbon, nitrogen and vitamins for the growth of most non-fastidious microorganisms (e.g., enterobacteria, staphylococci). The absence of sodium chloride limits the swarming of *Proteus* spp. The formulation of the Nutrient Agar complies with the recommendations of ISO 6579[1] and ISO 10273[2]. Nutrient Agar was one of the first media utilised in microbiology and can still be used for the examination of water and food for preparing stock cultures, for the preliminary cultivation of a sample undergoing successive bacteriological examinations, and for the isolation of microorganisms in pure culture.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 23 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Generally Nutrient Agar is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of samples.

TEST PROCEDURE

Allow plates or the tubes to come to room temperature.
Inoculate and streak the specimen with a loop over the four quadrants of the plate or over the slanted medium to obtain well isolated colonies.
Routinely, incubate at 35-37°C in aerobic conditions for 18-24 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the organisms to be cultivated and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of various morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium	ATCC	14028	35-37°C / 18-24H / A	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth
Y. enterocolitica	ATCC	23715	29-31°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin the self-prepared plates can be stored at +2°C /+8°C for up to 6-8 weeks while the tubed and bottles media can be store +2°C /+8°C for up to 6 months.[3]

Ready-to-use medium in plates, tubes and flasks

Store the products in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp.
2. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Nutrient Agar	Dehydrated medium	4018102 4018104	500 g (21.7 L) 5 kg (217 L)
Nutrient Agar	Ready-to-use plates	541810	2 x 10 plates ø 90 mm
Nutrient Agar	Ready-to-use tubes	551810	20 glass tubes with slanted medium
Nutrient Agar	Ready-to-use flasks	5118102	6 x 100 mL; 6 glass bottles with flat bottom and aluminium screw-cap

IFU rev 2, 2022/05

NUTRIENT AGAR N°2

Dehydrated culture medium

INTENDED USE

General purpose medium for the cultivation, sub-culture and purification of colonies of a wide variety of microorganisms.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptic digest of animal tissue	10 g
Beef extract	10 g
Sodium chloride	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

There are several proposed formulations of Nutrient Agar in the literature. The Atlas and Parks review[1] lists more than 30 of them. Biolife includes 4 formulations in its product portfolio: the classic formulation (Nutrient Agar REF 401810) with 3 g/L beef extract and 5 g/L meat peptone, Nutrient Agar No. 3 (REF 401814) in which NaCl 5 g/L is added to the classic formulation, Nutrient Agar w/NaCl (REF 401811) which includes beef extract 1 g/L, meat peptone 5 g/L, yeast extract 2 g/L and NaCl 5 g/L and finally Nutrient Broth n°2 (REF 401813) which originates from Nutrient Broth n°2 supplemented with 15 g/L of agar and containing 10 g/L of beef extract, 10 g/L of peptic digest of animal tissue and 5 g/L of NaCl. Depending on their operational needs, the end users will be able to choose the formulation they consider most appropriate. Due to the higher concentrations of peptones, Nutrient Agar No. 2 is more productive and can be used for the growth of moderately fastidious microbial strains.
Beef extract and peptic digest of animal tissue provide essential nitrogen- and carbon-based nutrients and trace elements for the growth of a wide variety of microorganisms. Sodium chloride is a source of electrolytes and maintains osmotic balance; agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Generally Nutrient Agar N° 2 is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of samples.

TEST PROCEDURE

Allow plates or the tubes to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate or over the slanted medium to obtain well isolated colonies.
Routinely, incubate at 37°C or 30°C in aerobic conditions for 24 hours. Use pure cultures for biochemical and serological confirmatory tests.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the organisms to be cultivated and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of various morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli	ATCC	25922	37°C / 24H / A	good growth
S. aureus	ATCC	25923	37°C / 24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin the self-prepared plates of nutrient agar media may be stored at +2°C /+8°C for 6-8 weeks whereas the nutrient agar in tubes/flasks may be stored at +2°C /+8°C for 6 months.[2]

REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Nutrient Agar n° 2	Dehydrated medium	4018132	500 g (12.5 L)

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NUTRIENT AGAR N°3

Dehydrated culture medium

Nutrient Agar n° 3: *Salmonella* Typhimurium

INTENDED USE

General purpose medium for the cultivation, sub-culture and purification of colonies of non-fastidious microorganisms to be submitted to confirmatory tests.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Meat extract	3.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar	11.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Nutrient Agar n° 3 is a culture medium based on meat peptones, used for the cultivation of non-fastidious microorganisms.
The formulation of Nutrient Agar n° 3 corresponds to Nutrient Agar included in some ISO Standards for the subculture of colonies to be submitted to biochemical and serological confirmatory tests: *Enterobacteriaceae,* according to ISO 21528[1,2], *Salmonella*, according to 6579[3], *Yersinia*, according to ISO 10273[4]
Meat extract and peptone provide essential nitrogen- and carbon-based nutrients and trace elements for the growth of most non-fastidious microorganisms. Sodium chloride is a source of electrolytes and maintains osmotic balance; agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 24 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Generally Nutrient Agar N° 3 is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of samples.

TEST PROCEDURE

Allow plates or the tubes to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate or over the slanted medium to obtain well isolated colonies.
Routinely, incubate at 37°C or 30°C in aerobic conditions for 24 hours. Use pure cultures for biochemical and serological confirmatory tests.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the organisms to be cultivated and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of various morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium	ATCC	14028	37°C / 24H / A	good growth
E. coli	ATCC	25922	37°C / 24H / A	good growth
Y. enterocolitica	ATCC	23715	30°C / 24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place. According to ISO 21528 and ISO 6579, the self-prepared plates can be stored protected from drying, at 2-8°C for up to four weeks.[1-3]

REFERENCES

1. ISO 21528-1:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae.
2. ISO 21528-2:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae —Part 2: Colony-count technique.
3. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp.
4. ISO 10273:2017 Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica.

PACKAGING

Product	Type	REF	Pack
Nutrient Agar n° 3	Dehydrated medium	4018142	500 g (20.8 L)

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NUTRIENT AGAR WITH NaCl

Dehydrated and ready-to-use medium

INTENDED USE

General purpose medium for the cultivation, sub-culture and purification of colonies of a wide variety of non-fastidious microorganisms.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED AND READY-TO-USE PLATES

Beef extract	1.0 g
Yeast extract	2.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

There are several proposed formulations of Nutrient Agar in the literature. The Atlas and Parks review[1] lists more than 30 of them. Biolife includes 4 formulations in its product portfolio: the classic formulation (Nutrient Agar REF 401810) with 3 g/L beef extract and 5 g/L meat peptone, Nutrient Agar No. 3 (REF 401814) in which NaCl 5 g/L is added to the classic formulation, Nutrient Agar n°2 (REF 401813) which originates from Nutrient Broth n°2 supplemented with 15 g/L of agar and containing 10 g/L of beef extract, 10 g/L of meat peptone and 5 g/L of NaCl and finally Nutrient Agar w/NaCl (REF 401811). Depending on their operational needs, the end users will be able to choose the formulation they consider most appropriate.
The formulation of Nutrient Agar W/NaCl corresponds to Nutrient Agar recommended by ISO 16266[2] for the subculture of colonies of suspected *Pseudomonas aeruginosa* to be submitted to confirmatory tests.
This medium may be enriched with other ingredients such as blood, serum, sugars, etc., for specific purposes.
Meat extract and peptone provide essential nitrogen- and carbon-based nutrients and trace elements for the growth of most non-fastidious microorganisms. Sodium chloride is a source of electrolytes, maintains osmotic balance and prevents haemolysis when blood is added; agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 28 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Generally Nutrient Agar W/NaCl is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of samples.

TEST PROCEDURE

Allow plates or the tubes to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate or over the slanted medium to obtain well isolated colonies.
Routinely, incubate at 37°C in aerobic conditions for 24 hours. Use pure cultures for biochemical and serological confirmatory tests.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the organisms to be cultivated and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of various morphology and size. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. aeruginosa	ATCC	10145	37°C / 24H / A	good growth
S. aureus	ATCC	25923	37°C / 24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place. According to ISO 16266 the self-prepared plates can be stored in the dark protected from desiccation at 2-8 °C for up to 1 month.[2]

Ready-to-use plates

Store plates in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997.
2. ISO 16266:2006 Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration.

PACKAGING

Product	Type	REF	Pack
Nutrient Agar W/NaCl	Dehydrated medium	4018112	500 g (17.8 L)
Nutrient Agar W/NaCl	Ready-to-use plates	541811	2 x 10 plates ø 90 mm

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NUTRIENT BROTH

Dehydrated and ready-to-use culture medium

Nutrient Broth
From left: un-inoculated tube, growth of *E. faecalis*

INTENDED USE

In vitro diagnostics. General purpose medium for the cultivation of a wide variety of non-fastidious microorganisms isolated from clinical and non-clinical specimens.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Beef extract	3 g
Peptone	5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Nutrient Broth was one of the first culture medium used in microbiology and has been included in numerous Standards for the examination of water, food, dairy products. It is not a recommended bacteriological medium in later editions of these publications. Nutrient Broth can be used for the sub-culture of colonies grown on other media, for purification of colonies to be subjected to bacteriological and serological tests.
Beef extract and peptone are sources of carbon, nitrogen and minerals for the growth of non-fastidious microorganisms (e.g., Enterobacteriaceae, staphylococci, enterococci, etc).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 8 g in 1000 mL of cold purified water. Heat to dissolve, if necessary, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	light brown, fine, homogeneous, free-flowing powder
Solution appearance	very light yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

Nutrient Broth should not be used for the direct inoculation of specimens. The samples consist of microbial colonies grown on other culture media.

TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube or bottle with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2° C for 18-24 is adequate for cultivation of common aerobes and facultative anaerobes.

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of growth is closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ t / ATM	EXPECTED RESULTS
E. faecalis	ATCC	19433	35-37°C / 18-24H / A	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Nutrient Broth is not suitable for the cultivation of fastidious microorganisms and for the cultivation of anaerobes.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin the self-prepared tubes/flasks may be stored at +2°C /+8°C for 6 months.[7]

Ready-to-use medium in tubes

Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. AOAC Bacteriological Analytical Manual. 8th ed.,1985.
2. AOAC Official methods of analysis of AOAC International, 16th ed.,1995
3. APHA Standard Methods for Examination of Water and Wastewater 14th ed.,1975
4. APHA Compendium of methods for the microbiological examination of foods, 3rd ed.,1992.
5. APHA Standard methods for the microbiological examination of dairy products, 16th ed.,1993
6. APHA Standard methods for the examination of water and wastewater, 19th ed.,1995.
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Nutrient Broth	Dehydrated medium	4018152 4018154	500 g (62.5 L) 5 kg (625 L)
Nutrient Broth	Ready-to-use tubes	551815	20 x 9 mL

IFU rev 2, 2022/05

NUTRIENT BROTH 13 G/L

Dehydrated culture medium

INTENDED USE

General purpose medium for the cultivation of a wide variety of non-fastidious microorganisms.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Beef extract	1.0 g
Yeast extract	2.0 g
Peptone	5.0 g
Sodium chloride	5.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

There are several proposed formulations of Nutrient Broth in the literature. The Atlas and Parks review[1] lists more than 10 of them. Biolife includes 4 formulations in its product portfolio: the classic formulation (Nutrient Broth REF 401815) with 3 g/L beef extract and 5 g/L meat peptone, Nutrient Broth n°2 (REF 401812) intended for the preparation of Preston Broth, Nutrient Broth AOAC (REF 401816) used for the determination of the phenol coefficient of disinfectants and finally Nutrient Broth 13 G/L (REF 401818). Depending on their operational needs, the end users will be able to choose the formulation they consider most appropriate.

Nutrient Broth 13 G/L is a general-purpose medium with superior fertility characteristics to Nutrient Broth. It corresponds in formulation to the preparations of Loeffler and other microbiologists of the European school. Compared to the Nutrient Broth (401815) typical of North American microbiologists, this liquid medium also contains yeast extract and has superior fertility characteristics. This medium may be enriched with other ingredients such as blood, serum, sugars, etc., for specific purposes.

Beef extract, peptone and yeast extract provide essential nitrogen- and carbon-based nutrients, vitamins and trace elements for the growth of most non-fastidious microorganisms. Sodium chloride is a source of electrolytes and maintains osmotic balance.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 13 g in 1000 mL of cold purified water. Mix well and heat to dissolve if necessary. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Generally Nutrient Broth 13 G/L is used for the sub-culture of microorganisms isolated on other culture media and is not used for the direct inoculation of samples.

TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube or bottle with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2° C for 18-24 is adequate for cultivation of common aerobes and facultative anaerobes.

READING AND INTERPRETATION
The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of growth is closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli	ATCC	25922	37°C / 24H / A	good growth
E. faecalis	ATCC	19433	37°C / 24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Nutrient Broth 13 G/L is not suitable for the cultivation of fastidious microorganisms and for the cultivation of anaerobes.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared nutrient broth media in tubes/flasks may be stored at 2-8°C for 6 months.[2]

REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
Nutrient Broth 13 G/L	Dehydrated medium	4018182	500 g (38.5 L)

IFU rev 1, 2022/11

NUTRIENT BROTH AOAC
Dehydrated culture medium

INTENDED USE
General purpose medium for the determination of the phenol coefficient of disinfectants.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Beef extract	5 g
Peptozimatic	10 g
Sodium chloride	5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
There are several proposed formulations of Nutrient Broth in the literature. The Atlas and Parks review[1] lists more than 10 of them. Biolife includes 4 formulations in its product portfolio: the classic formulation (Nutrient Broth REF 401815) with 3 g/L beef extract and 5 g/L meat peptone, Nutrient Broth n°2 (REF 401812) intended for the preparation of Preston Broth, Nutrient Broth 13G (REF 401818) prepared with 5 g/L peptone, 1 g/L beef extract, 2 g/L yeast extract and 5 g/L NaCl and finally Nutrient Broth AOAC (REF 401816). Depending on their operational needs, the end users will be able to choose the formulation they consider most appropriate.
Nutrient broth AOAC is an equivalent medium recommended by AOAC as a culture growth medium for testing disinfectants, using the test strains *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* Typhi.[2,3]
Beef extract and peptozimatic provide essential nitrogen- and carbon-based nutrients, vitamins and trace elements for the growth of most non-fastidious microorganisms. Sodium chloride is a source of electrolytes and maintains osmotic balance.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 20 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS
Pure cultures of test organisms.

TEST PROCEDURE
Consult AOAC publications for details of operating procedures.[2,3]

READING AND INTERPRETATION
The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of growth is closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538	37°C / 24H / A	good growth
S. Typhimurium ATCC 14028	37°C / 24H / A	good growth
P. aeruginosa ATCC 27853		

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Nutrient Broth AOAC is not suitable for the cultivation of fastidious microorganisms and for the cultivation of anaerobes.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared nutrient broth media in tubes/flasks may be stored at 2-8°C for 6 months.[4]

REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997.
2. Official Methods of Analysis AOAC International, Volume 1. Edited by Horwitz W. Published by AOAC International. USA. Chapter 6. Disinfectants Subchapter 1. Phenol coefficient methods AOAC official methods 955.1, 2000 17th edition
3. US Environmental Protection Agency Standard Operating Procedure for AOAC Use Dilution Method for Testing Disinfectants. SOP Number: MB-05-07 Date Revised: 08-18-09.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Nutrient Broth AOAC	Dehydrated medium	4018162	500 g (25 L)

IFU rev 1, 2022/11

NUTRIENT BROTH N° 2
PRESTON ANTIMICROBIC SUPPLEMENTS
CAMPYLOBACTER GROWTH SUPPLEMENT
(Preston broth)

Dehydrated culture medium and supplements

INTENDED USE

General purpose liquid medium the cultivation of fastidious and non-fastidious microorganisms; medium base for the preparation of Preston broth for the enrichment of *Campylobacter* spp. in food and water samples.

COMPOSITION*

DEHYDRATED NUTRIENT BROTH N° 2 (REF 401812)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of animal tissues	10 g
Peptone	10 g
Sodium chloride	5 g

CAMPYLOBACTER GROWTH SUPPLEMENT (REF 4240021)
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Sodium pyruvate	125 mg
Sodium metabisulfite	125 mg
Ferrous sulphate	125 mg

PRESTON ANTIMICROBIC SUPPLEMENT II (REF 4240022)
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Polymyxin B	2500 IU
Amphotericin B	5 mg
Rifampicin	5 mg
Trimethoprim	5 mg

PRESTON ANTIMICROBIC SUPPLEMENT (REF 4240017)
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Polymyxin B	2500 IU
Cycloheximide	50 mg
Rifampicin	5 mg
Trimethoprim	5 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Preston broth, prepared with Nutrient Broth n° 2 and the addition of Preston Antimicrobic Supplement, Campylobacter Growth Supplement and lysed horse blood, corresponds to the medium proposed by Bolton *et al.* in 1982[1,2] for estimating small numbers of campylobacters in water.
The current method described by ISO 10272-2[3] for food chain samples, recommends Bolton Broth as well as Preston broth, with or without Campylobacter Growth Supplement and with cycloheximide substituted by amphotericin B. Preston broth is recommended as a selective enrichment medium for samples with low numbers of campylobacters and high level of background microflora (ISO detection procedure B). There is a similar ISO method for water (ISO17995)[4] which uses Preston broth (with Campylobacter Growth Supplement) as well as Bolton broth.
The addition of growth supplement to Preston Broth enhances recovery of *Campylobacter* spp. and some strains will not grow without it.[3]
The use of Preston broth overcomes problems with background flora resistant to third generation β-lactams, like cefoperazone in Bolton broth.
Beef extract and peptone provide nitrogen, carbon, minerals and amino acids for the microbial growth. Sodium chloride maintains the osmotic balance. Sodium pyruvate aids in resuscitation of stressed cells and, together with sodium metabisulfite and sodium sulphate, enhances the isolation and the oxygen tolerance of *Campylobacter* spp.[5] Lysed blood horse provides specific and essential nutritional factors for the growth of campylobacters. The selective agents of the medium are polymyxin B, active against Gram-negative bacteria, trimethoprim which mainly suppresses the growth of *Proteus* spp. and other Gram-negative bacteria, rifampicin active against Gram-negative and Gram-positive bacteria and amphotericin B, included as an antifungal compound.
Nutrient Broth N°2 without the addition of supplements may be used as a general-purpose liquid medium for the cultivation of fastidious and non-fastidious microorganisms. It gives good growth from small inocula and is particularly suitable for subculture of staphylococci for the coagulase test.

DIRECTIONS FOR MEDIA PREPARATION

General purpose medium

Suspend 25 g in 1000 mL of purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

Preston broth

Suspend 25 g in 945 ml of purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Sterilize by autoclaving at 121°C for 15 minutes.

Cool to 47-50°C and add 50 ml of lysed horse blood (REF 90HLX100) and the contents of two vials of Preston Antimicrobic Supplement II (REF 4240022 with amphotericin B) or Preston Antimicrobic Supplement (REF 42540017 with cycloheximide) reconstituted with 2 mL of 50% acetone/sterile purified water. If required add also the contents of 2 vials of Campylobacter Growth Supplement (REF 4240021), reconstituted with 5 mL of sterile purified water. Mix well and distribute into sterile tubes or flasks.

PHYSICAL CHARACTERISTICS

Nutrient Broth n°2

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Prepared tubes appearance	dark red, limpid
Final pH at 20-25 °C	7.4 ± 0.2

Preston Antimicrobic Supplement (with cycloheximide)

Freeze-dried supplement appearance	short, red-brown pastille
Reconstituted supplement appearance	red limpid solution

Preston Antimicrobic Supplement II (with amphotericin B)

Freeze-dried supplement appearance	short, red-brown pastille
Reconstituted supplement appearance	red-orange opalescent solution

Campylobacter Growth Supplement

Freeze-dried supplement appearance	short, friable, green-grey pastille
Reconstituted supplement appearance	grey-green limpid solution

SPECIMENS

Water, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable International Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice.[3,4]

TEST PROCEDURE

Food samples

1. According to ISO 10272-1, Preston broth is used as selective enrichment broth for the detection of *Campylobacter* in samples with low numbers of campylobacters and high level of background microflora, e.g., raw meats (including poultry) or raw milk.
2. In general, for preparing the initial suspension, combine a quantity of 10 g or 10 mL of the test portion with 90 ml of the enrichment medium (Preston broth), so as to obtain a 1 in 10 dilution, and homogenize.
3. Incubate the initial suspension in a microaerobic atmosphere at 41.5 ± 1 °C for 24 h ± 2 h.
4. Using the culture obtained in the enrichment medium, inoculate with a sterile 10 µl loop the surface of the isolation medium, mCCD agar*.
5. Incubate the selective solid medium at 41.5 °C in a microaerobic atmosphere and examine after 44 h to detect the presence of typical and/or suspect *Campylobacter* colonies.

Water samples

1. In general, the detection of *Campylobacter* in water according to ISO 17995[4] requires enrichment followed by isolation of colonies and their confirmation.
2. Samples are inoculated either directly or after concentration using membrane filtration into one of two selective enrichment broths depending on the expected level of background microorganisms: Bolton broth^ for clean water and Preston broth for more heavily contaminated water. A single sample volume is processed for *Campylobacter* detection and, where necessary, at least three 10-fold volumes (for example 10 mL, 100 mL and 1000 mL) are used for a semi-quantitative determination. For a quantitative (MPN) determination, volumes of 500 mL, 5 x 100 mL, 5 x 10 mL and, where counts may be high, smaller volumes are used or the initial sample is diluted. The broths are then incubated microaerobically at 37 ± 1 °C for 44 ± 4 h.
3. From the enrichment broth cultures, liquid selective media are inoculated onto mCCDA agar* and the plates are incubated at 41.5 ± 1 °C for 44 ± 4 h in a microaerobic atmosphere.

Notes

*mCCDA agar: Campylobacter Blood Free Medium Base Bolton REF 401282 + Bolton CCDA Antimicrobic Supplement REF 42400120

^Bolton broth: Campylobacter Bolton Broth Base REF 401286B2 + Bolton Broth Selective Supplement REF 4240025.

READING AND INTERPRETATION

Microbial growth in Preston broth is evidenced by the development of turbidity.

After incubation of isolation plated media, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

Campylobacter colonies usually are greyish on mCCD Agar, often with a metallic sheen, and are flat and moist, with a tendency to spread. Colonies tend to spread less on drier agar surfaces. Other forms of colonies may occur.

The suspect *Campylobacter* colonies are examined for morphology and motility using a microscope and sub-cultured on a non-selective blood agar, and then confirmed by detection of oxidase activity and an aerobic growth test at 25°C.

Characteristics of *Campylobacter*:

morphology: small curved bacilli, motility: characteristic corkscrew darting, aerobic growth at 25°C: negative, oxidase test: positive

C. coli, *C. jejuni, C. lari, C. upsaliensis* can be differentiated by catalase test, hydrolysis of hippurate and indoxyl acetate test

As an alternative, or in addition, to the confirmation and identification tests, other tests such as PCR test, serological methods, MALDI-TOF-MS analysis, can be used.

For a complete explanation of the identification criteria and methods, refer to the quoted references.[3,4]

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Depending on the intended use (e.g., detection of *Campylobacter* in water or foodstuffs), apply the quality control required by the Standards.[3,4,8]

LIMITATIONS OF THE METHOD

- The procedures described above target the thermotolerant *Campylobacter* spp. relevant for human health. The most frequently encountered strains are *C. jejuni* and *C. coli*. However, other species have been described (*C. lari, C. upsaliensis* and others).[3]
- The recognition of colonies of *Campylobacter* is to a large extent a matter of experience and their appearance can vary somewhat.[3]

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Freeze-dried supplements

Store the products in the original package at +2°C /+8°C away from direct light.

According to ISO 10272[3] and ISO 17995[4] the complete Preston broth should be stored at 5 ± 3°C for not more than 7 days.

REFERENCES

1. Bolton FJ, Hinchliffe PM, Coates D, Robertson L. A most probable number method for estimating small numbers of campylobacters in water. J Hyg (Lond). 1982 Oct; 89(2):185-90.
2. Bolton FJ, Robertson L. A selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol 1982; 35(4):462-7.
3. ISO 10272-1:2017+ A1: 2023. Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. - Part 1: Detection method.
4. ISO 17995:2019 Water quality — Detection and enumeration of thermotolerant Campylobacter spp.
5. Hoffman PS, George HA, Krieg NR, Smibert RM. Studies of the microaerophilic nature of Campylobacter fetus subsp. jejuni. II. Role of exogenous superoxide anions and hydrogen peroxide. Can J Microbiol 1979 Jan; 25(1):8-16.
6. ISO 11133:2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

PACKAGING

Product	Type	REF	Pack
Nutrient Broth n° 2	Dehydrated medium	4018122	500 g (20 L)
Preston Antimicrobic Supplement	Freeze-dried supplement	4240017	10 vials, each for 500 mL of medium
Preston Antimicrobic Supplement II	Freeze-dried supplement	4240022	10 vials, each for 500 mL of medium
Campylobacter Growth Supplement	Freeze-dried supplement	4240021	10 vials, each for 500 mL of medium

IFU rev 1, 2023/02

OF HUGH LEIFSON BASE

Dehydrated culture medium

OF Hugh Leifson Glucose Agar
from left: *E. coli, P. aeruginosa, A. faecalis*

INTENDED USE

In vitro diagnostic. For the differentiation of Gram-negative bacilli, isolated from clinical samples or other materials, on the basis of the oxidative or fermentative metabolism of carbohydrates.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	2.00 g
Sodium chloride	5.00 g
Dipotassium phosphate	0.30 g
Bromothymol blue	0.03 g
Agar	2.50 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

O/F Hugh Leifson Base, prepared according to the formula proposed by Hugh and Leifson[1], is a medium to which various carbohydrates can be added for the study of oxidative or fermentative metabolism of microorganisms. The medium is intended for the differentiation of gram-negative bacilli isolated from clinical specimens and other materials.[2,3]

Bacteria utilise glucose and other carbohydrates through various metabolic pathways; some are oxidative routes but others involve fermentation reactions. During the anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids at high concentration, depending on the type of fermentation. Certain non-fermenting Gram-negative bacteria metabolize glucose using aerobic respiration and therefore only produce a small amount of weak acids during glycolysis and Krebs cycle. The complete medium will contain a high concentration of carbohydrate with a low content of peptone to avoid the utilisation of peptone by an aerobic organism and the resultant production of an alkaline reaction which would neutralise slight acidity produced by an oxidative organism.[3]

O/F Medium Base, supplemented with the suitable carbohydrate, allows to distinguish between the two metabolic pathways. The medium contains bromothymol blue as a pH indicator: the high concentration of acid produced during fermentation will turn the bromothymol blue indicator from green to yellow in the presence or absence of oxygen. The persistence, after incubation, of a green colour or the appearance of a blue colour, due to an alkaline transformation of the medium, indicates that the test is negative and that there was no degradation of the carbohydrate.

O/F Hugh Leifson Base is a semi-solid medium: the presence of agar at a concentration of 0.25% enables the determination of motility in addition to OF test and also aids in preventing the distribution of any acid produced towards the surface of the medium, with a consequent dilution.

Dipotassium phosphate promotes carbohydrate fermentation and acts as a pH control buffer.[1] t

Tryptone provides carbon, nitrogen and trace elements for microbial growth. Sodium chloride maintains the osmotic balance.

Glucose is the carbohydrate most frequently used for the OF test; however, there are organisms being tested unable to metabolize glucose, which can attack other carbohydrates; a battery consisting of glucose, lactose and sucrose should then be employed and, sometimes, maltose, mannitol and xylose.[2,4,5]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 9.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute in screw cap tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and add a sterile solution of the chosen carbohydrate at 1% (w/v) final concentration. Alternatively, and depending on heat stability, add 10 g/L of carbohydrate prior to sterilisation.
For each strain to be examined, prepare two test tubes and, after inoculation, cover one of them with liquid paraffin to create anaerobic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	green, limpid
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

The sample consists of bacterial cultures isolated from clinical samples or other materials, purified on Tryptic Soy Agar or blood agar or other suitable medium.

TEST PROCEDURE

For each carbohydrate used, inoculate lightly a pair of OF tubes for each organism being tested, by inserting a straight needle vertically to approximately ¼ inch from the bottom.
Cover one tube of each pair with 3 cm layer of liquid paraffin to create anaerobic condition, leaving the other tube open to the air.
Also set up control sets: one inoculated set with no carbohydrate added and one uninoculated set with carbohydrate.
Incubate, with loose caps, at 35-37°C for 44-48 hours; slow-growing bacteria require longer incubations (3-4 days or even up to 14 days)[3]

READING AND INTERPRETATION

Examine the tubes daily for colour change.
Oxidation: acid in aerobic tube only (yellow colour in aerobic tube, green in anaerobic tube)
Fermentation: acid in both tubes (yellow colour), with gas production (aerogenic strain) or without (anaerogenic strain).
No degradation of sugar (asaccharolytic strain): no yellow change of both test tubes that remain green (covered test tube) or turn green-blue (open test tube).
The following table, adapted from MacFaddin[3], summarizes the reactive patterns on OF Hugh Leifson Base supplemented with glucose.

Reaction	Tube with reaction	Reaction in open tube	Reaction in covered tube
Oxidation e.g. *P. aeruginosa*	Open	Yellow (A)	Green (-)
Fermentation			
Anaerogenic e.g. *S. dysenteriae*	Covered	Yellow (A)	Yellow (A)
Aerogenic e.g. *E. coli*	Covered	Yellow and gas (AG)	Yellow and gas (AG)
Neither fermentation nor oxidation e.g. *A. faecalis*	Neither*	Blu or Green (-)	Green (-)
Both Fermentation and oxidation e.g. *Citrobacter*	Both	Yellow (A o AG)	Yellow (A o AG)

A: acid production; G: gas production *: Uninoculated carbohydrate control reading: no change in colour

OF Hugh Leifson is also useful for detecting bacterial mobility (diffuse growth starting from the inoculum line).

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

TEST STRAINS	INCUBATION T°/ T	EXPECTED RESULTS	
		OPEN TUBE	COVERED TUBE
P. aeruginosa ATCC 14207	35-37°C / 44-48 h	yellow	green
A. faecalis NCTC 655	35-37°C / 44-48 h	green/blue	green
E. coli ATCC 25922	35-37°C / 44-48 h	yellow	yellow

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Type Culture Collection of the UK Health Protection Agency

LIMITATIONS OF THE METHOD

- Mineral oil is not recommended because it is a heavy liquid petroleum which allows oxygen diffusion in the medium.[2,3]
- Some organisms are unable to grow on OF Hugh Leifson Medium; if this occurs, repeat the OF test with the medium enriched either with 2% serum or 0,1% yeast extract.[4]
- An organism that is neither oxidative or fermentative will produce a Slight alkalinity (blue-green) in the open tube but the covered tube will not exhibit a colour change (green).[3]
- Some bacteria produce an atypical reaction: acid in the closed tube but not in the open tube; *Chromobacterium violaceum* develops these atypical reactions with starch and maltose.[6]
- Some organisms require prolonged incubation before acid production is visible. Lederberg[7] states that the delayed reaction is due to the inability of a carbohydrate to penetrate the bacterial cell. He recommends to perform ONPG test to determine potential fermentative ability.
- A fermentative organism will exhibit an acid reaction throughout the medium in both tubes. However, acid production of an oxidative organism is evident only at the surface of the open tube and gradually spreads throughout the tube. If the oxidative reaction is delayed or weak, an alkalinity may be observed on the surface of the open tube, often resulting in a misinterpretation of the OF reaction as being negative. However, on prolonged incubation of several days, the alkaline reaction reverts to acid.[3]

- Hugh and Leifson[1] report that some so-called "paracolon bacilli" can have both an oxidative and fermentative metabolism. In these cases, oxidation is not evident unless the fermentation is slow or delayed. This group of Gram-negative bacilli (*Citrobacter*, *S. arizonae*) is capable of oxidation or fermentation of lactose, or both.
- Even if the microbial colonies are differentiated on the basis of OF test, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months.[3]

References

1. Hugh R, Leifson E. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J Bacteriol 1953; 66:24-26
2. Public Health England. Oxidation/Fermentation of Glucose Test. UK Standards for Microbiology Investigations. TP 27 Issue 3, 2015
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Barrow GI, Feltham RKA. Bacterial characters and characterization. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 1999
5. Finegold SM, Martin WJ, Scott EG. Bailey and Scots Diagnostic Microbiology, 5th ed., St. Louis: The C.V. Mosby Company, 1978, pp 124, 184, 453.
6. Sivendra R. One-tube oxidation-fermentation methods: limitations posed by atypical fermentative reactions. Appl Environ Microbiol 1976: 31(5):778-80
7. Lederberg J. The beta-D-galactosidase of Escherichia coli, strain K-12. J. Bacteriol 1950; 60: 381

Packaging
Product	Type	REF	Pack
O/F Hugh Leifson Base	Dehydrated medium	4018362	500 g (51 L)

IFU rev 2, 2022/03

OGYE AGAR BASE
OXYTETRACYCLINE ANTIMICROBIC SUPPLEMENT
GENTAMICIN ANTIMICROBIC SUPPLEMENT
Dehydrated culture medium and selective supplements

Intended use
For the detection and enumeration of yeasts and moulds in foods.

Composition *
OGYE Agar Base, dehydrated medium
typical formula (after reconstitution with 1 L of water)

Yeast Extract	5.0 g
Glucose	20.0 g
Agar	13.0 g

Oxytetracycline Antimicrobic Supplement
(Vial contents for 500 mL of medium)

Oxytetracycline HCl	50 mg

Gentamicin Antimicrobic Supplement
(Vial contents for 500 mL of medium)

Gentamicin	25 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
Traditionally, acidified media have been used to enumerate yeasts and moulds and may be appropriate for certain types of foods. However, media supplemented with antibiotics are more commonly used as they may facilitate resuscitation of injured cells .[1]
Mossel et al.[2] in 1962 demonstrated that acidic media were not completely suitable for counting yeasts and moulds in foods for two reasons:
1) heat-stressed yeast cells do not tolerate the acidic conditions necessary to inhibit bacterial contaminants; 2) yeast and mould growth is often limited by the presence of acid-tolerant bacterial flora. Mossel et al.[3] described Oxytetracycline-Glucose Yeast Extract (OGYE or OGY) Agar for the selective isolation and enumeration of yeasts and moulds in foods and demonstrated that this medium improved fungal recovery compared to acidified agar media.
Under certain experimental conditions and when testing certain foods, the use of oxytetracycline alone was not sufficient to obtain reliable counts of yeasts and moulds.[4] Mossel et al.[5] observed that, with high-protein foods, heavily contaminated with Gram-negative rods, it was necessary to use both oxytetracycline and gentamicin to achieve complete inhibition of contaminants.
OGYE Agar contains yeast extracts which supplies B-complex vitamins to stimulate bacterial growth. Glucose is a source of carbon and energy. Chloramphenicol and gentamicin are inhibitors of a wide range of Gram-negative and Gram-positive bacteria.

Directions for media preparation
A) Oxytetracycline Glucose Yeast Extract Agar
Suspend 19 g in 500 mL of cold purified water and heat to boiling to dissolve completely Autoclave at 115ºC for 15 minutes and cool rapidly to approximately 47-50ºC. Reconstitute under aseptic conditions one vial of Oxytetracycline Antimicrobic Supplement (code 4240000) with 5 mL of sterile purified water, and add to the base medium. Final concentration of oxytetracycline HCl: 100 mg/L
B) Oxytetracycline Gentamicin Glucose Yeast Extract Agar
Prepare the medium as described above and add the contents of one vial of Gentamicin Antimicrobic Supplement (code 4240004), reconstituted with 5 mL of sterile purified water. Final concentrations: gentamicin 50 mg/L, oxytetracycline 100 mg/L

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, clear
Oxytetracycline Antimicrobic Supplement	short, dense, pale-yellow pellet; pale yellow limpid solution after reconstitution
Gentamicin Antimicrobic Supplement	short, dense, white pellet; colourless limpid solution after reconstitution
Final pH at 20-25 °C	6.6 ± 0.2

SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

For the enumeration of yeasts and moulds, the following scheme of culture media is recommended:
- OGYE Agar Base + oxytetracycline: general use on food with a low protein content and little contamination with Gram negative bacilli.
- OGYE Agar Base + oxytetracycline and gentamicin: foods with a high protein content in which yeasts and moulds are associated with Gram negative bacilli.

1. Prepare a series of suitable dilutions of the sample.
2. Transfer 1 mL of each sample dilution to empty sterile 9 cm Petri dish (2 plates per dilution).
3. Add approximately 15 mL of melted and cooled medium prepared as described above to each plate. Mix gently turning the plates.
4. Incubate between 22 °C and 25 °C for 5 days, but not longer.

The plates can also be inoculated using surface spreading technique.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Count the colonies in plates containing 50-100 colonies after 5 days or in any countable plates when aerial mycelia seem to obscure further readings after 2 days.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25 ± 1°C/ 5 days/A	growth
Aspergillus brasiliensis ATCC 16404	25 ± 1°C/ 5 days/A	growth
Escherichia coli ATCC 25922	25 ± 1°C/ 5 days/A	inhibited
Bacillus subtilis ATCC 6633	25 ± 1°C/ 5 days/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- OGYE Agar loses its bacteriostatic properties, especially towards *Bacillus* spp., when medium is heavy inoculated, when incubation is prolonged, or temperature is increased to 35-37°C, or when exposed to proteinaceous substrates.[6]
- Moulds do not grow on OGYE Agar when contamination is low.[6]
- Rapid growth of some moulds may mask colonies of slow growing organisms.[6]
- The medium is not suitable for cultivation of spores of *Byssochlamys* spp. It is recommended for this genus a chloramphenicol containing medium.[6]
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[7]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[7]
- Non-linearity of counts from dilution plating often occurs, i.e., 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[7]
- It is recommended to perform identification tests on isolates from pure cultures.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplements
Store the products in the original package at +2°C /+8°C away from direct light.
According to MacFaddin, OGYE Agar Base without antibiotics may be stored at 2-8°C in the dark for up to 6 months while the medium completed with antibiotics should be used immediately.

REFERENCES

1. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
2. Mossel DAA, Visser M, Mengerink WHJ. A comparison of media for the enumeration of moulds and yeasts in foods and beverages, Lab Pract 1962; 11:109.
3. Mossel DAA, Kleynen-Semmeling AM, Vincentie HM, Beerens H, Catsaras M. Oxytetracycline-glucose-yeast extract agar for selective enumeration of moulds and yeasts in foods and clinical material. J Appl Bacteriol 1970; 33:454-7.
4. Put HM The limitation of oxytetracycline as a selective agent in media for the enumeration of fungi in soil, feeds and foods in comparison with the selectivity obtained by globenicol (chloramphenicol). Arch. Lebensmittel Hyg. 1974; 25:73.
5. Mossel DAA, Vega CL, Put HM. Further studies on the suitability of various media containing antibacterial antibiotics for the enumeration of moulds in food and food environments J Appl Bacteriol 1975; 39:15-22.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.

PACKAGING

Product	Type	REF	Pack
OGYE Agar Base	Dehydrated medium	4013932	500 g (13.1 L)
Oxytetracycline Antimicrobic Supplement	Freeze dried supplement	4240000	10 vials, each for 500 mL of medium
Gentamicin Antimicrobic Supplement	Freeze dried supplement	4240004	10 vials, each for 500 mL of medium

IFU rev 3, 2022/11

OXACILLIN-SALT SCREEN AGAR

Ready-to-use plates

INTENDED USE

In vitro diagnostic. For the detection of *mec*A-mediated resistance to oxacillin of *Staphylococcus aureus* isolates.

COMPOSITION -TYPICAL FORMULA *

Beet extract	2.0 g
Acid digest of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Sodium chloride	40.0 g
Oxacillin	6.0 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important etiological agent of hospital and community acquired infections. Strains that are oxacillin and methicillin resistant, historically termed methicillin-resistant *S. aureus* (MRSA), are resistant to all ß-lactam agents, including cephalosporins and carbapenems, although they may be susceptible to the newest class of MRSA-active cephalosporins (e.g. ceftaroline).[1] MRSA is resistant to all β-lactams because of the presence of *mec*A, a non-native gene encoding for a novel penicillin-binding protein (PBP2a) with a significantly lower affinity for β-lactam antibiotics. This resistance allows cell-wall biosynthesis, the target of β-lactams, to continue even in the presence of typically inhibitory concentrations of antibiotic. Non *mec*A-mediated resistance (production of PBP protein by *mec*C gene, inactivation of the drug by increased production of β-lactamase, production of modified intrinsic PBPs with altered affinity for the drug) occurs only rarely in *S. aureus*.[2] A distinctive feature of methicillin/oxacillin resistance is its heterogeneous nature, with the level of resistance varying according to the culture conditions and the β-lactam antibiotics being used. All cells in a culture may carry the genetic information (*mec*A) for resistance, but only a small number may express the resistance *in vitro* (heteroresistance).[1,2]
In addition to broth microdilution testing, the Clinical and Laboratory Standards Institute (CLSI), recommends a plate containing 6 µg/ml of oxacillin in Mueller-Hinton agar supplemented with 4% NaCl or the cefoxitin disk diffusion test, as alternative methods of testing for MRSA.[3]
Oxacillin-Salt Screen Agar consists of Mueller Hinton Agar supplemented with sodium chloride for improving the growth of MRSA populations. Oxacillin is included as antistaphylococcal β-lactamase stable penicillin; its use is preferred because it is most resistant to degradation in storage and because it is more likely to detect heteroresistant staphylococcal strains.
Oxacillin-Salt Screen Agar is used for the detection of *mec*A-mediated resistance to oxacillin of *S. aureus* and the results applied to other β-lactams agents, ie, penicillins, β-lactam combination agents, cephems (with the exception of ceftaroline), and carbapenems.[3]

PHYSICAL CHARACTERISTICS

Prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Oxacillin-Salt Screen Agar is inoculated with pure cultures of clinical isolates presumptively identified as a *S. aureus*. It is not intended for the microbial isolation from clinical specimens.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
- Prepare the inoculum suspension by selecting colonies from overnight growth on a non-selective agar plate.
- Transfer the colonies to Trypic Soy Broth or saline solution to produce a suspension to obtain 0.5 McFarland turbidity.
- Using a 1-µL loop that was dipped in the suspension, spot an area 10 to 15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.
- Incubate for a full 24 hours at 33-35°C in aerobic conditions (MRSA may not be detected with incubation at temperature above 35°C)

READING AND INTERPRETATION

Examine the incubated plates carefully with transmitted light.
- Resistant strain (MRSA): presence of more than 1 colony or light film of growth.
- Susceptible strain: absence of colonies or presence of only1 colony.

MRS are resistant to all β-lactam agents with the exception of ceftaroline; other β-lactam agents should be reported as resistant or should not be reported.[3]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus	ATCC	29213	33-35°C / 24H / A	Susceptible: absence of growth
S. aureus	ATCC	43300	33-35°C / 24H / A	Resistant: presence of growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- When performed properly, the oxacillin agar screening method will detect most *mec*A-positive *S. aureus* strains. Occasionally a heteroresistant *mec*A-positive strain is not detected, in part due to a low frequency of resistance expression.[4,5]
- The medium may not detect borderline-resistant strains with non-*mec*A-mediated resistance.[2]
- Cells expressing heteroresistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35°C.[1]
- Occasionally, *S. aureus* isolates with borderline resistant MICs may fail to grow within 24 hours. It is recommended confirming any ambiguous results demonstrated on the screening plate, by a standard MIC test.
- Oxacillin-Salt Screen Agar cannot be used for detecting oxacillin resistance in coagulase negative staphylococci.[6]
- Together with Oxacillin-Salt Screen Agar, inoculate a blood agar plate to evaluate the viability and purity of the culture.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Centers for Disease Control. Methicillin-resistant Staphylococcus aureus (MRSA): Laboratory Testing. https://www.cdc.gov/mrsa/lab/index.html. Page last reviewed: February 6, 2019.
2. Limbago BM, Swenson JM. Special phenotypic methods for detecting antimicrobial resistance. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 30th ed. CLSI supplement M100-S30. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
4. Cavassini M, Wenger A, et al. Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination Kit, for rapid detection of Methicillin Resistance in Staphylococcus aureus. J Clin Microbiol 1999; 37:1591
5. Resende CA, Figueiredo AM. Discrimination of methicillin-resistant Staphylococcus aureus from borderline-resistant and susceptible isolates by different methods. J Med Microbiol 1997; 46:145
6. Tenover FC, Jones RN, Swenson JM, Zimmer B, McAllister S, Jorgensen JH. Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. J Clin Microbiol 1999; 37:4051

PACKAGING

Product	Type	REF	Pack
Oxacillin-Salt Screen Agar	Ready-to-use plates	549510	2 x 10 plates ø 90 mm

IFU rev 1, 2020/10

Oxacillin-Salt Screen Agar: growth of MRSA strain

PEPTONE (TRYPTONE) WATER

Dehydrated and ready-to-use medium

INTENDED USE

Liquid medium for indole production test.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Tryptone	10 g
Sodium chloride	5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Peptone (Tryptone) Water is prepared according to the formula described by ISO 7251[1] and is recommended for use in the detection of *Escherichia coli* in food and water samples based on indole production.
Bacteria that possess the enzyme tryptophanase are capable of hydrolysing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia.[2] The indole test is based on the formation of a red colour complex when indole reacts with aldehyde group of p-dimethylamino benzaldehyde of Kovacs' Reagent, under acidic conditions.
The indole test is used as part of the IMViC procedures, a battery of tests designed to distinguish among members of the family *Enterobacteriaceae*.[2] Indole production is an important characteristic in the identification of many microorganisms, being particularly useful in separating *E. coli* (positive) from members of the *Klebsiella-Enterobacter-Hafnia-Serratia* group (mostly negative).
The chief requirement for culturing an organism prior to perform the indole test is that the medium contains a sufficient quantity of tryptophan.[3]
The medium contains tryptone which is rich in tryptophan, carbohydrates free, and a source of nitrogen, carbon and minerals for microbial growth.
Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 15 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Dispense the medium in quantities of 5 mL to 10 mL into tubes and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

The specimens consist of cultures in selective broths or colonies grown on plated media.

TEST PROCEDURE

Test for detection and enumeration of presumptive *E. coli*.[1]

1. Inoculate tubes of Lauryl Pepto Bios Broth (REF 401580) at single and double strength.
2. For enumeration, follow the MPN scheme with 3 single-strength and 3 double-strength tubes inoculated with the sample suspension and its decimal dilutions. For detection method inoculate one tube with single strength medium or one tube with the double strength medium with the initial suspension, depending of the limit required.
3. Incubate at 37 °C ± 1 for 24 h ± 2 h. If, at this stage, neither gas production nor opacity preventing the observation of gas production is observed, incubate for up to 48 h ± 2 h.
4. From each of the incubated tubes with single strength and double-strength Lauryl Pepto Bios Broth showing opacity, cloudiness or any visible gas, inoculate with a sampling loop a tube of EC Broth (REF 401425).
5. Incubate the EC Broth tubes at 44 °C ± 1°C for 24 h ± 2 h. If, at this stage, there is no visible gas in the EC Broth, extend the incubation up to a total of 48 h ± 2 h.
6. For the confirmatory test of *E. coli* proceed as following:
7. After incubation, if visible gas is observed, inoculate a tube of Peptone (Tryptone) Water, preheated to 44 °C, using a sampling loop.
8. Incubate for 48 h ± 2 h at 44 °C.
9. Add 0.5 mL of Kovacs' Reagent (REF 19171000) to the incubated tubes of Peptone (Tryptone) Water.
10. Mix well and examine after 1 min. A red colour in the alcoholic phase indicates the presence of indole.
11. Consider as positive for *E. coli* each tube of double-strength or single-strength Lauryl Pepto Bios Broth that has given rise to any visible gas in the tube of EC Broth and to indole production in the tube of Peptone (Tryptone) Water.

Indole test using pure cultures

1. Inoculate Peptone (Tryptone) Water tubes using a light inoculum of an 18-24 h pure culture and incubate at 44°C or 37 °C for 24 h.
2. Add 0.5 mL of Kovacs' Reagent and shake gently.

READING AND INTERPRETATION

Indole positive result: formation of a pink to red colour within 1 minute (occurring normally within a few seconds)
Indole negative result: no colour change, the reagent layer remains yellow or slightly cloudy

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.
Positive control: *Escherichia coli* ATCC 25922
Negative control: *Enterobacter aerogenes* ATCC 13048

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Indole test is an aid in the differentiation of microorganisms. For complete identification further suitable tests are recommended.
- Change in colour of the Kovacs Reagent from yellow to brown indicates improper storage, which may cause weaker reactions.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the tubed medium prepared by the user can be stored at 2-8°C for 6 months.[3]

Ready-to-use medium in tubes

Store tubes in their original pack at 2-8°C away from direct light

REFERENCES

1. ISO 7251. Microbiology-General Guidance for enumeration of E. coli - Most Probable Number Technique. 1993-12-15
2. Maria P. MacWilliams. Indole Test Protocol. ASM, 08 December 2009, American Society for Microbiology © 2016.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Peptone (Tryptone) Water	Dehydrated culture medium	4018912 4018914	500 g (33.3 L) 5 kg (333 L)
Peptone (Tryptone) Water	Ready-to-use tubes	551891	20 x 9 mL

IFU rev 2, 2022/09

PEPTONE YEAST EXTRACT AGAR

Dehydrated culture medium

INTENDED USE

For the selective isolation of yeasts, moulds and dermatophytes.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Soy peptone	10.00 g
Yeast extract	5.00 g
Glucose	40.00 g
Streptomycin sulphate	0.03 g
Chloramphenicol	0.05 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Antibiotic-containing media are superior to acidified media and are widely used for the isolation of yeasts and moulds from clinical and non-clinical samples.

Peptone Yeast Extract Agar is a modification of the classical formula of Sabouraud dextrose agar, devised by Carmichael and Kraus primarily to selectively recover *Trichophyton verrucosum*, one of the species associated with ringworm.[1,2]

Peptone Yeast Extract Agar is useful for the isolation of yeasts and moulds and for the early detection of dermatophytes.

Soy peptone and yeast extract provide the nutrients for microbial growth. Glucose is a source of carbon and energy for enhancing dermatophytes growth. Chloramphenicol and streptomycin inhibit bacterial growth and assist isolation of dermatophytes and other fungi.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 70 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C mix well and pour into sterile Petri dishes. Avoid overheating.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellowish, limpid
Final pH at 20-25°C	6.6 ± 0.2

SPECIMENS

Non-clinical and clinical cutaneous specimens such as nails, hair, skin. Refer to appropriate references and standard procedures for collection, transport and storage of the specimens.[3-5]

TEST PROCEDURE

Allow plates to reach room temperature.

General procedure

1. Streak specimen onto the medium as to obtain isolated colonies.
2. Incubate aerobically at 25°C or 35°C, if necessary for up to 4 weeks.
3. Examine after 48 hours and intermittently thereafter.

Dermatophyte detection

1. Press cutaneous specimens by gently pressing lightly the samples onto the agar surface.
2. Incubate aerobically, at 25-30°C or at 30-37°C if *T. verrucosum* is suspected.
3. Examine microscopically after 48 and 72 hours to observe growth of microcolonies.
4. If microcolonies are observed, they must be transferred to new plates before overgrowth develops.
5. Re-incubate plates for up to 14 days and observe intermittently for growth.

The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

After incubation observe the bacterial growth and record the specific morphological and chromatic characteristics of the surface and reverse of the colonies. Dermatophytes develop in the form of fuzzy colonies of various colours depending on the species and may require a long incubation period.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
T. mentagrophytes ATCC 9533	25°C / up to 72 h / A	growth
C. albicans ATCC 10231	25°C / up to 72 h / A	growth
E. coli ATCC 25922	25°C / up to 72 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Care must be taken in handling culture plates since moulds can form spores which are easily released.
- When looking for dermatophytes in samples collected from certain body sites, *Candida* overgrowth can be a problem.
- Additional physiological or biochemical tests may be needed for complete identification of isolates.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Carmichael JW, Kraus HJ. Alberta Med Bull 1959; 24:201.
2. Carmichael JW. Mycopathologia 1961; 14:129.
3. Public Health England. Investigation of dermatological specimens for superficial mycoses. SMI B 39, Issue no: 3.1, 2016.
4. McGowan K. Specimen Collection, Transport and Processing: Mycology. In Jorgensen JH, Pfaller et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; Vol.2, 2015.
5. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

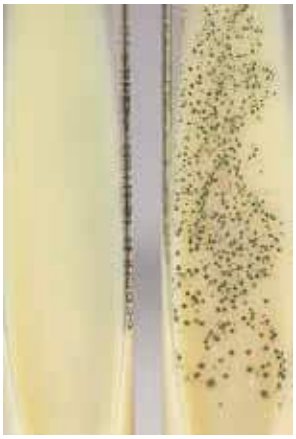
PACKAGING

Product	Type	REF	Pack
Peptone Yeast Extract Agar	Dehydrated medium	4018952	500 g (7.1 L)

IFU rev 1, 2023/02

PERGOLA MEDIUM

Ready-to-use tubes



Pergola Medium: un-inoculated tube and colonies of *Corynebacterium diphtheriae*

INTENDED USE

In vitro diagnostic device. For the cultivation and isolation of *Corynebacterium diphtheriae* from clinical specimens.

COMPOSITION -TYPICAL FORMULA *

Horse serum	600 mL
Saline solution	184 mL
Egg yolk	200 mL
Potassium tellurite 1% solution	16 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Pergola medium is one of the media that can be used for the cultivation and isolation of *C. diphtheriae* from clinical specimens, together with Loeffler's medium, Serum Tellurite Agar, Tinsdale agar and blood agar. Pergola Medium is coagulated by exposure at 80°C for 60 minutes in a slanted position. The medium contains horse serum and egg yolk to stimulate and support the growth of corynebacteria; potassium tellurite inhibits the growth of most normal Gram-negative bacteria of the upper respiratory tract[1]; it is reduced by corynebacteria and other microorganisms with the formation of grey or black colonies.

PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, opaque
Final pH at 20-25°C	Not applicable

SPECIMENS

Pergola Medium can be directly inoculated with clinical specimens; in case of respiratory diphtheria, material for culture should be obtained on a swab (either cotton or polyester tipped swab) from the inflamed area of nasopharynx; if membranes are present and can be removed, they should also be sent to the laboratory.[2] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information. Collect specimens before antimicrobial therapy where possible.

TEST PROCEDURE

Inoculate the sample directly onto the surface of the slope. Incubate in aerobic conditions at 35-37°C for 18-24 hours. If negative re-incubate for a total of 4 days before reporting as negative.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.

The typical colonies of *C. diphtheriae* are raised, shiny, of a light ashy colour, while the colonies of the generally associated cocci are of an intense black colour. Colonies of *C. diphtheriae* also tend to darken with aging.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. diphtheriae ATCC 11913	35- 37°C / 18-24H / A	growth with light ashy colonies

A: Aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Tellurite inhibits the growth of many non-coryneform bacteria but even a few *C. diphtheriae* strains are sensitive to potassium tellurite and therefore do not grow on Pergola Medium.[2]
- The growth on Pergola Medium and the reduction of tellurite are not specific for *C. diphtheriae* since many other coryneforms and other Gram-positive bacteria may also produce black colonies.[2]
- The medium is not inhibitory to Gram-positive bacteria: pseudodiphtheria, staphylococci, streptococci, micrococci, listeriae can grow with white-grey-black colonies. *Candida* grows with small greyish-white colonies.[3]
- It is advisable to inoculate, together with Pergola Medium, other plated or tubed media such as Blood Agar and Loeffler's Medium.[1-3]
- It is recommended that suitable identification and susceptibility tests be performed on isolates. For the detailed procedures consult the appropriate reference.[2]

STORAGE CONDITIONS

Store tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Bernard KA. Coryneform Gram-positive rods. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
3. Comitato Regionale per l'Ordinamento dei Servizi di Patologia (1977) Manuale di Tecniche Batteriologiche. Giunta Regionale della Lombardia Ass. Sanità.

PACKAGING

Product	Type	REF	Pack
Pergola Medium	Ready-to-use tubes	554002	20 glass tubes with slanted medium, 17x125 mm,

IFU rev 0, 2021/05

PHARMACOPOEIA DILUENT

(BUFFERED SODIUM CHLORIDE-PEPTONE SOLUTION)

EP NEUTRALISING DILUENT

Dehydrated and ready-to-use culture medium

INTENDED USE

Liquid medium for the test strains and samples suspension and dilution in the microbiological examination of non-sterile pharmaceutical products according to European Pharmacopoeia.

COMPOSITION *

PHARMACOPOEIA DILUENT, DEHYDRATED MEDIUM AND READY-TO-USE FLASKS
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Potassium dihydrogen phosphate	3.56 g
Disodium hydrogen phosphate anhydrous	5.76 g^
Sodium chloride	4.30 g
Tryptone	1.00 g

EP NEUTRALISING DILUENT, READY-TO-USE TUBES AND FLASKS
TYPICAL FORMULA

Potassium dihydrogen phosphate	3.56 g
Disodium hydrogen phosphate anhydrous	5.76 g^
Sodium chloride	4.30 g
Tryptone	1.00 g
Polysorbate (Tween) 80	30.00 g
Lecithin (egg)	3.00 g
Histidine HCl	1.00 g
Purified water	1000 mL

^ Equivalent to 7.2 g of disodium hydrogen phosphate dihydrate
*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Pharmacopoeia Diluent, also known as "buffered sodium chloride-peptone solution", is an isotonic diluent prepared according to the formulation reported by European Pharmacopoeia.[1]
It is recommended: a) to make the microbial suspension for growth promotion test, b) to dissolve and dilute the product to be tested, c) supplemented with specific compounds, for the removal of the antimicrobial activity of disinfectants.
EP Neutralising Diluent ready-to-use in flasks and tubes is supplemented with compounds for removing antimicrobial activity of disinfectants.
Pharmacopoeia Diluent and EP Neutralising Diluent maintain the viability of microorganisms during sample preparation without supporting growth.

Medium base contains a low concentration of peptone and sodium chloride that provides osmotic stability. Phosphates are used as buffering agents to control the pH in the medium.

Directions for medium preparation (Pharmacopoeia Diluent, dehydrated medium)

Suspend 14.6 g in 1000 mL of cold purified water. Heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes.
If required add the suitable neutralising compounds. A typical neutralising diluent has the following formulation:

Pharmacopoeia Diluent	1000 mL
Polysorbate 80	30 g
Lecithin (egg)	3 g
Histidine HCl	1 g

EP Neutralising Diluent in tubes and flasks

The medium is ready-to-use

Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Tubes and flasks appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.1

Specimens

Non-sterile pharmaceutical products and medical devices. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to European Pharmacopoeia.

Test procedure, reading and interpretation

Use Pharmacopoeia Diluent to make test strains suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2-8°C.
Dissolve o dilute (usually a 1:10 dilution is prepared) the sample in the medium. If necessary, prepare further dilutions in the same diluent.
For details on test methods for the examination of non-sterile pharmaceutical products, for information concerning inoculation and subculture procedure consult the current edition of European Pharmacopoeia and the instructions for use of relevant isolation and enumeration media.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of Pharmacopoeia Diluent.

Control strains	Incubation T°/ T / ATM	Expected results
S. aureus ATCC 6538	120 min at room temperature	± 30% original count (subculture in Tryptic Soy Agar)
E. coli ATCC 8739	120 min at room temperature	± 30% original count (subculture in Tryptic Soy Agar)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use medium in flasks and tubes
Store flasks and tubes in their original pack at +2°C/ +8°C away from direct light.

References

1. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631

Packaging

Product	Type	REF	Pack
Pharmacopoeia Diluent	Dehydrated medium	4013952	500 g (34.2 L)
Pharmacopoeia Diluent	Ready-to-use flasks	5113952	6 x 90 mL
EP Neutralising Diluent	Ready-to-use tubes	551395N	20 x 9 mL
EP Neutralising Diluent	Ready-to-use flasks	511395N2	6 x 90 mL

IFU rev 1, 2022/11

PHENOL RED BROTH BASE

Dehydrated culture medium

Phenol Red Broth w/Lactose: from left: uninoculated tube, *E. coli* (lac+), *S.* Enteritidis (lac-)

INTENDED USE

Basal medium to aid in differentiation between genera and species of bacteria by their ability to ferment (degrade) specific carbohydrates.

COMPOSITION - TYPICAL FORMULA*

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	10.000 g
Beef extract	3.000 g
Sodium chloride	5.000 g
Phenol red	0.018 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Phenol Red Broth Base, prepared according to a modification of the formula proposed by Vera[1] in 1950, is used for the differentiation between genera and species of bacteria, by their ability to ferment (degrade) specific carbohydrates incorporated in the basal medium [2]. Phenol Red Broth Base supplemented, in separate tubes, with 5 g/L of dulcitol, 10 g/L of sucrose and 10 g/L of lactose, is reported by FDA-BAM[3] among the tests for the identification of *Salmonella*.

The basal medium contains a peptone with a low carbohydrates content and beef extract which are sources of nitrogen, carbon and minerals for bacterial growth; sodium chloride maintains the osmotic balance; phenol red is a pH indicator: when Phenol Red Broth is prepared with a final concentration of 0.5-1 % carbohydrate, most of the end products of its fermentation are organic acids, which produce a colour change of the pH indicator from red to yellow; if gas is produced during the fermentation reaction, it is collected in the inverted Durham tube. If the test is negative, a catabolic attack of peptones will occur with the formation of ammonia, the alkalinisation of the medium and a colour change of phenol red from red-orange to reddish-pink.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 18 g in 1000 mL of cold purified water and add the chosen carbohydrate to the final concentration of 0.5-1% (5-10 g/L). Heat if necessary to dissolve the medium completely, dispense 3-5 mL in suitable tubes and insert Durham tubes when gas production must be recorded. Sterilize by autoclaving at 118°C for 15 minutes. Alternatively, to the autoclaved and cooled base, add a filter sterilized solution of carbohydrate so to obtain a final concentration of 0.5-1%.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	red-orange, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Phenol Red Broth Base is not intended for primary isolation from clinical specimens; it is inoculated with 18-24 h pure culture from solid media such as Tryptic Soy Agar or blood agar, isolated from clinical specimens or other materials.

TEST PROCEDURE

With a heavy inoculum, inoculate tubes of Phenol Red Carbohydrate Broth with pure culture using an inoculating loop or swab. Inoculate a carbohydrate-free test tube too.

Incubate tubes with loosened caps, aerobically or anaerobically depending on suspected microorganism(s) at 35-37°C for 18-48 hours.

Prolonged incubation may be required, up to 30 days to be considered a negative result.[2]

READING AND INTERPRETATION

After incubation observe the presence of growth (turbidity) and the colour change of the medium.

Positive reaction (carbohydrate degradation): the medium turns yellow and the formation of gas bubbles can be observed.

Negative reaction: the medium is turbid reddish-pink in colour.

No yellow colour should occur in the control tube.

After a positive reaction has been observed, discard the tube; by prolonging the incubation, an inversion of the reaction may be observed.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	GLUCOSE
E. coli ATCC 25922	35-37° / 18-24H /AE	AG
S. Typhimurium ATCC 14028	35-37° / 18-24H /AE	AG
P. aeruginosa ATCC 14207	35-37° / 18-24H /AE	K

AE: aerobic incubation; ATCC is a trademark of American Type Culture Collection; A: acid production, yellow colour; G: gas production; K: alkalinity, reddish-pink

LIMITATIONS OF THE METHOD

- "Carbohydrate" are collectively termed "sugars". However, the test can be performed with "real sugars" whose names end with "ose" (lactose, glucose, sucrose etc.) or with alcohols, whose names end with "ol" (dulcitol, mannitol). A few exceptions exist such as the sugar salicin (glycoside).

- The concentration of carbohydrates incorporated into the medium base is usually 1% (exception salicin and dulcitol: 0.5%). 1% concentration reduces the possibility of alkaline re-inversion of positive reactions.[2]
- Certain carbohydrates can withstand autoclaving at 116-118°C for 15 minutes with little or no breakdown. Autoclaving is not advisable for the following: arabinoses, lactose, maltose, salicin, sucrose, trehalose, xylose.[2]
- The medium after autoclaving, when hot, appears a light orange colour; this will change to an orangish-red colour after cooling.
- The addition of certain carbohydrates to the medium can cause a decrease in pH. If this occur add 0.1N NaOH drop by drop until the desired pH.
- The inverted Durham tube, in the examination of *Enterobacteriaceae*, is necessary only for the medium incorporating glucose: if the strain produces gas from glucose, it will also produce gas from all other degraded carbohydrates.[2]
- Even the observation of a single bubble makes the test positive for the production of gas (CO_2 and H_2) by the aerogenic strains.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks.[2]

References

1. Vera HD. Relation of peptones and other culture media ingredients to accuracy of fermentation tests. Am J Public Health 1950; 40:1267-1272.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019

Packaging
Product	Type	REF	Pack
Phenol Red Broth Base	Dehydrated medium	4019102	500 g (27,8 L)

IFU rev 2, 2022/05

PHENYLALANINE AGAR
Dehydrated culture medium

Intended use
In vitro diagnostic. For the differentiation of *Proteus, Morganella* and *Providencia* from other members of the *Enterobacteriaceae* by the ability to deaminate phenylalanine.

Composition - typical formula* (after reconstitution with 1 L of water)
Yeast extract	3 g
DL phenylalanine	2 g
Disodium hydrogen phosphate	1 g
Sodium chloride	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
Phenylalanine Agar is a differential medium developed by Buttieux[1] and subsequently modified by Ewing, Davis, and Reavis[2]. It is intended for the differentiation of the genera *Proteus, Providencia* and *Morganella* from the other members of *Enterobacteriaceae* by the ability to oxidatively deaminate phenylalanine to phenyl pyruvic acid and ammonia (NH_3) by the enzymatic activity of phenylalanine deaminase. The phenylpyruvic acid is detected by adding a few drops of 10% ferric chloride which acts as a chelating agent; a green coloured complex is formed between these two compounds indicating a positive test. If the medium remains a pale yellow, the organism is negative for phenylalanine deaminase production. Yeast extract is a source of carbon, nitrogen, vitamins for bacterial growth, sodium phosphate acts as a buffer system, sodium chloride maintains the osmotic balance of the medium, phenylalanine serves as the substrate for the enzyme.

Directions for medium preparation
Suspend 26 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute in tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool in slanted position to obtain a long slant and a short butt.

Physical characteristics
Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, limpid
Final pH at 20-25 °C	7.2 ± 0.2

Specimens
Phenylalanine Agar is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

Test procedure
Using a loopful of inoculum from an 18-24 hours pure culture, heavily streak the slant surface using a fishtail motion.
Incubate aerobically at 35-37°C for 18-24 hours. If tube is heavy inoculates, a 4-hour incubation period should be sufficient.

Reading and interpretation
After incubation, add 4-5 drops of ferric chloride reagent to the growth on the slant and gently rotate the tube.
A positive test (deamination of phenylalanine) is indicated by the development of a light green to bright green colour on the slant and on the reagent, within 1-5 minutes after applying the ferric chloride reagent.
A negative test is indicated by the absence of green colour: the reagent remains yellow.
Proteus spp., *Providencia* spp. and *Morganella* spp. are positive for the phenylalanine deamination test while the other *Enterobacteriaceae* are negative.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. mirabilis ATCC 25933	35-37°C / 18-24H / A	development of a light green to bright green colour after $FeCl_3$ reagent addition
E. coli ATCC 25922	35-37°C / 18-24H / A	the reagent remains yellow

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- A positive phenylalanine reaction must be interpreted within 5 minutes upon addition of reagent as the green colour fades quickly.[3]
- Rolling the reagent over the slant aids in obtaining a faster reaction and a more intense colour.[3]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months.[3]

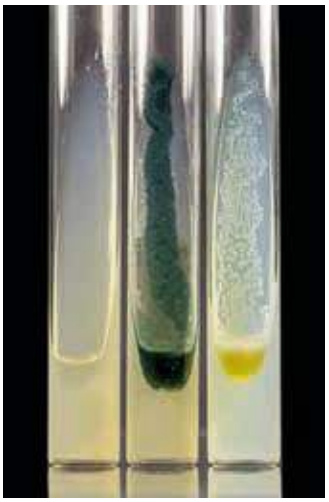
REFERENCES

1. Buttiaux R, Osteux R, Fresnoy R, Moriamez J. Les propriétés biochimiques charachéristiques di genre Proteus: inclusion souhaitable des Providencia dans celui-ca. Ann Inst Pasteur Lille 1954; 87:375.
2. Ewing WH, Davis BR Reavis RW. Phenylalanine and malonate media and their use in enteric bacteriology. Publ Health Lab 1975; 15:153.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Phenylalanine Agar	Dehydrated medium	4019162	500 g (19.2 L)

IFU rev 2, 2022/03



Phenylalanine Agar: from left: uninoculated tube,
P. mirabilis +, *E coli* -

PLATE COUNT AGAR: see TYRIPTIC GLUCOSE YEAST AGAR

PLATE COUNT AGAR WITH SKIM MILK

Dehydrated culture medium

INTENDED USE
For microbial plate count in milk and dairy products.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Skim milk	1.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Plate Count Agar supplemented with skim milk is recommended by ISO Standards[1-3] for the enumeration of mesophilic or psychrotrophic microorganisms in milk and dairy products.
This test is based on the assumption that each viable cell, pair of cells, or small cluster of cells will form a visible colony, named a colony-forming unit (CFU), when mixed with the growth medium.[4]
Enumeration of microorganisms requires diluting samples to achieve a population that is countable by the chosen method.
Several techniques have been described and are available for aerobic plate counts: pour plate technique, surface spread plate method, membrane filtration, spiral plate method, calibrated loop method, drop plate method.[4] The choice of the most appropriate method must take into account the requirements of the regulatory authorities, the type of sample to be analysed, the expected microorganisms and level of contamination.
The International Standard ISO 4833-1 specifies a pour plate method for the enumeration of mesophilic organisms and is applicable to products that require a reliable count when a low limit of detection is specified or to products expected to contains spreading colonies.[1]
ISO 4833-2 specifies a surface plating technique applicable to products containing heat sensitive organisms or obligately aerobic bacteria.[2]
ISO 17410 describes a surface plating method for the enumeration of psychrotrophic microorganisms with incubation at 6.5°C.[3]
The formulation of Plate Count Agar with Skim Milk complies with ISO Standards.[1-3] Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose is a source of carbon and energy. The skimmed milk included in the formulation is tested free of antibiotics.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 24.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale beige, clear or slightly opalescent
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS
Milk and dairy products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards.[1-3]

TEST PROCEDURE
Colony count by the pour plate technique.[1]
1. Using a sterile pipette, dispense 1 mL of the liquid test sample, or 1 mL of an initial suspension in the case of other products, into an empty Petri dish and mix with the molten medium pre-cooled to 44-46°C.
2. Prepare the other plates under the same conditions using decimal dilutions of the test sample or of the initial suspension.
3. Incubate the plates under aerobic conditions at 30 °C for 72 h.

Colony count by the surface plating technique.[2,3]
1. Dry the prepared plates before the use.
2. Using a sterile pipette, transfer 0.1 mL of the test sample, if the product is liquid, or of the initial suspension in the case of other products, to the centre of a Plate Count Agar with Skim Milk plate.
3. Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
4. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.
5. Incubate the plates under aerobic conditions at 30 °C for 72 h for the enumeration of mesophilic organisms or at 6.5°C for 10 days for the enumeration of psychrotrophic microorganisms.

Consult the appropriate International Standard for the details of the procedures.[1-3]

READING AND INTERPRETATION
After incubation, count all colonies obtained in the plates containing fewer than 300 colonies and calculate the number of microorganisms per gram or per millilitre of the test sample.
Follow recommended procedures for the counting of colonies and the reporting of results.[1-3]

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 8739	30°C/72H-A	good growth
S. aureus ATCC 6538	30°C/72H-A	good growth
B. subtilis ATCC 6633	30°C/72H-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.[4,5]
- A potential source of error in plate count can result from the stack-pouring Petri dishes: in a stack of 3 plates, the middle and the top plates took too longer to cool, thereby resulting in lower counts. [4,6]
- Increasing the holding time of the dilutions in the diluent leads to higher count. [4,7]
- The Aerobic Plate Count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.[4]

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO Standards, self-prepared flasks can be stored at +2 °C to +8 °C for up to 3 months and the self-prepared plates can be stored at +2 °C to +8 °C for up to 4 weeks.[1,3]

REFERENCES

1. ISO 4833-1:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 °C by the pour plate technique.
2. ISO 4833-2:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 °C by the surface plating technique.
3. ISO 17410:2019. Microbiology of the food chain — Horizontal method for the enumeration of psychrotrophic microorganisms
4. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
5. Berry JM, McNeill DA, Witter LD. Effect of delay in pour plating on bacterial counts. J Dairy Sci 1969; 52:1456-1457
6. Koburger JA. Stack pouring of Petri plates: a potential source of error. J Food Prot. 1980; 43:561-562.
7. Huhtanen CN Brazis AR, Arledge WL et al. Effects of time of holding dilutions on counts of bacteria from raw milk. J Milk Food Technol. 1972; 35:126-130.

PACKAGING

Product	Type	REF	Pack
Plate Count Agar with Skim Milk	Dehydrated medium	4019182	500 g (20.4 L)

IFU rev 1, 2022/11

POLYMYXIN BASE AGAR A 9, POLYMYXIN SEED AGAR A 10: see ANTIBIOTIC MEDIA

POTATO DEXTROSE AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

General purpose medium for the isolation, cultivation and enumeration of yeasts and moulds.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Potato extract	5.0 g
Glucose	20.0 g
Agar	17.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Potato Dextrose Agar (PDA) is a general-purpose medium for the isolation, cultivation and enumeration of yeasts and moulds. It meets harmonized EP, USP, JP performance specifications[1], where applicable, and corresponds to FDA-BAM medium 127.[2]
PDA is recommended by FDA-BAM[3] for the purification of the colonies enumerated with DG18 agar or DMRC and with the addition of chlortetracycline for the enumeration of yeasts and moulds in cosmetics.[4]
APHA recommends the use of PDA for the detection and enumeration of heat-resistant moulds in foods, because they are not-fastidious in their nutrient requirements and because they will easily form fruiting bodies which enables quick phenotype-based identification.[5]
PDA and PDA with 50 mg/L of chloramphenicol are recommended by ISO 18416[6] and ISO 16212[7] as alternative media to Sabouraud Dextrose Agar with and without chloramphenicol for suitability test and for the detection of *C. albicans* and enumeration of yeasts and moulds in cosmetics.
Potato extract encourages luxuriant fungal growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria. Glucose, at high concentration, is a carbon and energy source.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 42 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and pour into sterile Petri dishes. Medium should not be re-melted more than once.
For cosmetics, cool medium to 47-50°C after autoclaving and add 4 mL of filter sterilised 1% chlortetracycline HCl solution (1 g/100 mL) per litre of medium or add 4 mL of the contents of one vial of Dermatophyte Antimicrobic Supplement (REF 4240024) reconstituted with 5 mL of sterile purified water (final concentration in the medium: 40 mg/L). Alternatively add the contents of one vial of Chloramphenicol Antimicrobic Supplement (REF 424003) to 1 litre of medium before autoclaving (final concentration in the medium: 50 mg/L).

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	very pale yellow, opalescent
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Foods and cosmetics. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[1-6]

TEST PROCEDURE, READING AND INTERPRETATION

Enumeration of yeasts and moulds in cosmetics[4]

1. Use spread plate technique to facilitate recognition of different colony types. Decimally dilute the cosmetic preparation to obtain a complete dilution series from 10^{-1} to 10^{-3}. Mix the dilutions thoroughly and perform all plating in duplicate.
2. Aseptically pipet 0.1 mL of each dilution on pre-poured, solidified Potato Dextrose Agar plates supplemented with 40 mg/L of chlortetracycline and spread inoculum with a sterile, bent glass rod.
3. After the inoculum is absorbed by the medium, incubate the plates at 30 ± 2°C (Do not invert and do not stack more than 3 plates high).
4. Count colonies after 5 days of incubation. If there is no growth after 5 days, re-incubate for another 48 h.

Enumeration of heat-resistant moulds[5]

1. Heat the homogenized sample 30 minutes at 75°C-80°C and cool rapidly to 55°C.
2. Mix thoroughly the cooled suspension with an equal volume of warm (45°C) double strength Potato Dextrose Agar and dispense in 150 mm diameter Petri dishes.
3. Loosely seal the Petri dishes and incubate at 30°C for at least 14 days. Colonies formed by most activated ascospores will be visible in 7-10 days while heat injured or debilitated ascospores require additional time to form colonies.

For other applications, the user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	25°C / 72 h / A	good growth, white yeast-like colonies
A. brasiliensis ATCC 9642	25°C / 72 h / A	good growth, colonies with black hyphae

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Potato Dextrose Agar has poor selective properties; where bacterial overgrowth may be a problem, chloramphenicol (50 mg/L) or chlortetracycline (40 mg/L) are recommended.
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[8]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[8]
- Non-linearity of counts from dilution plating often occurs, i.e., 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[8]
- For complete identification of isolated microorganisms, it is recommended to perform appropriate tests using pure cultures.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the plated medium prepared by the user can be stored at 2-8°C for 6-8 weeks protected from dessication.[9]

Ready-to-use plates

Store plates in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
2. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Media M127: Potato Dextrose Agar. Content current as of: 10/17/2017.
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Chapter 18: Yeasts, Molds and Mycotoxins. Content current as of: 11/07/2022.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Chapter 23: Methods for Cosmetics. Content current as of: 12/23/2021.
5. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
6. ISO18416:2015. Cosmetics — Microbiology —Detection of Candida albicans.
7. ISO 16212:2017 Cosmetics — Microbiology — Enumeration of yeast and mould.
8. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Potato Dextrose Agar	Dehydrated medium	4019352 4019354	500 g (11.9 L) 5 kg (119 L)
Potato Dextrose Agar	Ready-to-use plates	541935D	2 x 10 plates ø 90 mm. Non-vented dishes.

IFU rev 2, 2022/11

PRESTON BROTH: see NUTRIENT BROTH N° 2

PSEUDOMONAS AGAR BASE
CN PSEUDOMONAS SUPPLEMENT
CFC PSEUDOMONAS SUPPLEMENT
PP PSEUDOMONAS SUPPLEMENT
PSEUDOMONAS CN SELECTIVE AGAR
PSEUDOMONAS CFC AGAR

Dehydrated and ready-to-use culture medium, selective supplement

INTENDED USE
For the isolation and enumeration of *Pseudomonas* spp. in waters, foodstuffs and environmental samples.

COMPOSITIONS*

PSEUDOMONAS AGAR BASE – DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of gelatin	16.0 g
Acid digest of casein	10.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g
Agar	11.5 g

PSEUDOMONAS CN SELECTIVE AGAR
TYPICAL FORMULA (READY-TO-USE PLATES 55 AND 90 MM)

Pseudomonas Agar Base	49 g
Cetrimide	200 mg
Nalidixic Acid	15 mg
Glycerol	10 mL
Purified water	1000 ml

PSEUDOMONAS CFC AGAR
TYPICAL FORMULA (READY-TO-USE PLATES 90 MM)

Pseudomonas Agar Base	49 g
Cetrimide	10 mg
Fusidic acid	10 mg
Cephalosporin	50 mg
Purified water	1000 ml

CN PSEUDOMONAS SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Cetrimide	100.0 mg
Nalidixic acid	7.5 mg

CFC PSEUDOMONAS SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Cetrimide	5 mg
Fusidic acid	5 mg
Cephalosporin	25 mg

PP PSEUDOMONAS SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Penicillin G	50,000 UI
Pimaricin	5 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Pseudomonas aeruginosa is an opportunistic environmental pathogen characterized by a high degree of adaptability, capable of growing in waters with very low nutrients concentrations and surviving in disinfected water.[1] *Pseudomonas* spp. are among the most frequently reported psychotropic bacteria in raw milk and are the most common cause of the meat spoilage at refrigeration temperature.[2]
Pseudomonas Agar Base is a modification of King's A Medium[3] in which the concentration of peptones is significantly increased and magnesium chloride and potassium sulphate are present to enhance pigments production.
The use of cetrimide at 10 mg/L and nalidixic acid at 15 mg/L, (CN Pseudomonas Supplement), was described by Goto and Enomoto[4] after the studies of Lowbury and Collins[5] on the selective properties of cetrimide against a broad range of Gram-positive and some Gram-negative organisms other than *P. aeruginosa*. The combination of cetrimide and nalidixic acid strongly suppresses the growth of *Klebsiella, Proteus* and *Providencia* spp.
CFC Supplement is based on the formulation devised by Mead and Adams[6] who demonstrated that the reduction of cetrimide to 10 mg/L improved the recovery of pigmented and non- pigmented psychrophilic pseudomonads associated with poultry meat spoilage. To enhance the medium selectivity, they added a cephalosporin and fusidic acid.
PP Supplement is based on the formulation included in ISO 11059[7] and contains penicillin as inhibitory agent for Gram positive bacteria and the antifungal compound pimaricin (natamycin).
Pseudomonas Agar Base supplemented with glycerol and CN Supplement corresponds to the medium recommended by ISO 16266[8] for the isolation and enumeration *of P. aeruginosa* in water samples.
The medium base supplemented with CFC Supplement corresponds to the medium recommended by ISO 13720[7] for the isolation and enumeration of *Pseudomonas* spp. in meat products.
The medium base supplemented with PP Supplement corresponds to the medium recommended by ISO/TS 11059[9] for the isolation and enumeration of *Pseudomonas* spp. in milk and milk products.

DIRECTIONS FOR MEDIA PREPARATION
Suspend 24.5 g in 500 mL of cold purified water and add 5 mL of g Glycerol (REF 421025) for the preparation of CN Pseudomonas Agar (ISO 16266); omit the glycerol for the preparation of CFC and PP Media. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to approximately 45-50°C and add the suitable selective supplement as follows:
CN Supplement (ISO 16266) Add the contents of one vial of CN Pseudomonas Supplement (REF 4240046) reconstituted with 2 mL of sterile purified water/ethanol (1:1) to 500 mL of pre-cooled Pseudomonas Agar Base. Mix well and pour into sterile Petri dishes.
CFC Supplement (ISO 13720) Add the contents of one vial of CFC Pseudomonas Supplement (REF 4240075) reconstituted with 2 mL of sterile purified water/ethanol (1:1) to 500 mL of pre-cooled Pseudomonas Agar Base. Mix well and pour into sterile Petri dishes.
PP Supplement (ISO/TS 11059) Add the contents of one vial of PP Pseudomonas Supplement (REF 4240048) reconstituted with 2 mL of sterile purified water to 500 mL of pre-cooled Pseudomonas Agar Base. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS
Pseudomonas Agar Base

Dehydrated medium appearance	white, fine, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid or slightly opalescent

CN Supplement	
Freeze-dried supplement appearance	high, white pellet
Reconstituted supplement appearance	colourless, limpid
CFC Supplement	
Freeze-dried supplement appearance	high, white pellet
Reconstituted supplement appearance	colourless to pale yellow, limpid
PP Supplement	
Freeze-dried supplement appearance	high, white pellet
Reconstituted supplement appearance	whitish, turbid
CN medium: final pH at 20-25 °C (ISO 16266)	7.1 ± 0.2
CFC and PP media: final pH at 20-25 °C (ISO 13720/ISO/TS 11059)	7.2 ± 0.2

SPECIMENS

Water samples, meat and meat products, milk and milk products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[7-9]

TEST PROCEDURE - READING AND INTERPRETATION

Waters (ISO 16266)[8]

1. Filter through a membrane filter of 0.45 µm the appropriate volume of water (bottled water or a spring water: 250 mL, other waters, including pool waters and water for human consumption: 100 mL).
2. Place each membrane on a plate prepared with CN Supplement, ensuring no air is trapped beneath the membrane.
3. Incubate at 36 ± 2 °C for 44 ± 4 h. Examine the membranes for growth after 22 ± 2 h and 44 ± 4 h.
4. Count all colonies that produce green/blue (pyocyanin) colour as confirmed *P. aeruginosa*.
5. Examine the membrane under UV radiation. Count all non-pyocyanin producing colonies and reddish-brown pigmented colonies that fluoresce as presumptive *P. aeruginosa* and confirm their identity using the oxidase test (Oxidase Test Strip, REF 191040ST), acetamide broth (Acetamide Broth, REF 5510101), and King's B medium (Pseudomonas Agar F, REF 401961).

Meat products (ISO 13720)[9]

1. Transfer on the surface of one plate prepared with CFC Supplement 0.1 mL of the initial suspension. Repeat this operation with subsequent dilutions taking two other CFC agar plates, using a new sterile pipette for each decimal dilution (if only one dilution is performed two plates shall be used).
2. Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dray.
3. Incubate the dishes at 25 °C ± 1 °C for 44 h ± 4 h. Count the colonies on the plates containing less than 150 colonies and select random five colonies from each retained plate for confirmation tests.
4. Confirm the presence of *Pseudomonas* with oxidase test. Colonies showing a positive oxidase reaction shall be considered as *Pseudomonas* colonies.

Milk and milk products (ISO/TS 11059)[7]

1. Transfer on the surface of the plate prepared with PP Supplement 0.1 mL of the initial suspension. Repeat this operation with subsequent dilutions taking other PPA agar plates, using a new sterile pipette for each decimal dilution (if only one dilution is performed two plates shall be used).
2. Spread the liquid over the surface of the agar with a sterile spreader until the surface is completely dray.
3. Incubate the dishes at 25 °C ± 1 °C for 48 h ± 2 h. Count the colonies on the plates containing less than 150 colonies and select random five colonies from each retained plate for confirmation tests.
4. Confirm the presence of *Pseudomonas* with oxidase test and fermentation of glucose on Purple Glucose Agar (REF 401970).
5. Colonies showing a positive oxidase reaction and absence of glucose fermentation shall be considered as *Pseudomonas* colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
Pseudomonas Agar Base + CN Supplement			
P. aeruginosa	ATCC 10145	36°C / 40-48 H / A	yellow-green, fluorescent colonies
E. coli	ATCC 25922	36°C / 40-48 H / A	inhibited
Pseudomonas Agar Base + CFC Supplement			
P. fluorescens	ATCC 13525	25°C / 40-48 H / A	green-blue, fluorescent colonies
E. coli	ATCC 25922	25°C / 40-48 H / A	inhibited
Pseudomonas Agar Base + PP Supplement			
P. aeruginosa	ATCC 27853	25°C / 40-48 H / A	yellow-green, fluorescent colonies
P. fluorescens	ATCC 13525	25°C / 40-48 H / A	green-blue, fluorescent colonies
E. coli	ATCC 25922	25°C / 40-48 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Where large numbers of presumptive *P. aeruginosa* are isolated, the spreading nature of colonies can hinder precise quantitative assessment.[8]
- The pigment pyocyanin (blue-green) is produced by more than 90 % of *P. aeruginosa* strains.[8]
- The identification of *Pseudomonas* spp. or *P. aeruginosa* should be confirmed by suitable tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +10 °C / +30 °C away from direct light in a dry place.

Selective supplements

Store the product in the original package at +2 °C/ + 8°C away from direct light.

According to ISO 16266 the self-prepared CN agar plates can be kept in the dark protected from desiccation for not more than 4 weeks at 5 °C ± 3 °C. Do not keep the agar molten for more than 4 hours. [8]

According to ISO 13720 the self-prepared CFC agar plates can be kept in the dark protected from desiccation for not more than 4 weeks at 5 °C ± 3 °C.[9]

According to ISO 11059, the self-prepared PP agar plates can be kept in the dark at 5 °C ± 3 °C for no longer than 1 day.[7]

Ready-to-use plates
Store plates in their original pack at +2 °C/ + 8°C away from direct light

References

1. Briancesco R, Paduano S, Semproni M, Vitanza L, Bonadonna L. Behavior of Pseudomonas aeruginosa and Enterobacter aerogenes in Water from Filter Jugs. Int. J. Environ. Res. Public Health 2020; 17: 8263.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015
3. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 1954; 44:301-7.
4. Goto S. Enomoto S. Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of Pseudomonas aeruginosa. Jpn J Microbiol 1970; 14: 65-72
5. Lowbury EJ, Collins AG. The use of a new cetrimide product in a selective medium for Pseudomonas pyocyanea. J Clin Pathol 1955; 8:47-8.
6. Mead GC, Adams BW. A selective medium for the rapid isolation of pseudomonads associated with poultry meat spoilage. Br Poult Sci 1977; 18: 661-70.
7. ISO/TS 11059:2009 Milk and milk products — Method for the enumeration of Pseudomonas spp.
8. ISO 16266:2006 Water quality – Detection and enumeration of Pseudomonas aeruginosa by membrane filtration.
9. ISO 13720:2010 Meat and meat products — Enumeration of presumptive Pseudomonas spp

Packaging

Product	Type	REF	Pack
Pseudomonas Agar Base	Dehydrated medium	4019602	500 g (10.2 L)
CN Pseudomonas Supplement	Freeze-dried supplement	4240046	10 vials, each for 500 mL of medium
CFC Pseudomonas Supplement	Freeze-dried supplement	4240075	10 vials, each for 500 mL of medium
PP Pseudomonas Supplement	Freeze-dried supplement	4240048	10 vials, each for 500 mL of medium
Pseudomonas CN Selective Agar	Ready-to-use plates	541960	2 x 10 plates ø 90 mm
Pseudomonas CN Selective Agar	Ready-to-use plates	491960	3 x 10 plates ø 55 mm
Pseudomonas CFC Agar	Ready-to-use plates	541959	2 x 10 plates ø 90 mm

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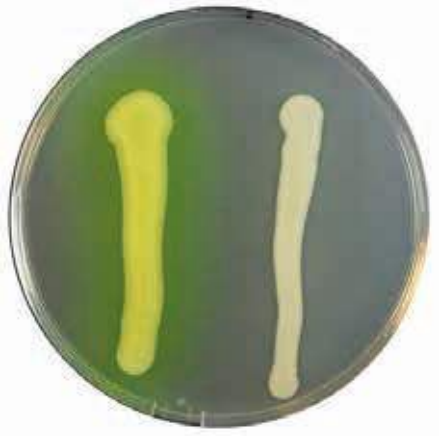
Pseudomonas CN Selective Agar: colonies of *P. aeruginosa* on a membrane filter

PSEUDOMONAS AGAR F

Dehydrated and ready-to-use culture medium



From left: *P. aeruginosa* and *B. cepacea* on Pseudomonas Agar F

Intended use

Pseudomonas Agar F (King Medium B) is intended for the differentiation of *Pseudomonas aeruginosa* by the ability to produce fluorescein.

Composition *

Pseudomonas Agar F, dehydrated medium
typical formula (after reconstitution with 1 L of water)

Tryptone	10.00 g
Peptone	10.00 g
Dipotassium hydrogen phosphate	1.50 g
Magnesium sulphate	1.50 g
Agar	15.00 g

Pseudomonas Agar F, ready-to-use plates
Typical formula

Pseudomonas Agar F	38 g
Glycerol	10 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Pseudomonas spp. are aerobic, non-spore-forming, Gram-negative rods that are straight or slightly curved, 0.5 to 1.0 by 1.5 to 5.0 µm; they have a very strict aerobic respiratory metabolism with oxygen but in some cases, nitrate may be used as an alternative that allows anaerobic growth.[1] They are usually motile with one or several polar flagella. *Pseudomonas* spp. are catalase positive and most species of clinical interest are oxidase positive (except *P. luteola* and *P. oryzihabitans*).[1]
P. aeruginosa is widely distributed in superficial water, waste and marine waters, on the soil, on vegetation and in all moist environments; moreover, it is able to grow in purified water, to survive in disinfectants, in cosmetics and to contaminate food. *P. aeruginosa* is considered an opportunistic

pathogen especially in immunocompromised patients and is characterized by multi-resistance to antibiotics, thus representing a health risk in hospital environments. *P. aeruginosa* can cause ventilator-associated pneumonia, urinary tract infections, burns and wound infections, corneal ulcers and keratitis, septicaemia, gastroenteritis in new-borns, abscesses, and meningitis.[2]
Other characteristics that may be associated with *P. aeruginosa* species (with some exceptions) include secretion of pyoverdine (fluorescein), a fluorescent yellow-green siderophore under iron-limiting conditions.[2] Additional types of siderophores, such as pyocyanin (blue) pyorubine (red) or pyomelanin (brown) may also be produced by *P. aeruginosa* and thioquinolobactin by *P. fluorescens*.[3]
King, Ward, and Raney[4] in 1954 described two media for pigment detection in *P. aeruginosa*: medium A enhancing the production of pyocyanin and medium B enhancing the production of fluorescein.
Pseudomonas Agar F, also known as King's Medium B or Flo Agar, is a modification of the formula described by King, Ward and Raney[4], it conforms to the formulation recommended by ISO16266[5] and by FDA-BAM[6] and is used for the fluorescein production test for the differentiation of *P. aeruginosa*.
Potassium phosphate has a stimulatory effect on fluorescein production and an inhibitory effect on pyocyanin; meat and casein peptones in equal quantities contribute to the optimal production of fluorescein, activated by the presence of Mg cations of magnesium sulphate; glycerol, added to the base medium, is a source of carbon for the growth and production of the pigment.[7]
A fluorescein-producing *Pseudomonas* will grow with yellow-green colonies, fluorescent under Wood's lamp.
Pseudomonas Agar F, combined with Pseudomonas Agar P, allows to perform the conventional phenotypic test for the differentiation of *P. aeruginosa* from other species of the genus *Pseudomonas*, isolated from clinical specimens.[8]
ISO 16266[5] Standard recommends the fluorescein production on Pseudomonas Agar F (+) as a confirmation test of *P. aeruginosa* colonies isolated from water, together with the oxidase test (+) and the ability to produce ammonia in Acetamide Broth (+).
FDA-BAM[6] recommends the pyocyanin and fluorescein production tests on Pseudomonas Agar P (+) and Pseudomonas Agar F (+) for the confirmation of *P. aeruginosa* colonies isolated from cosmetics, together with arginine dehydrolase (+), citrate and malonate utilisation (+), nitrate reduction (+), motility (+) and growth at 42°C (+).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 38 g in 1000 mL of cold purified water and add 10 mL of glycerol (REF 421015). Heat to boiling stirring constantly and sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes. Pseudomonas Agar F can also be distributed in tubes and let solidify in slanted position with a short slant.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution appearance	whitish, lightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

The sample consists of pure cultures of bacteria. The isolate should be Gram-stained and examined to confirm that morphology is appropriate for *Pseudomonas*.

TEST PROCEDURE

Inoculate the medium in a plate or in a test tube with a single colony taken from the primary isolation medium, smear onto the medium surface with a single line streak; do not stab the butt when slanted tubes are used.
Incubate plates or tubes, with loosened caps, at 35 ± 2°C for 18-24 hours. If the isolate fails to grow or grows slowly, re-incubate at 25-30°C for 1-2 days and observe for growth and pigment production.
ISO 16266: incubate plates aerobically at 36 ± 2°C for up to 5 days, examining the cultures daily.[5]
FDA-BAM recommends an incubation temperature at 25°C for at least 3 days. [6]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Examine the growth under Wood's lamp daily: record as positive any appearing fluorescence.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. aeruginosa	ATCC 14207	36 ± 2°C / 18-24H / A	greenish-yellow growth, fluorescent under Wood's lamp
B. cepacia	ATCC 25415	36 ± 2°C / 18-24H / A	colourless not fluorescent growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The presence of colourless colonies does not completely exclude the presence of *P. aeruginosa*.[7]
- Mucoid isolates of *P. aeruginosa* may undergo several phenotypic changes including the loss of pigment production.[8]
- Occasionally a *Pseudomonas* strain will produce small quantities of pyocyanin which, normally, should be inhibited on resulting in a yellow-green colour on the medium.[7]
- Pseudomonas Agar F should not be used as an isolation medium, but only as a differential medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place. According to ISO 16266 the tubed medium prepared by the user can be stored at +2°C/+8°C in the dark for up to 3 months.[5]
Ready-to-use plates
Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Public Health England- Identification of Pseudomonas species and other Non-Glucose Fermenters. UK Standards for Microbiology Investigations. ID 17 Issue 3, 2015
2. Istituto Superiore di Sanità. Metodi analitici per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. Metodi microbiologici. A cura di Lucia Bonadonna e Massimo Ottaviani 2007, iv, 204 p. Rapporti ISTISAN 07/5
3. Meyer JM, Geoffroy VA, Baida N, Gardan L, Izard D, Lemanceau P, et al. Siderophore typing, a powerful tool for the identification of fluorescent and nonfluorescent pseudomonads. Appl Environ Microbiol 2002; 68:2745-53
4. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 1954; 44:301-7.
5. ISO 16266:2006 - Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Microbiological Methods for cosmetics. Updated 07/2017
7. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD.
8. Hoibi N, Ciofu O, Bjarnsholt T. Pseudomonas. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.

PACKAGING

Product	Type	REF	Pack
Pseudomonas Agar F	Dehydrated medium	4019612	500 g (13.2 L)
Pseudomonas Agar F	Ready-to-use plates	541961	2 x 10 plates ø 90 mm

IFU rev 3, 2023/03

PSEUDOMONAS AGAR P

Dehydrated culture medium

INTENDED USE

Pseudomonas Agar P (King Medium A) is intended for the differentiation of *Pseudomonas aeruginosa* by ability to produce pyocyanin.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	20.0 g
Potassium sulphate	10.0 g
Magnesium chloride	1.4 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Pseudomonas spp. are aerobic, non-spore-forming, Gram-negative rods that are straight or slightly curved, 0.5 to 1.0 by 1.5 to 5.0 µm; they have a very strict aerobic respiratory metabolism with oxygen but in some cases, nitrate may be used as an alternative that allows anaerobic growth.[1] They are usually motile with one or several polar flagella. *Pseudomonas* spp. are catalase positive and most species of clinical interest are oxidase positive (except *P. luteola* and *P. oryzihabitans*).[1]

P. aeruginosa is widely distributed in superficial water, waste and marine waters, on the soil, on vegetation and in all moist environments; moreover, it is able to grow in distilled water, to survive in disinfectants, in cosmetics and to contaminate food. *P. aeruginosa* is considered an opportunistic pathogen especially in immunocompromised patients and is characterized by multi-resistance to antibiotics, thus representing a health risk in hospital environments. *P. aeruginosa* can cause ventilator-associated pneumonia, urinary tract infections, burns and wounds infections, corneal ulcers and keratitis, septicaemia, gastroenteritis in new-borns, abscesses, and meningitis.[2]

Other characteristics that may be associated with *P. aeruginosa* species (with some exceptions) include secretion of pyoverdine (fluorescein), a fluorescent yellow-green siderophore under iron-limiting conditions.[2] Additional types of siderophores, such as pyocyanin (blue) pyorubine (red) or pyomelanin (brown) may be also produced by *P. aeruginosa* and thioquinolobactin by *P. fluorescens*.[3]

King, Ward, and Raney[4] in 1954 described two media for pigment detection in *P. aeruginosa*: medium A enhancing the production of pyocyanin and medium B enhancing the production of fluorescein.

Pseudomonas Agar P, also known as King's Medium A or Tech Agar, is a modification of the formula described by King, Ward and Raney[4], it conforms to the formulation recommended by FDA-BAM[5] and is used for the pyocyanin production test. Pyocyanin producing strains develop a blue to blue-green colour on the growth and on the medium around the growth.

Potassium sulphate and magnesium chloride enhance the production of pyocyanin and inhibit the production of fluorescein.[6] Peptone provides nitrogen and carbon compounds for bacterial growth and furthermore, thanks to a low phosphorus content, it has a reduced inhibitory effect on the production of pyocyanin; glycerol, added to the medium, is a carbon source for the growth and for the production of pyocyanin.[6]

Pseudomonas Agar P, combined with Pseudomonas Agar F, allows to perform the conventional phenotypic tests for the differentiation of *P. aeruginosa* from other species of the genus *Pseudomonas*, isolated from clinical specimens.[7]

FDA-BAM[5] recommends the pyocyanin and fluorescein production tests on Pseudomonas Agar P (+) and Pseudomonas Agar F (+) for the confirmation of *P. aeruginosa* colonies isolated from cosmetics, together with arginine dehydrolase (+), citrate and malonate utilisation (+), nitrate reduction (+), motility (+) and growth at 42°C (+).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 46.4 g in 1000 mL of cold purified water and add 10 mL of glycerol (REF 421015). Heat to boiling stirring constantly and sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes. Pseudomonas Agar P can also be distributed in tubes before sterilization and let solidify in slanted position with a short slant.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	whitish, lightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

The sample consists of pure cultures of bacteria. The isolate should be Gram-stained and examined to confirm that morphology is appropriate for *Pseudomonas*.

TEST PROCEDURE

Inoculate the medium in a plate or in a test tube with a single colony taken from the primary isolation medium, smear onto the medium surface with a single line streak; do not stab the butt when slanted tubes are used.
Incubate plates or tubes, with caps loosened, at 35 ± 2°C for 18-24 hours. If the isolate fails to grow or grows slowly, re-incubate at 25-30°C for 1-2 days and observe for growth and pigment production.[5]
FDA-BAM[5] recommends the incubation temperature at 25°C for at least 3 days.

READING AND INTERPRETATION

Examine daily and note the presence of growth colour: record as positive the presence of blue to blue-green colour on the growth diffusing into the medium.
FDA-BAM[5] recommends the following result reading: break up Pseudomonas Agar P with a glass rod in approximately equal amount of purified water and shake vigorously until water has removed as much pigment as possible. Decant into a separator. In a chemical hood, add 5-10 ml chloroform to water in separator and shake (venting occasionally to prevent internal pressure). The blue pyocyanin will migrate to chloroform. Draw off chloroform layer into a test tube. Add about 3 ml purified water. Add 1 drop 1N H_2SO_4. Pyocyanin becomes red and migrates to water.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. aeruginosa	ATCC 14207	35 ± 2°C / 18-24H / A	blue to blue-green growth
B. cepacia	ATCC 25415	35 ± 2°C / 18-24H / A	colourless not fluorescent growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The presence of colourless colonies does not completely exclude the presence of *P. aeruginosa*.[6]
- Mucoid isolates of *P. aeruginosa* from cystic fibrosis patients may undergo several phenotypic changes including the loss of pigment production.[7]
- Occasionally a *Pseudomonas* strains will produce small quantities of fluorescein which, normally, should be inhibited on resulting in a blue-green colour on the medium.[6]
- Pseudomonas Agar P should not be used as an isolation medium, but only as a differential medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months.[6]

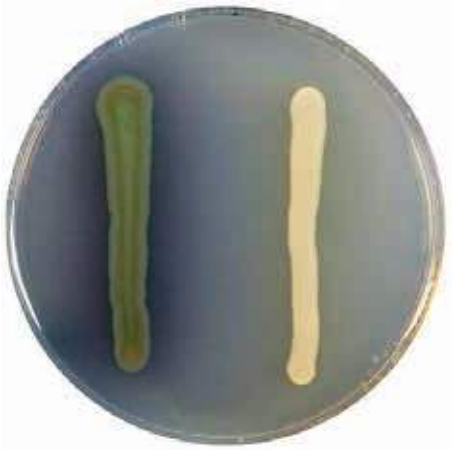
REFERENCES

1. Public Health England- Identification of Pseudomonas species and other Non-Glucose Fermenters. UK Standards for Microbiology Investigations. ID 17 Issue 3, 2015
2. Istituto Superiore di Sanità. Metodi analitici per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. Metodi microbiologici. A cura di Lucia Bonadonna e Massimo Ottaviani 2007, iv, 204 p. Rapporti ISTISAN 07/5
3. Meyer JM, Geoffroy VA, Baida N, Gardan L, Izard D, Lemanceau P, et al. Siderophore typing, a powerful tool for the identification of fluorescent and nonfluorescent pseudomonads. Appl Environ Microbiol 2002; 68:2745-53
4. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med1954; 44:301-7.
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Microbiological Methods for cosmetics. Updated 07/2017
6. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD.
7. Hoibi N, Ciofu O, Bjarnsholt T. Pseudomonas. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.

PACKAGING

Product	Type	REF	Pack
Pseudomonas Agar P	Dehydrated medium	4019622	500 g (10,8 L)

IFU rev 2, 2022/05



From left: *P. aeruginosa* and *B. cepacia*
on Pseudomonas Agar P

PSEUDOMONAS SELECTIVE AGAR

(Cetrimide Agar)

Dehydrated and ready-to-use culture medium

P. aeruginosa on Pseudomonas Selective Agar

INTENDED USE

In vitro diagnostics. For selective isolation and presumptive identification of *Pseudomonas aeruginosa* from clinical and non-clinical specimens.

COMPOSITION *

PSEUDOMONAS SELECTIVE AGAR, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of gelatine	20.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g
Cetrimide	0.3 g
Agar	14.0 g

PSEUDOMONAS SELECTIVE AGAR, READY-TO-USE PLATES AND FLASKS
TYPICAL FORMULA

Pseudomonas Selective Agar	45.7 g
Glycerol	10.0 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Pseudomonas spp. are aerobic, non-sporeforming Gram-negative rods that are straight or slightly curved, 0.5 to 1.0 by 1.5 to 5.0 µm; they have a very strict aerobic respiratory metabolism with oxygen but in some cases, nitrate has been used as an alternative that allows anaerobic growth.[1] They are usually motile with one or several polar flagella. *Pseudomonas* spp. are catalase positive and most species of clinical interest are oxidase positive (except *P. luteola* and *P. oryzihabitans*).[1]

P. aeruginosa is widely distributed in surface, waste and marine water, on soil, vegetation and in all humid environments; moreover, it is able to grow in distilled water, to survive in disinfectants, in cosmetics and to contaminate food. *P. aeruginosa* is considered an opportunistic pathogen especially in immunocompromised patients and is characterized by multi-resistance to antibiotics, thus representing a health risk in hospital environments. *P. aeruginosa* can cause ventilator-associated pneumonia, urinary tract infections, burns and wounds infections, corneal ulcers and keratitis, septicaemia, gastroenteritis in new-borns, abscesses, and meningitis.[2]

Other characteristics that tend to be associated with *Pseudomonas* species (with some exceptions) include secretion of pyoverdin (fluorescein), a fluorescent yellow-green siderophore under iron-limiting conditions.[2] Certain *Pseudomonas* spp. may also produce additional types of siderophore, such as pyocyanin (blue) pyorubin (red) or pyomelanin (brown) by *P. aeruginosa* and thioquinolobactin by *P. fluorescens*.[3]

King, Ward, and Raney[4] in 1954 have described two media, one of which (medium A) enhances the production of pyocyanin by *P. aeruginosa*, while the other (medium B) enhances the production of fluorescein. Pseudomonas Selective Agar is prepared according to the formulation of Tech Agar/Medium A with the addition of cetrimide for the inhibition of microorganisms other than *Pseudomonas*, originally proposed at the concentration 0.1% by Lowbury[5] and later decreased to 0.03% by Lowbury and Collins in 1955[6].

Pseudomonas Selective Agar meets cetrimide agar formulation and performance specifications described by EP, USP, JP harmonized method for the determination of absence of *P. aeruginosa* in non-sterile pharmaceutical products.[7] It is recommended by ISO Standard 22717[8] and by FDA-BAM[9] for the detection of *P. aeruginosa* in cosmetics.

Pancreatic digest of gelatin provides nitrogen and carbon for bacterial growth and, as reported by King et al.[4] and by Goto and Enomoto,[10] contributes to the pyocyanin and fluorescein production. Cetyltrimethylammonium bromide, acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane protein, causes the release of nitrogen and phosphorous from the cell and has bactericidal activity against a broad range of Gram-positive organisms and some Gram-negative organisms other than *P. aeruginosa*; magnesium chloride and potassium sulphate provide necessary cations for the activation and stimulation of fluorescein and pyocyanin production.[11] Glycerol is present in the medium as a carbon source for microbial growth and as a stimulant for the production of pyocyanin.[11] The presumptive identification of *P. aeruginosa* is obtained on the basis of the pigments production and the chromatic characteristics of the colonies.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour into sterile Petri dishes, under aseptic conditions.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Pseudomonas Selective Agar may be directly inoculated with clinical specimens collected from various normally non-sterile human sites such as respiratory secretions, damaged tissues, ear, eye, urine etc.[12] Collect specimens before antimicrobial therapy where possible and apply good laboratory practices for collection, transport and storage of the clinical specimens.

Non-clinical samples analysed with Pseudomonas Selective Agar include non-sterile pharmaceutical products and cosmetics. Consult the references for sample collection and preparation.[7-9]

TEST PROCEDURE

Allow plates to come to room temperature; the agar surface should be smooth and moist, but without excessive moisture.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate aerobically at 35-37°C and record the results after 18-24 hours. If typical colonies are not observed re-incubate an additional 24-36 hours (72 h in total). For cultures from cystic fibrosis patients, it is recommended that solid media plates be held at 35 to 37°C for 5 days since some strains from chronic infections grow very slowly.[12]
For the detection of *P. aeruginosa* in non-sterile pharmaceuticals products, the technique recommended by European Pharmacopoeia[7] and summarized below, should be followed:

- Prepare a sample using a 1:10 dilution of not less than 1 g of the product to be examined and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate the suitable amount of Tryptic Soy Broth. Mix and incubate at 30-35°C for 18-24 h.
- Subculture on a plate of Pseudomonas Selective Agar and incubate at 30-35 °C for 18-72 h.

The growth of colonies indicates the possible presence of *P. aeruginosa*. This is confirmed by identification tests.
For the detection of *P. aeruginosa* in cosmetics consult the references.[8,9]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
Any growth indicates a possible *Pseudomonas* species. Examine colonies under UV light (254 nm) for fluorescence.
Positive: bright yellow green colour diffuses into the agar producing a fluorescent zone in the agar surrounding the growth (*P. aeruginosa, P. fluorescens, P. putida).*
Examine colonies for pigmentation under normal light
The production of pyocyanin is indicated by the blue or green / blue colour of the colonies.
The production of fluorescein is indicated by the yellow-green colour of the colonies
The formation of pyorubin is indicated by the colour from light pink to red or dark brown of the colonies. Pyorubin is frequently formed simultaneously with pyocyanin and or fluorescein.
Fluorescein combines with pyocyanin, to give *P. aeruginosa* its characteristic bright green colour. These chromatic characteristics are typical of *P. aeruginosa*, together with the morphology of the colonies and the typical grape smell caused by the production of aminoacetophenone.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. aeruginosa ATCC 9027	35-37°C / 18-24H / A	good growth, green colonies
E. coli ATCC 25922	35-37°C / 18-24H / A	inhibited
S. aureus ATCC 25923	35-37°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection. User quality control of Pseudomonas Selective Agar used for *P. aeruginosa* detection in non-sterile pharmaceutical products and cosmetics should meet the requirements of EP[7] and ISO Standard.[8]

LIMITATIONS OF THE METHOD

- Inhibition of some strains of *P. aeruginosa* has been reported using a selective agar containing cetrimide.[12]
- A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. For an optimized recovery of *P. aeruginosa*, mainly in cystic fibrosis patients, it is recommended to use in addition to Pseudomonas Selective Agar, other selective and non-selective media such as Mac Conkey Agar, blood agar and chocolate agar.[12]
- No single medium can be depended upon to exhibit all pigment-producing *P. aeruginosa* strains.[4]
- Occasionally some enteric organisms (e.g. *Klebsiella, Enterobacter, Citrobacter, Proteus, Providencia*), *Alkaligenes* and *Aeromonas* will exhibit growth with a slight yellowing of the medium; however, this coloration is easily distinguished from slight fluorescein production since this yellowing does not fluoresce.[11]
- There are non-pigmented strains of *P. aeruginosa* that grow on the medium but do not produce the typical green-blue or yellow-green colour.
- Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. Some *Serratia* strains may exhibit a pink pigmentation.[11]
- Studies of Lowbury and Collins[6] showed that *P. aeruginosa* may lose its fluorescence under UV light if the cultures are left at room temperature for a short time. However, fluorescence reappears when plates are re-incubated.
- Some mucoid *P. aeruginosa* strains have a delayed oxidase positive reaction and therefore may require further confirmation tests.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 6-8 weeks.[11]
Ready-to-use plates and flasks
Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Public Health England- Identification of Pseudomonas species and other Non-Glucose Fermenters. UK Standards for Microbiology Investigations. ID 17 Issue 3, 2015
2. Istituto Superiore di Sanità. Metodi analitici per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. Metodi microbiologici. A cura di Lucia Bonadonna e Massimo Ottaviani 2007, iv, 204 p. Rapporti ISTISAN 07/5
3. Meyer JM, Geoffroy VA, Baida N, Gardan L, Izard D, Lemanceau P, et al. Siderophore typing, a powerful tool for the identification of fluorescent and nonfluorescent pseudomonads. Appl Environ Microbiol 2002; 68:2745-53
4. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 1954; 44:301-7.
5. Lowbury EJ. Improved culture methods for the detection of Pseudomonas pyocyanea J Clin Pathol 1951; 4:66-72
6. Lowbury EJ, Collins AG the use of a new cetrimide product in a selective medium for Pseudomonas pyocyanea. J Clin Pathol 1955; 8:47-8.
7. European Pharmacopoeia, current edition
8. ISO 21717:2015. Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa.
9. U.S. Food and Drug Administration. Bacteriological Analytical Manual BAM Chapter 23: Methods for Cosmetics. Content current as of:10/31/2017
10. Goto S. Enomoto S. Nalidixic Acid Cetrimide Agar. A New Selective Plating Medium for the Selective Isolation of Pseudomonas aeruginosa Jpn J Microbiol 1970 14 (1): 65.

11. MacFaddin, Jean F. (1985). Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. Williams & Wilkins, Baltimore, MD.
12. Hoibi N, Ciofu O, Bjarnsholt T. Pseudomonas. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.

PACKAGING

Product	Type	REF	Pack
Pseudomonas Selective Agar	Dehydrated medium	4019632 4019634	500 g (10.9 L) 5 kg (109 L)
Pseudomonas Selective Agar	Ready-to-use plates	541963	2 x 10 plates ø 90 mm
Cetrimide Agar USP-EP	Ready-to-use plates	491963	3 x 10 plates ø 55 mm
Pseudomonas Selective Agar	Ready-to-use flasks	5119632	6 x 100 mL

IFU rev 3, 2023/03

PSEUDOMONAS SELECTIVE BROTH

Dehydrated culture medium

INTENDED USE

For the enumeration of *Pseudomonas aeruginosa* and other *Pseudomonas* spp. in liquid samples by the membrane filtration method and for the confirmation test of *P. aeruginosa*.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of gelatin	16.0 g
Acid digest of casein	10.0 g
Potassium sulphate	10.0 g
Magnesium chloride	1.4 g
Cetrimide	0.2 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

King, Ward, and Raney[1] in 1954 described two media, one of which (medium A) enhances the production of pyocyanin by *P. aeruginosa*, while the other (medium B) enhances the production of fluorescein. Pseudomonas Selective Broth is prepared according to the formulation of Tech Agar/Medium A without agar and with the addition of cetrimide for the inhibition of microorganisms other than *Pseudomonas*, originally proposed at the concentration 0.1% by Lowbury[2] and later decreased by Lowbury and Collins in 1955[3].
Pseudomonas Selective Broth can be used for the enumeration of *Pseudomonas* spp. by membrane filtration technique and for the confirmation test of *P. aeruginosa* colonies cultivated on solid selective media.[4]
Pancreatic digest of gelatin and acid digest of casein provide nitrogen, carbon and amino acids for bacterial growth and contributes to the pyocyanin and fluorescein production. Cetrimide has bactericidal activity against a broad range of Gram-positive organisms and some Gram-negative organisms other than *P. aeruginosa*. Magnesium chloride and potassium sulphate provide necessary cations for the activation and stimulation of fluorescein and pyocyanin production.[5] Glycerol is present in the medium as a carbon source for microbial growth and as a stimulant for the production of pyocyanin.[5]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 37.6 g in 1000 mL of cold purified water and add 10 mL of glycerol. Heat to boiling with frequent agitation, distribute 5 mL in tubes and sterilise by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Water and other liquid samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Membrane filtration method

5. Filter an appropriate volume of sample onto the membrane depending on the expected *Pseudomonas* number. When the bacterial density is unknown, filter several volumes or dilutions to achieve a countable plate.
6. Using aseptic technique, place a sterile absorbent pad in each culture dish and pipet at least 2 mL of broth. Carefully remove any excess of liquid from culture dish by decanting plate.
7. Roll the membrane filter used to collect the sample onto the surface of the pad, so as to avoid the formation of air bubbles between the filter and the pad.
8. Incubate the inverted Petri dish for 24 - 72 hours at 25-35° C. Incubation at 35° C during 24 hours is favourable to *P. aeruginosa*, 25° C for *P. fluorescens*.

Confirmation technique[4]

Select five typical colonies cultivated onto primary selective medium and subculture into tubes of Pseudomonas Selective Broth and perform cytochrome oxidase test. Incubate the tubes of Pseudomonas Selective Broth at 42 ± 0.5°C for 24 hours. The oxidase positive colonies that grow on Pseudomonas Selective Broth tubes are identified and enumerated as *P. aeruginosa*

READING AND INTERPRETATION

Enumerate the number of colonies per plate and calculate the microbial count.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
P. aeruginosa ATCC 9027	37°or 42°C / 24-48 H-A	good growth
E. coli ATCC 25922	37° or 42°C/ 24-48 H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Inhibition of some strains of *P. aeruginosa* has been reported using a selective agar containing cetrimide.[6]
- Occasionally some enteric organisms (e.g., *Klebsiella, Enterobacter, Citrobacter, Proteus, Providencia*), *Alkaligenes* and *Aeromonas* will exhibit growth with a slight yellowing of the medium; however, this coloration is easily distinguished from slight fluorescein production since this yellowing does not fluoresce.[5]
- There are non-pigmented strains of *P. aeruginosa* that grow on the medium but do not produce the typical green-blue or yellow-green colour.
- Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. Some *Serratia* strains may exhibit a pink pigmentation.[5]
- Studies of Lowbury and Collins[3] showed that *P. aeruginosa* may lose its fluorescence under UV light if the cultures are left at room temperature for a short time. However, fluorescence reappears when tubes are re-incubated.
- Some mucoid *P. aeruginosa* strains have a delayed oxidase positive reaction and therefore may require further confirmation tests.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 1954; 44:301-7.
2. Lowbury EJ. Improved culture methods for the detection of Pseudomonas pyocyanea. J Clin Pathol 1951; 4:66-72
3. Lowbury EJ, Collins AG. The use of a new cetrimide product in a selective medium for Pseudomonas pyocyanea. J Clin Pathol 1955; 8:47-8.
4. Manuel Suisse des Denrées Alimentaires (MSDA). Chapitre 56, Microbiologie. Juillet 2000.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Hoibi N, Ciofu O, Bjarnsholt T. Pseudomonas. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015

PACKAGING

Product	Type	REF	Pack
Pseudomonas Selective Broth	Dehydrated medium	4019642	500 g (13.3 L)

IFU rev 1, 2022/12

PURPLE GLUCOSE AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

For the confirmation test of *Pseudomonas* spp.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Enzymatic digest of casein	10.0 g
Yeast extract	1.5 g
Glucose	10.0 g
Sodium chloride	5.0 g
Agar	12.2 g
Bromocresol purple	15.0 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Purple Glucose Agar is a medium recommended by ISO 11059[1] for the confirmation test of *Pseudomonas* spp. after isolation on a selective medium. Enzymatic digest of casein and yeast extract provide nitrogen, vitamins, minerals and amino acids for microbial growth. Glucose is the fermentable carbohydrate and a source of carbon and energy. Sodium chloride maintains the osmotic balance. Bromocresol purple is a pH indicator. After incubation, the glucose-fermenting microorganisms exhibit a yellow colour throughout the content of the tube.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 38.7 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute in 10 mL amounts into test tubes and sterilise by autoclaving at 121ºC for 15 minutes. Leave the tubes in a vertical position. The medium may be stored for up to one week at 2-8°C. In order to remove oxygen, just before the use, heat the medium in boiling water or flowing steam for 15 minutes, then cool rapidly to the incubation temperature.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	violet, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	violet, clear
Final pH at 20-25 °C	7.0 ± 0.2

Specimens
The sample consists of bacterial cultures isolated from dairy products or other materials, purified on Nutrient Agar or other suitable medium.

Test procedure
Obtain pure isolated colonies by subculturing from isolation medium to Nutrient Agar plates.
Stab, by means of a needle, colonies from the nutrient agar medium into tubes containing Purple Glucose Agar.
Incubate at 25 °C ± 1 °C for 24 h ± 3 h without hermetically closing the tubes.

Reading and interpretation
If a yellow colour develops in the entire contents of the tube, the reaction is considered positive: presence of glucose fermentation.
Consider the test to be negative (absence of glucose fermentation) when growth can be observed but no yellow colour develops throughout the content of the tube.
Consider colonies showing a positive oxidase reaction and absence of glucose fermentation as *Pseudomonas* colonies.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t - ATM	Expected results
P. aeruginosa ATCC 27053	25°C/ 24h/A	growth, no colour change to yellow
E. coli ATCC 25922	25°C/ 24h/A	growth, colour change to yellow

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- Some *Pseudomonas* strains may develop a yellow colour at the agar surface resulting from glucose oxidation.

Storage conditions
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 21528:2004[2] withdrawn, the self-prepared tubes can be stored at +2°C +8°C for up to one weeks. In order to remove oxygen, just before the use, heat the medium in boiling water or flowing steam for 15 minutes, then cool rapidly to the incubation temperature.[1]
Ready-to-use medium in tubes
Store tubes in their original pack at 2-8°C away from direct light.

References

1. ISO/TS 11059: 2009. Milk and milk products -Method for the enumeration of Pseudomonas spp.
2. ISO 21528-1: 2004 Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of Enterobacteriaceae -- Part 1: Detection and enumeration by MPN technique with pre-enrichment

Packaging
Product	Type	REF	Pack
Purple Glucose Agar	Dehydrated medium	4019702	500 g (13 L)
Purple Glucose Agar	Ready-to-use tubes	551970	20 x 10 mL

IFU rev 2, 2022/11

R2A AGAR

Dehydrated and ready-to-use culture medium

Intended use
For heterotrophic plate count in water samples.

Composition - typical formula * (after reconstitution with 1 L of water)
DEHYDRATED MEDIUM AND READY-TO-USE TUBES AND FLASKS

Yeast extract	0.5 g
Proteose peptone	0.5 g
Acid digest of casein	0.5 g
Glucose	0.5 g
Soluble starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Sodium pyruvate	0.3 g
Magnesium sulphate anhydrous	24.0 mg^
Agar	14 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
^ Equivalent to 50 mg of magnesium sulphate heptahydrate

Principle of the method and explanation of the procedure
R2A Agar was devised by Reasoner and Geldreich[1] for bacteriological plate count of treated potable water. Results from their study with spread, membrane filter, and pour plate procedures, showed that R2A medium yielded significantly higher bacterial counts than did plate count agar and longer incubation time, up to 14 days, at 20°C, yielded higher counts and increased detection of pigmented bacteria.[1]
This low-nutrient agar with a longer incubation temperature can improve the recovery of stressed and chlorine-tolerant bacteria.[2]
The medium formulation is based on the principle that many bacteria, living in natural waters with limited nutrients and at temperatures close to room temperature, grow best on culture media with reduced peptone concentrations at room temperature.
R2A Agar is recommended by European Pharmacopoeia for the determination of the total microbial count in water for injections in bulk, purified water in bulk and in containers.[3]

R2A Agar is included in the APHA methods for the heterotrophic plate count with pour-plate, spread plate and membrane filter methods in potable treated waters.[2]
Proteose peptone and acid digest of casein provide nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose is a source of carbon and energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium. Sodium pyruvate and starch aid in resuscitation of stressed cells. Magnesium ions enhance microbial growth.

Directions for medium preparation (dehydrated medium)
Suspend 17.12 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

Directions for medium preparation (Ready-to-use flasks/tubes)
Liquefy the contents of the flask/tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle or the tube may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale beige, clear
Final pH at 20-25 °C	7.2 ± 0.2

Specimens
Water samples: treated potable water, water for injections in bulk, purified water in bulk and in containers. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[2,3]

Test procedure
Colony count by the pour plate technique (treated water).[2]
1. Using a sterile pipette, dispense 0.1 to 1 mL of the liquid test sample, into an empty Petri dish and mix with the molten R2A Agar pre-cooled to 44-46°C.
2. Prepare the other plates under the same conditions using decimal dilutions of the test sample.
3. Incubate the plates under aerobic conditions at 20-28°C for 5 to 7 days.

Colony count by the surface plating technique (treated water).[2]
1. Dry the prepared plates before the use.
2. Using a sterile pipette, transfer 0.1 to 0.5 mL of the test sample to the centre of a R2A Agar plate.
3. Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
4. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.
5. Incubate the plates under aerobic conditions at 20-28°C for 5 to 7 days.

Membrane filter method (pharmaceutical water and treated water)[2,3]
This method can be used to analyse large volumes of low-turbidity water and is the method of choice for samples with low number of heterotrophic organisms (<1 to 10 CFU/mL).[2]
1. Filter a suitable volume of water (e.g. 200 mL) through a membrane (≤ 0.45µm). The size of the sample is to be chosen in relation to the expected result.
2. Using aseptic technique, roll the membrane filter onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
3. Incubate the plates under aerobic conditions for 5 days at 30-35 °C [3] or at 20-28°C for 5 to 7 days[2].

Consult the appropriate International Standard for the details of the procedures.[1-7]

Reading and interpretation
After incubation, count all colonies obtained in the plates containing fewer than 300 colonies (pour-plate and spread plate) or 200 colonies (MF) and calculate the number of microorganisms per millilitre of the test sample.
Follow recommended procedures for the counting of colonies and the reporting of results.[2,3]

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

Control strains	Incubation T°/ t / ATM	Expected results
B. subtilis ATCC 6633	30-35°C/3 days-A	good growth
P. aeruginosa ATCC 9027	30-35°C/3 days-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the methods
- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.[4,5]
- Increasing the holding time of the dilutions in the diluent leads to higher count. [4,6]
- The aerobic plate count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.[4]

Storage conditions
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to APHA, self-prepared plates can be stored in sealed plastic bags at +2 °C to +8 °C for up to 2 weeks.[4]
Ready-to-use medium in flasks and tubes
Store flasks/tubes in their original pack at +2°C/ +8°C away from direct light.

References

1. Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol. 1985 Jan; 49: 1-7.
2. American Public Health Association. Standard Methods for the Examination of Water, 23rd ed. 2017. APHA, Washington, DC.
3. European Pharmacopoeia 11th Edition, 2022, Vol. III.
4. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
5. Berry JM, McNeill DA, Witter LD. Effect of delay in pour plating on bacterial counts. J Dairy Sci 1969; 52:1456-1457
6. Huhtanen CN, Brazis AR, Arledge WL et al. Effects of time of holding dilutions on counts of bacteria from raw milk. J Milk Food Technol. 1972; 35:126-130.

PACKAGING

Product	Type	REF	Pack
R2A Agar	Dehydrated medium	4019962	500 g (29 L)
R2A Agar	Ready-to-use medium in tubes	551996Q	20 x 15 mL
R2A Agar	Ready-to-use medium in flasks	5119963	6 x 200 mL

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RAPPAPORT VASSILIADIS (RV) BROTH

Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. Liquid medium for the selective enrichment of *Salmonella* in food, environmental and clinical samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Tryptone	4.540 g
Potassium dihydrogen phosphate	1.450 g
Sodium chloride	7.200 g
Magnesium chloride anhydrous	13.300 g
Malachite green oxalate	0.036 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Rappaport Vassiliadis (RV) Broth is prepared according to the R25/37 formulation proposed by Rappaport in 1956[1] and modified by Vassiliadis in 1976[2], and called R10/43. Two major modifications were introduced in the composition and use: one modification consisted of the reduction to one-third of the amount of malachite green and the other in the incubation at 43°C instead of 37°C

From 1977 to 1981, in eleven studies, the RV enrichment broth has been compared to the standardized Muller-Kauffmann Tetrathionate broth (MK broth) recommended as a reference method by the International Standards Organization; in all these studies the RV broth was superior to the MK broth in the isolation of salmonellas from naturally contaminated meat products, sewage and faeces of healthy pigs, after pre-enrichment in buffered peptone water.[3]

Rappaport Vassiliadis (RV) Broth is reported by the FDA BAM[4] as a selective enrichment broth for the isolation of *Salmonella*.

The ISTISAN 96/35[5] Report indicates the following scheme for the selective enrichment of *Salmonella* in food:

Non-regulated food: Rappaport Vassiliadis (RV) (42°C) + Selenite Cystine Broth (37°C)

Milk and derivatives: Muller Kauffmann (42°C) + Selenite Cystine Broth (37° C)

Fresh eggs: Rappaport Vassiliadis (RV) (42°C) + Muller Kauffmann (42°C)

Molluscs: MSRV (42°C)

The medium is also recommended as a selective enrichment broth for *Salmonella* spp. other than *Salmonella* Typhi in human stool samples by Kist et al.[6] and by Burkhardt[7].

Tryptone is a source of nitrogen and carbon for microbial growth; malachite green is inhibitory towards coliforms; the high osmotic pressure of the medium due to the high concentrations of magnesium chloride, together with the acid pH, act as inhibitors of the saprophytic flora, favouring the development of *Salmonella* in the broth. Magnesium chloride suppresses the toxic effects of malachite green towards *Salmonellae*; potassium dihydrogen phosphate acts as a buffer system.

An extensive review of the scientific papers published on the Rappaport Vassiliadis Broth was published by Vassiliadis in 1983.[3]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 26.5 g in 1000 mL of cold purified water. Heat to dissolve, distribute 10 mL into screw-cap tubes and sterilise by autoclaving at 115°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-green, fine, homogeneous, free-flowing powder
Medium appearance	blue, limpid
Final pH at 20-25 °C	5.2 ± 0.2

SPECIMENS

Rappaport Vassiliadis (RV) Broth may be inoculated with human clinical specimens such as faeces or rectal swab. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied. For food and environmental samples refer to the quoted references.[4,5]

TEST PROCEDURE

Faeces:

- Inoculate the tube of Rappaport Vassiliadis (RV) Broth with a substantial loop of faeces or with 50 - 100 µL of liquid faeces.
- Rectal swabs received fresh or in transport medium should be rinsed thoroughly in 1 mL of saline.
- Incubate the inoculated tubes in aerobic atmosphere at 42 ± 1°C for 18-24 hours.

Food:

- Inoculate 25 g of sample in 225 ml of Buffered Peptone Water (code 401278) and incubate at 35-37°C for 16-20 hours
- Transfer 0.1 mL to 10 mL of Rappaport Vassiliadis (RV) Broth and incubate at 42 ± 1°C for 24 hours
- From the tubes of Rappaport Vassiliadis Broth transfer a loopful of growth on a plate of XLD Agar (code 402208) and on another selective medium for *Salmonella*.

For a detailed description of methods for detecting *Salmonella* in food, refer to the cited literature.[4,5]

READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by a milky appearance of the broth or by turbidity.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Enteritidis ATCC 13076	42 ± 1°C / 18-24h / A	good growth after subculture to Tryptic Soy Agar plate
S. Typhimurium ATCC 14028	42 ± 1°C / 18-24h / A	good growth after subculture to Tryptic Soy Agar plate
E. coli ATCC 25922	42 ± 1°C / 18-24h / A	growth partially inhibited after subculture to Tryptic Soy Agar plate

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Rappaport Vassiliadis (RV) Broth is inhibitory for S. Typhi. The medium is therefore not indicated for the diagnosis of typhoid fevers.
- For the enrichment of human faecal specimens, the most recommended media by microbiological manuals and procedures are selenite containing broths.[8,9]
- After the enrichment in Rappaport Vassiliadis (RV) Broth, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

Ready-to-use medium in tubes

Store tubes in their original pack at +2°C/+8°C away from direct light.

According to Baird RM et al. the medium may be stored in screw-capped bottles at +2/+8°C for up to 6 months (10 mL are transferred to test tubes when needed).[10]

REFERENCES

1. Rappaport, F., N. Konforti, and B. Navon. (1956) A new enrichment medium for certain salmonellae. J. Clin. Pathol. 9:261-266.
2. Vassiliadis, P., Pateraki, E., Papiconomou, N., Papadakis, J. and Trichopoulos, D. (1976) Nouveau procède d'enrichissement de salmonella. Ann. Microb. Irist, Pasteur 127 B, 195.
3. Vassiliadis P. (1983) The Rappaport Vassiliadis enrichment Broth for the isolation of salmonellas: an overview. J. App. Bact. 54, 69
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 07/2020
5. Rapporto ISTISAN 96/35. ISSN 1123-3117. Metodi di analisi per il controllo microbiologico degli alimenti. Raccolta a cura di D. De Medici, L. Fenicia, L. Orefice e A. Stacchini.
6. Kist, M., et al. 2000. Infektionen des Darmes. In: Mauch, H., Lüttiken, R., and S. Gatermann (eds.): MiQ - Qualitätsstandards in der mikrobiologisch-infektiologischenDiagnostik, vol. 9. Urban & Fischer, Munich, Germany.
7. Bockemühl, J. 1992. Enterobacteriaceae. In: Burkhardt, F. (ed.). MikrobiologischeDiagnostik. ThiemeVerlag, Stuttgart, New York.
8. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
9. Public Health England- UK Standards for microbiology investigations (UK SMI): SMI B 30: investigation of faecal specimens for enteric pathogens. Issue 8.1, 04/2014.
10. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:254-5.

Product	Type	REF	Pack
Rappaport Vassiliadis (RV) Broth	Dehydrated medium	4019802 4019804	500 g (18.8 L) 5 kg (188 L)
Rappaport Vassiliadis (RV) Broth	Ready-to-use tubes	551980	20 x 10 mL

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Rappaport Vassiliasis (RV) Broth
from the left: un-inoculated tube and *S.* Enteritidis growth.

RAPPAPORT VASSILIADIS ENRICHMENT SALMONELLA BROTH EP

Dehydrated culture medium

INTENDED USE

Liquid medium for the selective enrichment of *Salmonella* in non-sterile pharmaceutical products.

COMPOSITION - TYPICAL FORMULA ^* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Soy peptone	4.500 g
Sodium chloride	8.000 g
Potassium dihydrogen phosphate	0.600 g
Dipotassium hydrogen phosphate	0.400 g
Magnesium chloride anhydrous	13.580 g ^
Malachite green oxalate	0.036 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
^ corresponding to magnesium chloride hexahydrate 29.0 g/L

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Rappaport in 1956[1] devised an enrichment broth for *Salmonella* that included malachite green and magnesium chloride as inhibitors. Vassiliadis in 1976[2] modified the Rappaport medium by reducing to one-third the concentration of malachite green and incubating at 43°C instead of 37°C. Van Schothorst and Renaud[3] reported that the use of soy peptone instead of animal peptone improved recovery rates of *Salmonella*. Rappaport Vassiliadis Enrichment Salmonella Broth EP is based on the formulation of Van Schothorst[3] and Renaud and meets the requirements of European Pharmacopoeia.[4]
The medium is used as a selective enrichment medium for the isolation of *Salmonella* from non-sterile pharmaceutical products with after the pre-enrichment in Tryptic Soy Broth, according to the method reported by European Pharmacopoeia.[4]
Rappaport Vassiliadis Enrichment Salmonella Broth EP differs from the ISO standard medium (REF 401781) by slightly different concentrations of sodium chloride and phosphate buffer.
The efficiency of this enrichment medium is based on the ability of *Salmonella* spp. to multiply at relatively high osmotic pressures, at relatively low pH values, at a high temperature and with reduced nutritional requirements.[5]
Essential growth factors are provided by soy peptone; malachite green is inhibitory to organisms other than salmonellae; the high osmotic pressure of the medium due to the high concentrations of magnesium chloride, together with the acid pH, act as inhibitors of the saprophytic flora, favouring the growth of *Salmonella* in the broth. Magnesium chloride in addition counteracts the toxic effect of malachite green for salmonellae. Phosphates are used as buffering agents to control the pH in the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 27.1 g in 1000 mL of cold purified water. Heat gently to dissolve, distribute 10 mL into screw-cap tubes and sterilise by autoclaving at 115°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-green, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	blue, limpid
Final pH at 20-25 °C	5.2 ± 0.2

SPECIMENS

Non-sterile pharmaceutical products. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to European Pharmacopoeia.[4]

TEST PROCEDURE

The following method is a summary of the technique recommended by European Pharmacopoeia.[4]

- Inoculate not less than 10 g o 10 mL into 90 mL of Tryptic Soy Broth (REF 402155), mix and incubate at 30-35°C for 18-24 hours.
- Transfer 0.1 mL of the culture to 10 mL of Rappaport Vassiliadis Salmonella Enrichment Broth EP and incubate at 30-35 °C for 18-24 h.
- Subculture on plates of XLD agar (REF 402208) and incubate at 30-35 °C for 18-48 h.

READING AND INTERPRETATION

After incubation, the growth of organisms in the enrichment broth is indicated by a milky appearance of the broth or by turbidity.
The possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centres on XLD Agar plates. This is confirmed by identification tests.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028	30-35 °C/ 18-24h/ A	good growth
S. aureus ATCC 25923	30-35 °C/ 18-24h/ A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- RVS Broth is inhibitory for *S*. Typhi.
- After the selective enrichment, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579 prepared tubes and flasks of RVS Broth may be stored at 2-8°C for up to three months.[6]

REFERENCES

1. Konforti K, Navon B, Rappaport F. A new enrichment medium for certain salmonellae. J Clin Pathol 1956; 9:261-266.
2. Vassiliadis P, Pateraki E, Papiconomou N, Papadakis J, Trichopoulos D. Nouveau procède d'enrichissement de salmonella. Ann. Microb. Irist, Pasteur 1976; 127 B: 195.
3. Van Schothorst M, Renaud AM. Dynamics of salmonella isolation with modified Rappaport's medium (R10). J Appl Bacteriol 1983; 54:209
4. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
5. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:254-255.
6. ISO 6579:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella —Part 1: Detection of Salmonella spp

PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis Enrichment Salmonella Broth EP	Dehydrated medium	4019792	500 g (18.4 L)

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RAPPAPORT VASSILIADIS SEMI-SOLID MEDIUM MODIFIED (MSRV)
NOVOBIOCIN ANTIMICROBIC SUPPLEMENT
Dehydrated culture medium and selective supplement

MSRV medium: migration of *Salmonella* Typhimurium

INTENDED USE

Basal medium and supplement for selective enrichment/isolation of mobile *Salmonella* strains from food and animal feed, animal faeces and environmental samples from primary production stage.

COMPOSITION*

MSRV MEDIUM – DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Enzymatic digest of animal and plant tissue	4.6 g
Acid digest of casein	4.6 g
Sodium chloride	7.3 g
Potassium dihydrogen phosphate	1.5 g
Magnesium chloride anhydrous	10.9 g
Agar	2.7 g
Malachite green oxalate	40.0 mg

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT - VIAL CONTENT

Novobiocin	10 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Modified Semisolid Rappaport Vassiliadis (MSRV) medium is a semisolid modification of Rappaport Vassiliadis enrichment broth, originally described by De Smedt *et al*[1] in 1986 with a novobiocin concentration of 20 mg/L. MSRV medium enables the sensitive determination of motile *Salmonella* strains in contaminated food or other samples. The use of this medium following pre-enrichment or selective enrichment gave better results in the isolation of *Salmonella* than conventional methods.[2-4]
Veenman et al.[5] demonstrated the presence of larger migration zones on MSRV medium with a lower concentration of novobiocin and the influence of novobiocin on bacterial motility.
MSRV medium with a novobiocin concentration of 10 mg/L is recommended by ISO 6579-1,[6] for the determination of *Salmonella* in food, animal feed samples, environmental samples from the food production area, as an alternative to RVS broth and as the only selective enrichment medium for samples from the primary production stage.
The principle of the method is based on the ability of salmonellae to move from the point of inoculation through the selective medium, more rapidly than other competitive microorganisms, producing opaque halos of growth.
Essential growth factors are provided by peptones. Malachite green, the high osmotic pressure due to the high concentration of magnesium chloride, the presence of novobiocin and the acid pH, act as inhibitors of the saprophytic flora, promoting the growth of motile *Salmonella* strains. Magnesium chloride in addition counteracts the toxic effect of malachite green for salmonellae. Phosphates are used as buffering agents to control the pH in the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 31.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to approximately 47-50°C.

ISO 6579-1 formulation

Add the contents of one vial of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 10 mg/L

Original De Smedt formulation

Add the contents of two vials of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 20 mg/L

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	blue, clear, semisolid medium
Freeze-dried selective supplement	short, dense, white pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	5.2 ± 0.2

SPECIMENS

Products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling, samples from the primary production stage such as animal faeces, dust, and swabs. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to the applicable International Standards.

TEST PROCEDURE

Summary of ISO 6579 procedure for food, animal feed samples, and environmental samples from the food production area.[6]

- Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. In general, an amount of test portion is added to a quantity of pre-warmed Buffered Peptone Water (REF 401278) to yield a tenfold dilution (e.g., 25 g test portion is mixed with 225 mL of Buffered Peptone Water).
- Incubate between 34 °C and 38 °C for 18 h ± 2 h.
- Transfer 0.1 mL of the culture obtained in Buffered Peptone Water to the surface of a MSRV Agar plate or to a tube containing 10 mL of RVS broth (REF 401981). Inoculate the MSRV agar with one to three equally spaced spots on the surface of the medium.
- Transfer 1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of Muller Kauffmann Tetrathionate Novobiocin Broth (REF 401745 - MKTTn Broth).
- Incubate the inoculated MSRV Agar plates (or RVS broth tubes) at 41.5 °C ± 1 for 24 h ± 3 h. Do not invert the plates.
- Incubate the inoculated MKTTn Broth between 34 °C and 38 °C for 24 h ± 3 h.
- From MSRV medium (or RVS broth) and MKTTn broth transfer a loopful of growth on a plate of XLD Agar ISO Formulation (code 402208) and on another selective medium for *Salmonella* based on different diagnostic characteristics to those of XLD agar (e.g. Chromogenic Salmonella Agar REF 405350). With MSRV medium positive plates use a 1 µL loop, with MKTT Broth use a 10 µL loop.
- Incubate the XLD plates inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use.

Summary of ISO 6579 procedure for samples from the primary production stage.[6]

After pre-enrichment in Buffered Peptone Water inoculate only the MRSV medium as described above. However, the sensitivity of the method can be improved by using a second selective enrichment procedure, e.g. MKTTn broth incubated at 41.5 °C for 24 h.

NOTES

After incubation, it is permissible to store the pre-enriched sample and selective enrichment at 2-8 °C for a maximum of 72 h.[6]
In dried milk products and cheese, *Salmonella* may be sub lethally injured. Incubate the selective enrichment media from these products for an additional 24 h ± 3 h. When investigating outbreak samples, this additional incubation time may also be beneficial.[6]
Refer to the ISO Standard for the detailed procedures.

READING AND INTERPRETATION

Suspect MSRV plates will show a grey-white, turbid zone extending out from the inoculated drop. If the plates are negative after 24 hours, re-incubate for a further 24 h ± 3 h.
Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of β-galactosidase (optional), indole detection (optional).[6] Serological confirmation includes the detection of the presence of *Salmonella* O- and H-antigens.
Biochemical confirmation can be substituted with the rapid MUCAP Test (REF 191500). All the colonies MUCAP Test positive must be serologically confirmed.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[6]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. Enteritidis ATCC 13076	41.5 °C ± 1 °C / 24-48 h / A	growth with turbid zone
S. Typhimurium ATCC 14028	41.5 °C ± 1 °C / 24-48 h / A	growth with turbid zone
E. faecalis ATCC 29212	41.5 °C ± 1 °C / 48 h / A	inhibited
E. coli ATCC 25922	41.5 °C ± 1 °C / 48 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The combination of malachite green, magnesium chloride and low pH does not allow the growth of certain *Salmonella* serovars such as *Salmonella* Typhi and *Salmonella* Paratyphi A.
- The medium is not suitable for detecting non-motile strains of *Salmonella*, which occur in any case with a very low incidence (less than 0.1%). If the presence of non-motile strains is suspected, a conventional method with pre-enrichment and selective enrichment is recommended.
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light.

Freeze-dried supplement

Store the product in the original package at 2-8°C away from direct light.
According to ISO 6579-1 the self-prepared plates can be stored with surface upwards, and protected from drying for up to two weeks at +2°C +8°C in the dark.[6]

REFERENCES

1. De Smedt JM, F Bolderdijk RF, Rappold H, Lautenschlaeger D. Rapid Salmonella Detection in Foods by Motility Enrichment on a Modified Semi-Solid Rappaport-Vassiliadis Medium. J Food Prot. 1986 Jul;49(7):510-514.
2. De Smedt JM, Chartron S, Cordier JL, Graff E, Hoekstra H, Lecoupeau JP, Lindblom M, Milas J, Morgan RM, Nowacki R, et al. Collaborative study of the International Office of Cocoa, Chocolate and Sugar Confectionery on Salmonella detection from cocoa and chocolate processing environmental samples. Int J Food Microbiol. 1991 Aug;13(4):301-8.

3. De Smedt JM, Bolderdijk R, Milas J. Salmonella detection in cocoa and chocolate by motility enrichment on modified semi-solid Rappaport-Vassiliadis medium: collaborative study. J AOAC Int . 1994 Mar-Apr;77(2):365-73.
4. Perales I, Erkiaga E. Comparison between semisolid Rappaport and modified semisolid Rappaport-Vassiliadis media for the isolation of Salmonella spp. from foods and feeds. Int J Food Microbiol. 1991 Oct;14(1):51-7
5. Veenman C, Korver H, Mooijman KA. Improvements in the method for detection of Salmonella spp. In animal faeces. National Institute for Public Health and the Environment, Bilthoven, the Netherlands. RIVM report 330300 010. 2007.
6. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp. - ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC

PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis Semisolid Medium (MSRV)	Dehydrated medium	4019822	500 g (15.8 L)
Novobiocin Antimicrobic Supplement	Freeze-dried supplement	4240045	10 vials, 10 mg/vial

IFU rev 1, 2022/11

RAPPAPORT VASSILIADIS SOY (RVS) BROTH

Dehydrated and ready-to-use culture medium

INTENDED USE

Liquid medium for the selective enrichment of *Salmonella* in food, water and environmental samples.

COMPOSITION - TYPICAL FORMULA ^* (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES AND FLASKS

Soy peptone	4.500 g
Sodium chloride	7.200 g
Potassium dihydrogen phosphate	1.260 g
Dipotassium hydrogen phosphate	0.180 g
Magnesium chloride anhydrous	13.400 g
Malachite green oxalate	0.036 g

^ The reported formulation uses anhydrous ingredients to better preserve the powder. The Biolife formulation per litre corresponds to the ISO Standard formulation, which refers to a total volume of 1110 mL.
*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Rappaport in 1956[1] devised an enrichment broth for *Salmonella* that included malachite green and magnesium chloride as inhibitors. Vassiliadis in 1976[2] modified the Rappaport medium by reducing to one-third the concentration of malachite green and incubating at 43°C instead of 37°C. Van Schothorst and Renaud[3] reported that the use of soy peptone instead of animal peptone improved recovery rates of *Salmonella*. Rappaport Vassiliadis Soy (RVS) Broth is based on the formulation of Van Schothorst and Renaud and meets the requirements of ISO 6579-1[4] and ISO 19250[5].
Rappaport Vassiliadis Soy (RVS) Broth is used as a selective enrichment medium for the isolation of *Salmonella* from food, water and environmental specimens with incubation at 41.5 °C, after the pre-enrichment in Buffered Peptone Water.
The efficiency of this enrichment medium is based on the ability of *Salmonella* spp. to multiply at relatively high osmotic pressures, at relatively low pH values, at a high temperature and with reduced nutritional requirements.[6]
Essential growth factors are provided by soy peptone; malachite green is inhibitory to organisms other than salmonellae; the high osmotic pressure of the medium due to the high concentrations of magnesium chloride, together with the acid pH, act as inhibitors of the saprophytic flora, favouring the growth of *Salmonella*. Magnesium chloride in addition counteracts the toxic effect of malachite green for salmonellae. Phosphates are used as buffering agents to control the pH in the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 26.6 g in 1000 mL of cold purified water. Heat to dissolve, distribute 10 mL into screw-cap tubes and sterilise by autoclaving at 115 °C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	blue-green, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	blue, clear
Final pH at 20-25 °C	5.2 ± 0.2

SPECIMENS

Food, water and environmental specimens. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.[4,5]

TEST PROCEDURE

Food samples

The following method is a summary taken from the ISO 6579-1.[4]

1. Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. In general, an amount of test portion is added to a quantity of pre-warmed Buffered Peptone Water (REF 401278) to yield a tenfold dilution (e.g., 25 g test portion is mixed with 225 mL of Buffered Peptone Water).
2. Incubate between 34 °C and 38 °C for 18 h ± 2 h.
3. Transfer 0.1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of the RVS broth or to the surface of a MSRV Agar plate (REF 401982).
4. Transfer 1 mL of the culture obtained in Buffered Peptone Water to a tube containing 10 mL of Muller Kauffmann Tetrathionate Novobiocin Broth (REF 401745 - MKTTn Broth).
5. Incubate the inoculated RVS Broth (or MSRV plates) at 41.5 °C ± 1 for 24 h ± 3 h.
6. Incubate the inoculated MKTTn Broth between 34 °C and 38 °C for 24 h ± 3 h.

7. From RVS Broth or MSRV medium and MKTT Broth transfer a loopful of growth on a plate of XLD Agar (code 402208) and on another selective medium for *Salmonella* based on different diagnostic characteristics to those of XLD agar (e.g. Chromogenic Salmonella Agar REF 405350). With MSRV medium positive plates use a 1 µL loop, with MKTTn Broth use a 10 µL loop.
8. Incubate the XLD plates inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use.

NOTES

After incubation, it is permissible to store the pre-enriched sample and selective enrichment at 2-8 °C for a maximum of 72 h.[4]
In dried milk products and cheese, *Salmonella* may be sub lethally injured. Incubate the selective enrichment media from these products for an additional 24 h ± 3 h. When investigating outbreak samples, this additional incubation time may also be beneficial.[4]
Refer to the ISO Standard for the detailed procedures.

Water samples

The following method is a summary taken from the ISO 19250.[5]

1. Non-selective pre-enrichment for volumes < 10 mL: inoculate 50 mL of Buffered Peptone Water with the sample or dilutions thereof and incubate at 36 ± 2 °C for 18 ± 2 h.
2. Non-selective pre-enrichment for volumes > 10 mL: filter a volume of water appropriate for the water being examined. Place the membrane filter into 50 mL of Buffered Peptone Water. Alternatively, add the sample to the same volume of double strength Buffered Peptone Water. Note that this latter procedure is not suitable for mineral waters with high salt content or sea water.
3. Incubate the cultures at 36 ± 2 °C for 18 ± 2 h.
4. Transfer 0.1 mL to 10 mL of RVS Broth and incubate at 41.5 °C ± 1 °C for 24 h ± 3 h and, if necessary for 48 ± 4 h.
5. From the tubes of RVS Broth transfer a loopful of growth on a plate of XLD Agar ISO Formulation (code 402208) and on another selective medium for *Salmonella* based on different diagnostic characteristics to those of XLD agar (e.g., Chromogenic Salmonella Agar REF 405350).
6. Incubate the XLD plates inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use.

NOTES

For waste water it has been shown that shorter incubation times of pre-enrichment culture or direct inoculation of the sample in selective medium produces better results.[5]
To detect slow growing *Salmonella* species, incubate the RVS broth for a total of 48 ± 4 hours.[5]
Refer to the ISO Standard for the detailed procedures.

READING AND INTERPRETATION

After incubation, the growth of organisms in RVS Broth is indicated by a milky appearance of the broth or by turbidity.
Refer to the instructions for use of the two plated media for the description of *Salmonella* colony characteristics.
Mark suspect colonies on each plate. Select suspect colony for subculture and confirmation.
Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of β-galactosidase (optional), indole detection (optional).[4] Serological confirmation includes the detection of the presence of *Salmonella* O- and H-antigens.
Biochemical confirmation can be substituted with the rapid MUCAP Test (REF 191500). All the colonies MUCAP Test positive must be serologically confirmed.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028 + *P. aeruginosa* ATCC 27853 + *E. coli* ATCC 25922	41.5 °C ± 1 °C / 24 h ± 3/ A	> 10 typical colonies after subculture on XLD Agar
E. faecalis ATCC 29212	41.5 °C ± 1 °C / 24 h ± 3/ A	< 100 colonies after subculture on TSA
E. coli ATCC 25922	41.5 °C ± 1 °C / 24 h ± 3/ A	partially inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The combination of malachite green, magnesium chloride and low pH does not allow the growth of certain *Salmonella* serovars such as *Salmonella* Typhi and *Salmonella* Paratyphi A.
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579-1 prepared tubes and flasks of RVS Broth may be stored at 2-8°C for up to three months.[4]

Ready-to-use medium in flasks/tubes

Store flasks/tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Konforti K, Navon B, Rappaport F. A new enrichment medium for certain salmonellae. J Clin Pathol 1956; 9:261-266.
2. Vassiliadis P, Pateraki E, Papiconomou N, Papadakis J, Trichopoulos D. Nouveau procède d'enrichissement de salmonella. Ann. Microb. Irist, Pasteur 1976; 127 B: 195.
3. Van Schothorst M, Renaud AM. Dynamics of salmonella isolation with modified Rappaport's medium (R10). J Appl Bacteriol 1983; 54:209
4. ISO 6579-1:2017 Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of Salmonella -- Part 1: Detection of Salmonella spp. - ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC
5. ISO 19250:2010. Water quality -- Detection of Salmonella spp.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:254-255.

PACKAGING

Product	Type	REF	Pack
Rappaport Vassiliadis Soy (RVS) Broth	Dehydrated medium	4019812	500 g (18.7 L)
RVS Broth	Ready-to-use tubes	551981	20 x 10 mL
RVS Broth	Ready-to-use flasks	5119812	6 x 100 mL

IFU rev 2, 2022/08

ROGOSA BIOS AGAR

Dehydrated and ready-to-use culture medium

L. rhamnosus on Rogosa Bios Agar

INTENDED USE

In vitro diagnostics. Culture medium for the selective isolation and enumeration of lactobacilli from clinical specimens and foodstuffs.

COMPOSITION *

ROGOSA BIOS AGAR, DEHYDRATED MEDIUM

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptozimatic	2.00 g
Tryptone	4.00 g
Yeast extract	9.00 g
Glucose	10.00 g
Arabinose	5.00 g
Sucrose	5.00 g
Sodium acetate	15.00 g
Ammonium citrate	2.00 g
Potassium dihydrogen phosphate	6.00 g
Magnesium sulphate	0.57 g
Manganous sulphate	0.12 g
Ferrous sulphate	0.03 g
Agar	15.00 g

ROGOSA BIOS AGAR, READY-TO-USE PLATES

TYPICAL FORMULA

Rogosa Bios Agar	73.7 g
Tween 80	1.00 mL
Acetic acid	1.32 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactobacilli are large, Gram-positive aerotolerant anaerobes or microaerophilic, rod-shaped, non-spore-forming bacteria. Lactobacilli are regarded as beneficial members of the human microbiota at a number of body sites, such as oral cavity, gastro-intestinal tract, and female genital system, but they can infrequently act as opportunistic pathogens in both children and adults.[1]

Lactobacilli are particularly associated with advanced dental caries where they are considered a secondary colonizer but probably play a role in exacerbating existing lesions and have been associated to a multitude of various infections including bacteriemia, endocarditis, peritonitis, chorioamnionitis, meningitis and intra-abdominal abscesses.[1] The depletion of lactobacilli from the vaginal microbiota and the increased bacterial diversity are characteristic feature of bacterial vaginosis.[1]

Rogosa Bios Agar is prepared according to a modification of the formula proposed by Rogosa, Mitchell and Wiseman[2,3] and is intended for the isolation and enumeration of lactobacilli from clinical specimens and foodstuffs.[1,4,5]

The medium contains two peptones and yeast extract as sources of nitrogen, carbon and vitamins, necessary for microbial growth. Dextrose, arabinose and sucrose provide carbon and are sources of energy. Tween 80 acts as surfactant and provides fatty acids required for the metabolism of lactobacilli. Ammonium citrate and sodium acetate inhibit the growth of streptococci, moulds, and other oral microbial flora and restrict *Proteus* swarming. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate, ferrous sulphate and manganous sulphate are sources of inorganic ions for the optimal growth of lactobacilli. Acetic acid reduces the pH of the medium to acidic values.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 73.7 g in 1000 mL of cold purified water. Add 1 mL of Tween 80 and 1.32 mL of 96% glacial acetic acid. Heat to boiling with frequent agitation, boil for 2-3 minutes. Do not autoclave. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	Yellowish, fine, free-flowing powder
Solution and plated medium appearance	yellow, limpid
Final pH at 20-25°C	5.4 ± 0.2

SPECIMENS

Rogosa Bios Agar plates can be directly inoculated with a variety of clinical specimens such as faeces, saliva, vaginal specimens.[4,5] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[1] Collect specimens before antimicrobial therapy where possible. Rogosa Bios Agar may be used for inoculation of foodstuffs: consult appropriate Standard Methods for detailed information.[6] The medium is not suitable for isolation of dairy lactobacilli.[4]

TEST PROCEDURE

Allow plates to come to room temperature. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For quantitative evaluation use appropriate inoculation techniques. Incubate for 3 days at 35°C or for 5 days at 30°C.[2] Lactobacilli prefer a microaerophilic atmosphere, therefore an incubation in a 5-10% CO_2-supplemented atmosphere or in anaerobic conditions are recommended by some authors.[2,3,4]

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Lactobacilli appear as large (2-3 mm in diameter), whitish, smooth, circular colonies.
Other lactic acid bacteria may also grow on this medium and produce similar types of colonies. Most other organisms are inhibited on Rogosa Bios Agar although enterococci and pediococci may show delayed growth. Both enterococci and pediococci will produce very small colonies with a diameter of 0.5 to 1.0 mm.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. rhamnosus ATCC 7469	35-37°C / 44-48 H / CO_2	growth
S. aureus ATCC 25923	35-37°C / 44-48 H / CO_2	inhibited

CO_2: 5-10% CO_2; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- In general *Lactobacillus* spp. can be selectively cultured using agar media with an acidic pH such as Rogosa medium, though some more fastidious strains may not grow on these media.[1]
- It is advisable to inoculate, together with Rogosa Bios Agar, conventional blood agar media.[1]
- The medium should not be used for maintenance of lactobacilli; transfer colonies for further tests as soon as possible.[4]
- The salt in the formulation makes the medium unsuitable for isolation of dairy lactobacilli: *L. lactis, L. bulgaricus* and *L. helveticus*.[4]
- Other organisms such as enterococci, pediococci and *Leuconostoc* species may grow on this medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS
Dehydrated medium
Store at +2°C/+8°C away from direct light in a dry place.
According to Baird RM et al. the self-prepared plates may be stored at +2°C/+8°C for up to 7 days.[7]
Ready-to-use plates
Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Butler-Wu SM, She RC. *Actinomyces, Lactobacillus, Cutibacterium* and other non-spore-forming Gram-positive rods. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Rogosa M, Mitchell JA, Wiseman RF. A selective medium for the isolation and enumeration of oral and fecal lactobacilli J Bact 1951; 62:132
3. Rogosa M, Mitchell JA, Wiseman RF. A selective medium for the isolation and enumeration of oral lactobacilli. J Dent Res 1951; 30(5):682
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997.
6. Hall, Ledenbach and Flowers. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C. 2001.
7. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:256:7.
8.

PACKAGING
Product	Type	REF	Pack
Rogosa Bios Agar	Dehydrated medium	4019852	500 g (6,8 L)
Rogosa Bios Agar	Ready-to-use plates	541985	2 x 10 plates ø 90 mm

IFU rev 1, 2022/03

ROGOSA BIOS BROTH
Dehydrated culture medium

INTENDED USE
Culture medium for the selective isolation and enumeration of lactobacilli.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Peptozimatic	2.00 g
Tryptone	4.00 g
Yeast extract	9.00 g
Glucose	10.00 g
Arabinose	5.00 g
Sucrose	5.00 g
Sodium acetate	15.00 g
Ammonium citrate	2.00 g
Potassium dihydrogen phosphate	6.00 g
Magnesium sulphate	0.57 g
Manganous sulphate	0.12 g
Ferrous sulphate	0.03 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Lactobacilli are large, Gram-positive aerotolerant anaerobes or microaerophilic, rod-shaped, non-spore-forming bacteria. Lactobacilli are regarded as beneficial members of the human microbiota at a number of body sites, such as oral cavity, gastro-intestinal tract, and female genital system, but they can infrequently act as opportunistic pathogens in both children and adults.[1]
Lactobacilli are particularly associated with advanced dental caries where they are considered a secondary colonizer but probably play a role in exacerbating existing lesions and have been associated to a multitude of various infections including bacteriemia, endocarditis, peritonitis, chorioamnionitis, meningitis and intra-abdominal abscesses.[1] The depletion of lactobacilli from the vaginal microbiota and the increased bacterial diversity are characteristic feature of bacterial vaginosis.[1]
Rogosa Bios Broth is prepared according to a modification of the formula proposed by Rogosa, Mitchell and Wiseman[2,3] and is intended for the isolation and enumeration of lactobacilli.[1,4,5]
The medium contains two peptones and yeast extract as sources of nitrogen, carbon and vitamins, necessary for microbial growth. Dextrose, arabinose and sucrose provide carbon and are sources of energy. Tween 80 acts as surfactant and provides fatty acids required for the metabolism of lactobacilli. Ammonium citrate and sodium acetate inhibit the growth of streptococci, moulds, and other oral microbial flora and restrict *Proteus* swarming. Potassium dihydrogen phosphate buffers the medium. Magnesium sulphate, ferrous sulphate and manganous sulphate are sources of inorganic ions for the optimal growth of lactobacilli. Acetic acid reduces the pH of the medium to acidic values.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 58.7g in 1000 mL of cold purified water. Add 1 mL of Tween 80 and 1.32 mL of 96% glacial acetic acid. Heat to boiling with frequent agitation, boil for 2-3 minutes. Do not autoclave.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	Yellowish, fine, free-flowing powder
Solution and plated medium appearance	yellow, limpid
Final pH at 20-25°C	5.4 ± 0.2

SPECIMENS

Rogosa Bios Broth tubes can be directly inoculated with a variety of specimens. Good laboratory practices for collection, transport and storage of the specimens should be applied. The medium is not suitable for isolation of dairy lactobacilli.[4]

TEST PROCEDURE

Inoculate the specimen directly into test tubes.
Incubate for 3 days at 35°C or for 5 days at 30°C.[2] Lactobacilli prefer a microaerophilic atmosphere, therefore an incubation in a 5-10% CO_2-supplemented atmosphere or in anaerobic conditions are recommended by some authors.[2,3,4]

READING AND INTERPRETATION

After incubation, observe the bacterial growth (turbidity of the medium in tubes).

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
L. rhamnosus ATCC 7469	35-37°C / 44-48 H	growth
S. aureus ATCC 25923	35-37°C / 44-48 H	inhibited

CO_2: 5-10% CO_2; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- In general *Lactobacillus* spp. can be selectively cultured using media with an acidic pH such as Rogosa medium, though some more fastidious strains may not grow on these media.[1]
- It is advisable to inoculate, together with Rogosa Bios Broth, conventional blood agar media.[1]
- The medium should not be used for maintenance of lactobacilli; transfer colonies for further tests as soon as possible.[4]
- The salt in the formulation makes the medium unsuitable for isolation of dairy lactobacilli: *L. lactis, L. bulgaricus* and *L. helveticus*.[4]
- Other organisms such as enterococci, pediococci and *Leuconostoc* species may grow on this medium.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +2°C /+8°C away from direct light in a dry place.
According to MacFaddin it is advisable to use the plates on the day of their preparation

REFERENCES

1. Butler-Wu SM, She RC. *Actinomyces, Lactobacillus, Cutibacterium* and other non-spore-forming Gram-positive rods. *In* Carrol KC, Pfaller MA *et al*. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Rogosa M, Mitchell JA, Wiseman RF. A selective medium for the isolation and enumeration of oral and fecal lactobacilli J Bact 1951; 62:132
3. Rogosa M, Mitchell JA, Wiseman RF. A selective medium for the isolation and enumeration of oral lactobacilli. J Dent Res 1951; 30(5):682
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Rogosa Bios Broth	Dehydrated medium	4019902	500 g (8.5 L)

IFU rev 1, 2022/05

ROSE BENGAL AGAR BASE

Dehydrated culture medium

INTENDED USE

For the enumeration of yeasts and moulds in foods, animal feeding stuffs and waters.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Mycological peptone	5.00 g
Dipotassium hydrogen phosphate	1.00 g
Magnesium sulphate	0.50 g
Glucose	10.0 g
Rose bengal	0.05 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Rose Bengal Agar is based on the formulation devised by Jarvis[1] and modified by Overcast and Weakley[2], in which chlortetracycline has been replaced by chloramphenicol. It is a selective medium at neutral pH for the enumeration of yeasts and moulds in foods and is recommended for fresh proteinaceous foods whose associated flora is composed mainly by Gram-negative bacilli.[3]
Rose Bengal Agar, supplemented with chlortetracycline, is recommended by APHA[4] for the enumeration of fungi in sewage and polluted waters with spread plate and membrane filter techniques.
The culture medium proposed here is a base to which an antibiotic supplement of the user's choice can be added, to avoid the risks associated with the use of powdered media containing antibiotics.
Mycological peptone provides nitrogen and minerals for microbial growth and colonies pigmentation. Glucose is a source of carbon and energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium. Magnesium sulphate enhances the microbial growth. Rose bengal not only restricts the size and height of mould colonies but assists their enumeration as the colour is taken up by the colonies. Chloramphenicol or chlortetracycline are used as the selective agents to suppress most Gram-positive and Gram-negative bacteria.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 16 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of Chloramphenicol Antimicrobic Supplement (REF 4240003) reconstituted with 3 mL of a mixture of sterile purified water-ethanol (1:1). Mix well and distribute into sterile Petri dishes. The chloramphenicol supplement can also be added to the culture medium before sterilisation. The basic medium can also be supplemented with chlortetracycline hydrochloride 70 mg/L (Dermatophyte Antimicrobic Supplement REF 4240024) after autoclaving.
Avoid exposure of the medium to light.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	bright pink, clear
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Foods, animal feeding stuffs and water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Prepare suitable decimal dilutions of the samples.
Add 1 mL to empty Petri dishes using two dishes for each dilution. Pour into each dish approximately 15 mL of melted medium, cooled to 44-47°C. Mix gently, allowing the medium to solidify.
Alternatively, directly inoculate the agar plates using surface spreading technique with 0.1 or 0.2 mL of decimal dilutions.
Alternatively use membrane filter technique: filter an appropriate volume of well-shaken sample or dilution through membrane filters with pore diameter of 0.45 µm and transfer to dishes.
Invert the plates and incubate at 22°C for 5-7 days.

READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies
Count colonies on plates that contain an estimated 50-100 colonies. Report as number of yeasts or moulds per gram of food by multiplying the number of colonies by the dilution factor.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25°C/ 72h/A	growth
Aspergillus brasiliensis ATCC 16404	25°C/ 72h/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25°C/ 72h/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[5]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[5]

- Non-linearity of counts from dilution plating often occurs, i.e., 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[5]
- For complete identification of isolated microorganisms, it is recommended to perform appropriate tests using pure cultures.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to Baird *et al.* the self-prepared plates with chloramphenicol can be stored in the dark at 2 to 8°C for 7 days.[6] According to APHA the plated medium with chlortetracycline may be held up to 4 weeks at 2 to 8°C.[4]

REFERENCES

1. Jarvis B. Comparison of an improved rose-bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J Appl Bacteriol 1973; 36: 723-727.
2. Overcast WW, Weakley DJ. An aureomycin-rose Bengal agar for the enumeration of yeasts and moulds in cottage cheese. J Milk Food Technol 1969; 32:442.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. APHA Standards Methods for the Microbiological of Water and Wastewater. 23rd, 2017. American Public Health Association, Washington D.C.,
5. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:261-262.

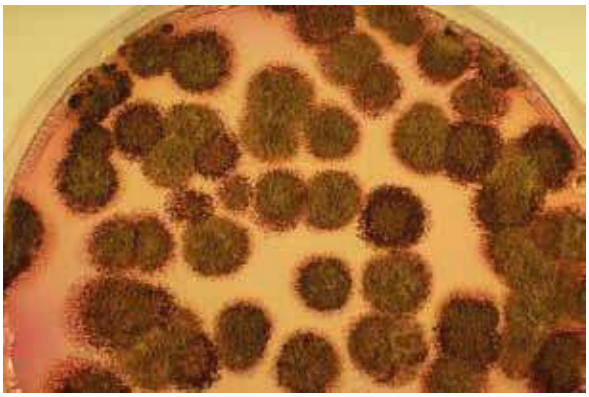
PACKAGING

Product	Type	REF	Pack
Rose Bengal Agar Base	Dehydrated medium	4019912	500 g (15.6 L)

IFU rev 2, 2022/09

ROSE BENGAL AGAR WITH CHLORAMPHENICOL

Dehydrated and ready-to-use culture medium



Rose Bengal Chloramphenicol Agar:
colonies of *Aspergillus brasiliensis*

INTENDED USE

For the enumeration of yeasts and moulds in foods and animal feeding stuffs.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Mycological peptone	5.00 g
Dipotassium hydrogen phosphate	1.00 g
Magnesium sulphate	0.50 g
Glucose	10.0 g
Rose bengal	0.05 g
Agar	15.00 g
Chloramphenicol	0.10 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Rose Bengal Chloramphenicol Agar is based on the formulation devised by Jarvis[1] and modified by Overcast and Weakley[2], in which chlortetracycline has been replaced by chloramphenicol. It is a selective medium at neutral pH for the enumeration of yeasts and moulds in foods and is recommended for fresh proteinaceous foods whose associated flora is composed mainly by Gram-negative bacilli.[3]
Mycological peptone provides nitrogen and minerals for microbial growth and colonies pigmentation. Glucose is a source of carbon and energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium. Magnesium sulphate enhances the microbial growth. Rose Bengal not only restricts the size and height of mould colonies but assists enumeration in that the colour is taken up by fungi. Chloramphenicol is used as the selective agent to suppress most Gram-positive and Gram-negative bacteria.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 32 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Cool to 47-50°C, mix well and distribute into sterile Petri dishes. Avoid exposure of the medium to light.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	bright pink, clear
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Prepare suitable decimal dilutions of the samples.
Add 1 mL to empty Petri dishes using two dishes for each dilution. Pour into each dish approximately 15 mL of melted medium, cooled to 44-47°C. Mix gently, allowing the medium to gel.
Alternatively, directly inoculate the agar plates using surface spreading technique with 0.1 or 0.2 mL of decimal dilutions.
Invert the plates and incubate at 22°C for 5 days.

READING AND INTERPRETATION
After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Count colonies on plates that contain an estimated 50-100 colonies. Report as number of yeasts or moulds per gram of food by multiplying the number of colonies by the dilution factor.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Saccharomyces cerevisiae ATCC 9763	25°C/72h/A	growth
Aspergillus brasiliensis ATCC 16404	25°C/72h/A	growth with limited colony spreading
Escherichia coli ATCC 25922	25°C/72h/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- The spores of moulds disperse in the air with a great facility, handle the Petri dishes with care to avoid development of satellite colonies which would give an overestimation of population in the sample.[4]
- Enumeration methods for yeasts and especially moulds are imprecise because they consist of a mixture of mycelium and asexual and sexual spores. Numbers of colony-forming units depend on the degree of fragmentation of mycelium and the proportion of spores able to grow on the plating medium.[4]
- Non-linearity of counts from dilution plating often occurs, i.e. 10-fold dilutions of samples often do not result in 10-fold reductions in numbers of colonies recovered on plating media. This has been attributed to fragmentation of mycelia and breaking of spore clumps during dilution in addition to competitive inhibition when large numbers of colonies are present on plates.[4]
- For complete identification of isolated microorganisms, it is recommended to perform appropriate tests using pure cultures.

STORAGE CONDITIONS
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to Baird *et al.* the self-prepared plates can be stored in the dark at 2-8°C for 7 days.[5]
Ready-to-use plates
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Jarvis B. Comparison of an improved rose-bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J Appl Bacteriol 1973; 36: 723-727.
2. Overcast WW, Weakley DJ. An aureomycin-rose Bengal agar for the enumeration of yeasts and moulds in cottage cheese. J Milk Food Technol 1969; 32:442.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. ISO 21527-1:2008. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0,95.
5. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:261-262.

PACKAGING
Product	Type	REF	Pack
Rose Bengal Agar with Chloramphenicol	Dehydrated medium	4019922	500 g (15.6 L)
Rose Bengal CAF Agar	Ready-to-use plates	541991C	2 x 10 plates ø 90 mm

IFU rev 1, 2022/09

RPMI AGAR
Ready-to-use plates

INTENDED USE
In vitro diagnostic device. Culture medium for quantitative determination of susceptibility to antifungal agents by gradient-based strips.

COMPOSITION - TYPICAL FORMULA *
RPMI 1640	10.4 g
MOPS **	34.5 g
Glucose	20.0 g
Agar	15.0 g
Purified water	1000 mL

* The formula may be adjusted and/or supplemented to meet the required performances criteria.
** 3-(N-morpholino) propanesulfonic acid

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
The reference tests for antifungal susceptibility testing are the broth microdilution assays devised by the Clinical and Laboratory Standards Institute (CLSI)[1] and by the European Committee on Antibiotic Susceptibility Testing (EUCAST) [2,3,4] These reference methods are time-consuming and poorly suited for the routine clinical laboratory setting.[5] To overcome these limitations some methods based on gradient strip testing have been developed and are commercially available for the laboratory. Such tests are based on the diffusion of a stable concentration gradient of an antimicrobial agent from a plastic or paper strip onto an agar medium. The medium devised for the detection of MICs with gradient-based strips is RPMI 1640 supplemented with agar, glucose and buffered with MOPS. This medium derives from the formulation recommended for the broth microdilution assays.[1]
RPMI 1640 was developed by Moore, Gerner, and Franklin[6] in 1967 at Roswell Park Memorial Institute, from where it derives its name. It contains vitamins, amino acids, salts, a pH indicator and it is widely used for cell cultures. When supplemented with MOPS, glucose and agar, RPMI 1640 demonstrated accurate MICs with antifungal agents on gradient-based strips, comparable with the results obtained with CLSI reference method.[4, 7-11]

PHYSICAL CHARACTERISTICS

Medium appearance	pink, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

RPMI Agar must be used with pure culture of fungal strains isolated from clinical specimens. RPMI Agar is not intended for microbial isolation directly from clinical specimens.

TEST PROCEDURE

Allow plates and antifungal gradient strips to equilibrate to room temperature. The surface of the agar should be dry before use.
Guidelines for inoculums preparation:
Inoculum for yeast: suspension in saline to 0.5 McFarland for *Candida* spp. and 1 McFarland for *C. neoformans*.
Inoculum for moulds: suspension of both conidia and hyphae (mature growth 5-7days) in saline with Tween to 0.5 McFarland for Aspergillus spp. e 1 McFarland for *Fusarium* and *Rhizopus* spp.
Dip a sterile cotton swab into the suspension and remove excess fluid by pressing and turning the swab against the inside of the tube.
With the cotton swab inoculate evenly the entire surface of the plate, taking care to check that t that there are no gaps between streaks. Dip the swab again and inoculate a second time. Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator.
As soon as the inoculum has been absorbed and the agar surface is dry, apply the gradient-strips. Make sure that the strips are in complete contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from strips is very rapid. The strips should be placed on the agar plate in a manner which does not result in overlapping zones of inhibition.
Guidelines for incubation:
Yeast: 35°C in air for 24-48 hours for *Candida* spp. and 48-72 hours for *C. neoformans*.
Aspergillus spp.: 35°C for 18-24 hours; *Fusarium* spp.: 35°C/24-48 hours, followed by room temperature for 24-48 hours; *Rhizopus* spp.: 35°C for 18-24 hours
For other species, extend the incubation time as needed, inspect plates daily for the formation of readable inhibition ellipse.
For the details of inoculation and incubation procedures consult the gradient-strips manufacturer's package insert.

READING AND INTERPRETATION

After incubation, read plates from the front with the lid removed and with reflected light.
A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light and the test must be repeated.
The growth should be evenly distributed over the agar surface to achieve a uniform inhibition ellipse.
Check that inhibition zones for quality control strain are within acceptable range.
Determination of the MIC is at the point at which the lower part of the bacterial growth ellipse intersects with the corresponding number on the test strip. For specific reading and interpretation instructions consult the gradient-strips manufacturer's package insert.

USER QUALITY CONTROL

All manufactured lots of RPMI Agar plates are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. For QC organisms testing, details are described in the strips manufacturer's package insert. At a minimum, at least one QC strain should be tested to ensure proper product functionality. Strain and the gradient-strips useful for the quality control: *C. parapsilosis* ATCC 22019 / amphotericin B, voriconazole. Refer to interpretation guidelines in IFU provided by manufacturer of antifungal gradient-strips.

LIMITATIONS OF THE METHOD

- Incorrect inoculum concentration, improper storage of antimicrobial strips, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.
- The inoculation, incubation and reading methods here described are to be considered as guidelines; strict adherence to the protocol suggested by gradient-strips manufacturer is required to ensure reliable results.
- Reading and interpretation require expertise and close adherence to the gradient-strips manufacturer instructions; problems can arise when inexperienced readers incorrectly interpret faint background growth of small colonies within the zones as resistance.[12]
- Agreement of gradient-strips testing and reference MICs medium may be species, drug and medium dependent.[13,14]
- This culture medium is intended as an aid in the treatment of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard CLSI document M27-A3. Clinical and Laboratory Standards Institute, Wayne, Pa. 2008.
2. Arendrup MC *et al*. EUCAST definitive document E.7.3.2 Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts. Valid from 22 April, 2020.
3. Guinea J *et al*. How to: EUCAST recommendations on the screening procedure E.Def 10.1 for the detection of azole resistance in Aspergillus fumigatus isolates using four-well azole-containing agar plates. Clin Microbiol Infect 2019; 25(6):681-687.
4. Arendrup MC *et al*. How to: perform antifungal susceptibility testing of microconidia-forming dermatophytes following the new reference EUCAST method E.Def 11.0, exemplified by Trichophyton. Clin Microbiol Infect 2021;27(1):55-60.
5. Ranque SL, Lachaud LM, Gari-oussaint L, Michel-Nguyen A, Mallié M, Gaudart J, Bertoutd S. Interlaboratory Reproducibility of Etest Amphotericin B and Caspofungin Yeast Susceptibility Testing and Comparison with the CLSI Method.J Clin Microbiol 2012; 50(7): 2305–2309. .
6. Moore GE, Gerner RE, Franklin HA. Culture of normal human leukocytes. JAMA 1967; 199 (8): 519–524
7. Pfaller MA, Messer SA, Mills K, Bolmström A, Jones RN. Evaluation of Etest method for determining caspofungin (MK-0991) susceptibilities of 726 clinical isolates of Candida species. J Clin Microbiol 2001;39 (12):4387-9.
8. Espinel-Ingroff A .. Etest for antifungal susceptibility testing of yeasts. Diagn Microbiol Infect Dis 1994;19(4):217-20
9. Pfaller MA, Messer SA, Houston A, Mills K, Bolmström A, Jones RN. Evaluation of the Etest method for determining voriconazole susceptibilities of 312 clinical isolates of Candida species by using three different agar media. J Clin Microbiol . 2000;38(10):3715-7.
10. Maxwell MJ, Messer SA, Hollis RJ, Diekema DJ,. Pfaller MA. Evaluation of Etest Method for Determining Voriconazole and Amphotericin B MICs for 162 Clinical Isolates of Cryptococcus neoformans. J Clin Microbiol. 2003; 41(1): 97–99.
11. Jong Hee Shin JH *et al*. Detection of amphotericin B resistance in Candida haemulonii and closely related species by use of the Etest, Vitek-2 yeast susceptibility system, and CLSI and EUCAST broth microdilution methods. J Clin Microbiol 2012;50(6):1852-5.
12. Johnson EM, Cavling-Arendrup M. Susceptibility Test Methods: Yeasts and Filamentous Fungi. *In*: Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

13. Shields RK, Nguyen MH, Press EG, Updike CL, Clany CJ. Anidulafungin and micafungin MIC breakpoints are superior to that of caspofungin for identifying FKS mutant candida glabrata atrains and echinocandin resitsance. Antimicrob Agents Chemoter 57:6361-6365
14. Axner-Elings M, Botero-Kleiven S, Jensen RH, Arendrup MC. Echinocandin susceptibility testing of Candida isolates collected during 1-year period in Sweden. J Clin Microbiol 2011; 49:2516-2521.

PACKAGING

Product	Type	REF	Pack
RPMI Agar	Ready-to-use plates	54RPMI90	2 x 10 plates ø 90 mm
RPMI Agar	Ready-to-use plates	54RPMI15	5 plates ø 150 mm

IFU rev 1, 2021/01

RPMI Agar: *Candida krusei* and voriconazole gradient-strip

SABOURAUD BROTH

Dehydrated culture medium

Sabouraud Broth – from left: uninoculated tube and tube with *Aspergillus brasiliensis*

INTENDED USE

In vitro diagnostics. Liquid medium for the cultivation of yeasts and moulds.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptic digest of animal tissue	5 g
Pancreatic digest of casein	5 g
Glucose	20 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Sabouraud Broth, also known as Sabouraud Dextrose Broth, is a modification proposed by Sabouraud to the Sabouraud Dextrose Agar medium, without agar and with half-concentration of glucose.[1,2]
Sabouraud Broth is a liquid medium intended for the cultivation of yeasts and moulds.[3] The medium is recommended for growth promotion test, for the preparation of the sample and its pre-enrichment in the procedure for the detection of *Candida albicans* in non-sterile products with EP harmonized method.[4] Sabouraud Broth complies with the quality specifications reported by European Pharmacopoeia.
Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen, carbon and trace elements for microbial growth. The low pH is favourable for the growth of fungi and aciduric microorganisms. Glucose, at high concentration is a carbon and energy source.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute in tubes or flasks and sterilize by autoclaving at 121°C for 15 minutes. Do not overheat.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25°C	5.6 ± 0.2

SPECIMENS

Sabouraud Broth is mainly intended for the cultivation of yeasts and moulds isolated on plate media from clinical and non-clinical specimens. In pharmaceutical microbiology, the samples consist of non-sterile products on which to detect *C. albicans*. Refer to the European Pharmacopoeia for sample collection and transport procedures.[4]

TEST PROCEDURE

Allow the tubes to reach room temperature.
General procedure
Inoculate each test strain or specimen into duplicate tubes. Incubate one tube at 22-25°C and the second at 35°C for 2-7 days.
The incubation conditions may vary according to the type of expected microorganisms and can be extended up to 30 days.
The user is responsible for choosing the appropriate incubation time, and temperature depending on the processed specimen or inoculated strain, the requirements of organisms to be recovered or cultivated and the local applicable protocols.
Detection of *C. albicans* in non-sterile pharmaceutical products.[4]
Prepare the sample suspension in 100 mL of Sabouraud Broth using at least 10 g or 10 mL of sample to be examined. Incubate this suspension at 30°C - 35°C for 3-5 days.
Using a loop, subculture from Sabouraud Broth onto Sabouraud Dextrose Agar plate (REF 402005) and incubate 30°C-35°C for 24-48 hours.
Growth of white colonies may indicate the presence of *C. albicans* to be confirmed by appropriate identification tests
The test is considered negative if such colonies are not present or if the identification tests are negative.

READING AND INTERPRETATION

After incubation, the presence of microbial growth is evidenced by the presence of turbidity compared to an un-inoculated control. The characteristic of the growth is closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans	ATCC 10231	20-25°C / up to 72 h / A	growth
A. brasiliensis	ATCC 16404	20-25°C / up to 72 h / A	growth
T. rubrum	ATCC 28188	20-25°C / up to 72 h / A	growth

For quality control in the pharmaceutical field refer to the current edition of European Pharmacopoeia.
A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Since Sabouraud Broth is a general-purpose medium with very poor selective properties, bacterial strains will also grow.
- If it is necessary to add antimicrobial compounds to the culture medium, it must be considered that their use may be inhibitory to certain fungi. *Cryptococcus neoformans, Trichosporum parapsilosis, Candida krusei, Candida tropicalis* are sensitive to the combination of cycloheximide,

penicillin and streptomycin. Cycloheximide is inhibitory towards saprophytic fungi. Chloramphenicol may be inhibitory towards some pathogenic fungi. Penicillin inhibits *Nocardia* and *Actinomyces*. The combination of cycloheximide and chloramphenicol is inhibitory to the growth of many pathogenic fungi.[5]

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin, the tubed and bottled medium prepared by the user can be stored at +2°C/+8°C for 6 months.[5]

Ready-to-use medium in tubes and flasks

Store flasks/flasks in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralite des trichophytons de l'homme. Ann Dermatol Syphil1892; 3:1061-1087.
3. Atlas R, Parks L. Media Handbook of Microbiological Media 2nd ed CRC Press, 1997
4. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Sabouraud Broth	Dehydrated medium	4020002 4020004	500 g (16.7 L) 5 kg (167 L)
Sabouraud Broth	Ready-to-use tubes	552000	20 x 9 mL
Sabouraud Broth	Ready-to-use flasks	5120002	6 x 100 mL

IFU rev 2, 2023/02

SABOURAUD DEXTROSE AGAR

Dehydrated and ready-to-use culture medium

Sabouraud Dextrose Agar: *Candida albicans*

INTENDED USE

In vitro diagnostics. General purposes medium for the isolation and cultivation of yeasts and moulds, from clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE PLATES AND FLASKS

Pancreatic digest of casein	5 g
Peptic digest of meat	5 g
Glucose	40 g
Agar	15 g
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The Italian lawyer and farmer Agostino Bassi in 1835 discovered the mycotic nature of an epidemic disease of silkworms called muscardine; this recognition of the relationship between fungi and disease was the basis for the development of medical mycology.[1] By the end of the 1890's, Raymond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for the their isolation and classification.[1,2]

Numerous experiments were performed to improve Sabouraud's formula with a variety of peptones and carbohydrates by Weidman and Spring[3] but the suitable medium was selected by Hodges[4]. In its final formulation, this medium contained a 1% peptone, 4% dextrose, and 1.8% agar, with a final pH of 5.0. This formulation was named Sabouraud medium and is the basic routine culture medium used to grow fungi in clinical laboratories.[1]

Sabouraud Dextrose Agar is a non-selective medium for the isolation and cultivation of yeasts and moulds, especially dermatophytes, from clinical specimens[5-7]. It is recommended for the total combined yeasts and moulds count and for the detection of *C. albicans* in non-sterile pharmaceutical products according to the harmonized EP, USP, JP method[8].

Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen, carbon and trace elements for microbial growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria. Glucose, at high concentration is a carbon and energy source.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 65 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and pour into sterile Petri dishes. Do not exceed the boiling and sterilization times and temperatures. Alternatively distribute in screw capped tubes before sterilization and solidify in slanted position.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates/flasks appearance	yellow, limpid
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Sabouraud Dextrose Agar can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimen types, related to specific infections.[5-7] Sabouraud Dextrose Agar is not suitable for direct inoculation of blood samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[5] For pharmaceutical samples, refer to the EP for details on sample collection and preparation.[8]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.
Inoculate each specimen in duplicate; incubate one set in aerobic condition at 22-25°C, the other at 33-37°C.[10]
For dermatophytes, examine cultures every 4-6 days for a period of up to 20 days; for others incubate 2-5 days. Plates should be incubated under conditions of increased humidity during prolonged incubation.
The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

For the detection of *C. albicans* in non-sterile pharmaceuticals products, the technique recommended by European Pharmacopoeia[8] and summarized below should be followed:

- Prepare a sample suspension in 100 mL of Sabouraud Broth using 10 mL of sample or the quantity corresponding to not less than 1 g of or 1 mL of the product to be examined. Mix and incubate at 30-35°C for 3-5 days.
- Subculture on a plate of Sabouraud Dextrose Agar and incubate at 30-35 °C for 24-48 hours.

READING AND INTERPRETATION

Clinical specimen: after incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and sub-culture to appropriate media for further identification tests.
Non-sterile pharmaceutical products: growth of white colonies indicates the possible presence of *C. albicans*; this is confirmed by identification tests. The test is to be considered negative if such colonies are not present or if the identification tests are negative.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	25-35°C / up to 72 h / A	good growth, white yeast-like colonies
T. mentagrophytes ATCC 9533	25-35°C / up to 72 h / A	good growth, white colonies with typical morphology

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Sabouraud Dextrose Agar has poor selective properties; a selective medium should be inoculated in parallel for isolation of fungi from potentially contaminated specimens.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin, the tubed and bottled medium prepared by the user can be stored at +2°C/+8°C for 6 months while the plated media can be stored at +2°C/+8°C for 6-8 weeks.[10]
Ready-to-use plates and flasks
Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralite des trichophytons de l'homme. Ann Dermatol Syphil1892; 3:1061-1087.
3. Weidman FD, Spring D. Comparison of ringworm culture ingredients: II and III. Arch Dermatol Syphilol 1928; 18:829–851.
4. Hodges RS. Cultures of ringworm fungi on Sabouraud's proof mediums and on mediums prepared with American peptones and sugars. Arch Dermatol Syphilol 1928; 18:852–856.
5. McGowan K. Specimen Collection, Transport and Processing: Mycology. *In* Jorgensen JH, Pfaller et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; Vol.2 2015.
6. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
7. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
8. European Pharmacopoeia, current edition
9. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

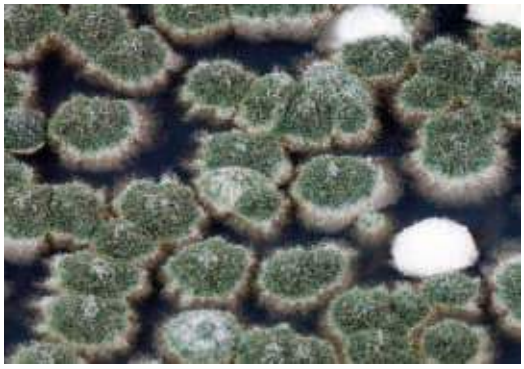
PACKAGING

Product	Type	REF	Pack
Sabouraud Dextrose Agar	Dehydrated medium	4020052 4020054	500 g (7.7 L) 5 kg (77 L)
Sabouraud Dextrose Agar	Ready-to-use plates	542005	2 x 10 plates ø 90 mm
Sabouraud Dextrose Agar	Ready-to-use flasks	5120052 5120053	6 x 100 mL 6 x 200 mL

IFU rev 2, 2022/11

SABOURAUD DEXTROSE AGAR w/ CAF 50 MG

Dehydrated and ready-to-use culture medium



A. restrictus on Sabouraud Dextrose Agar w/CAF 50 mg

INTENDED USE

In vitro diagnostics. Selective medium for the isolation and enumeration of yeasts and moulds in clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE PLATES, TUBES AND FLASKS

Pancreatic digest of casein	5.00 g
Peptic digest of meat	5.00 g
Glucose	40.00 g
Agar	15.00 g
Chloramphenicol	0.05 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

By the end of the 1890's, Raymond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for their isolation and classification.[1,2]
Numerous experiments were performed to improve Sabouraud's formula with a variety of peptones and carbohydrates by Weidman and Spring[3], but the suitable medium was selected by Hodges[4]. In its final formulation, this medium contained 1% peptone, 4% dextrose, and 1.8% agar, with a final pH of 5.0. This formulation was named Sabouraud medium and is the basic routine culture medium used to grow fungi in clinical laboratories. The components of Sabouraud Dextrose Agar conform to the recommendations of the current European Pharmacopoeia[5]. The addition of chloramphenicol is a modification designed to increase bacterial inhibition and improve the isolation of opportunistic fungi from contaminated specimens. Sabouraud Dextrose Agar w/ CAF 50 mg is a selective medium for the isolation of yeasts and moulds, mainly opportunist pathogens (*Aspergillus, Fusarium, Mucor, Rhizopus*, etc.), cycloheximide sensitive fungi such as *Cryptococcus neoformans* and *Allescheria boydii* and *Candida* spp. in clinical specimens and for the enumeration of yeasts and moulds in non-clinical samples such as cosmetics, as recommended by ISO 16212.[6]
Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen, carbon and trace elements for microbial growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria; the selective properties are increased by the presence of chloramphenicol, a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria. Glucose, at high concentration is a carbon and energy source.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 65 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.
Notes
Do not exceed the boiling and sterilization times and temperatures.
Alternatively distribute in screw capped tubes before sterilization and solidify in slanted position.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Sabouraud Dextrose Agar w/CAF 50 mg can be directly inoculated with many clinical specimens collected from various normally non-sterile human sites. Refer to the quoted literature for specimen types, related to specific infections.[7-8] Sabouraud Dextrose Agar w/CAF 50 mg is not suitable for direct inoculation of blood samples or other specimens from normally sterile sites. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[7] For cosmetics, refer to the ISO Standard for details of sample collection and preparation.[6]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.
Inoculate each specimen in duplicate; incubate one set in aerobic condition at 22-25°C, the other at 33-37°C.[9]
For dermatophytes, examine cultures every 4-6 days for a period of up to 20 days; for others incubate 2-5 days. Plates should be incubated under conditions of increased humidity during prolonged incubation.
The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

For the enumeration of yeasts and moulds in cosmetics, the technique recommended by ISO 16212[6] and summarized below for surface spread method, should be followed.
Spread over the surface of the medium a measured volume of not less than 0.1 ml of the initial suspension and/or sample dilution.
Incubate at 25 °C ± 2.5 °C for 3 to 5 days.
The ISO Standard describes also the pour-plate and the membrane filtration methods.

READING AND INTERPRETATION

Clinical specimen: after incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and sub-culture to appropriate media for further identification tests.
Cosmetics: after incubation, count the colonies in Petri dishes containing 15 colonies to 150 colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[10]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	20-25°C / ≤ 5 days/ A	good growth, white yeast-like colonies
T. mentagrophytes ATCC 9533	20-25°C / ≤ 5 days/ A	good growth, white colonies with typical morphology
A. brasiliensis ATCC 16404	20-25°C / ≤ 5 days/ A	good growth, white/black colonies with typical morphology
E. coli ATCC 25922	25-35°C / ≤ 5 days/ A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Chloramphenicol may inhibit pathogenic fungi.[9]
- Sabouraud Dextrose Agar w/CAF 50 has a poor efficacy in the isolation of *Histoplasma capsulatum* from potentially contaminated clinical specimens.[11]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of yeasts and moulds with lower selectivity such as Sabouraud Dextrose Agar or Potato Dextrose Agar and with higher selectivity such as Dermatophyte Test medium, should be used.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin, the tubed and bottled medium prepared by the user can be stored at +2°C/+8°C for 6 months while the plated media can be stored at +2°C/+8°C for 6-8 weeks.[12]
Ready-to-use plates, tubes and flasks
Store in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution à l'étude de la trichophytie humaine. Etude clinique, microscopique et bactériologique sur la pluralité des trichophytons de l'homme. Ann Dermatol Syphil 1892; 3:1061-1087.
3. Weidman FD, Spring D. Comparison of ringworm culture ingredients: II and III. Arch Dermatol Syphilol 1928; 18:829–851.
4. Hodges RS. Cultures of ringworm fungi on Sabouraud's proof mediums and on mediums prepared with American peptones and sugars. Arch Dermatol Syphilol 1928; 18:852–856.
5. European Pharmacopoeia, current edition
6. ISO16212:2017. Cosmetics -Microbiology -Enumeration of yeast and mould
7. McGowan K. Specimen Collection, Transport and Processing: Mycology. In Jorgensen JH, Pfaller et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; Vol.2 2015.
8. Public Health England- UK SMI B 17: tissues and biopsies from deep-seated sites and organs. 05.01.18
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
11. Unis G, da Silva VB, Severo LC. Disseminated histoplasmosis and AIDS. The role of culture medium for the bronchoscopic clinical specimens Rev Soc Bras Med Trop. 2004; 37:234-7
12. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

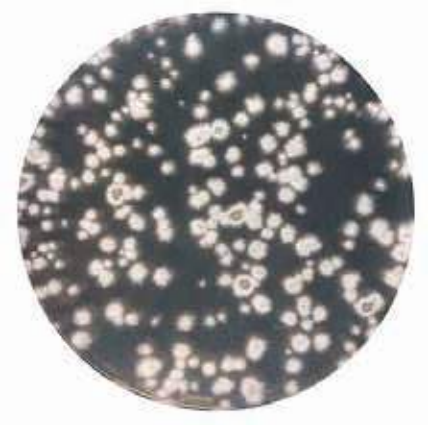
PACKAGING

Product	Type	REF	Pack
Sabouraud Dextrose Agar w/CAF 50 mg	Dehydrated medium	4020062 4020064	500 g (7.7 L) 5 kg (77 L)
Sabouraud Dextrose Agar + CAF	Ready-to-use plates	542006	2 x 10 plates ø 90 mm
Sabouraud Dextrose Agar + CAF	Ready-to-use tubes	552006	20 glass tubes with slanted medium, 17x125 mm,
Sabouraud Dextrose Agar w/CAF 50	Ready-to-use flasks	5120062 5120063	6 x 100 mL 6 x 200 mL

IFU rev 3, 2022/01

SABOURAUD DEXTROSE AGAR CAF 500

Dehydrated culture medium



Sabouraud Dextrose Agar CAF 500: *Aspergillus* sp.

INTENDED USE

In vitro diagnostic. Selective medium for the isolation of yeasts and moulds in clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	5.0 g
Peptic digest of meat	5.0 g
Glucose	40.0 g
Agar	15.0 g
Chloramphenicol	0.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

By the end of the 1890's, Raymond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for their isolation and classification.[1,2]
Numerous experiments were performed to improve Sabouraud's formula with a variety of peptones and carbohydrates by Weidman and Spring[3], but the suitable medium was selected by Hodges[4]. In its final formulation, this medium contained 1% peptone, 4% dextrose, and 1.8% agar, with a final pH of 5.0. This formulation was named Sabouraud medium and is the basic routine culture medium used to grow fungi in clinical laboratories.
The components of Sabouraud Dextrose Agar conform to the recommendations of the current European Pharmacopoeia[5]. The addition of chloramphenicol at the concentration of 500 mg/L is a modification designed to increase bacterial inhibition and improve the isolation of opportunistic fungi from contaminated specimens.
Sabouraud Dextrose Agar CAF 500 is a selective medium for the isolation of yeasts and moulds, mainly opportunist pathogens (*Aspergillus, Fusarium, Mucor, Rhizopus*, etc.), cycloheximide sensitive fungi such as *Cryptococcus neoformans* and *Allescheria boydii* and *Candida* spp. in clinical specimens.
Pancreatic digest of casein and peptic digest of meat provide nitrogen, carbon and trace elements for microbial growth. Glucose, at high concentration is a carbon and energy source. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria; the selective properties are increased by the presence of chloramphenicol, a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria. The higher concentration of chloramphenicol (500 mg/L) compared to standard formulations (50 or 100 mg/L) is not inhibitory of fungal growth and increases the selective properties of the medium towards bacterial contaminants, especially *Pseudomonas*.[6]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 65,5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.
Notes
Do not exceed the boiling and sterilization times and temperatures.
Alternatively distribute in screw capped tubes before sterilization and solidify in slanted position.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Sabouraud Dextrose Agar CAF 500 can be directly inoculated with many clinical specimens collected from various normally non-sterile human sites. Refer to the quoted literature for specimen types, related to specific infections.[7-8] Sabouraud Dextrose Agar CAF 500 is not suitable for direct inoculation of blood samples or other specimens from normally sterile sites. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[7]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.
Inoculate each specimen in duplicate; incubate one set in aerobic condition at 22-25°C, the other at 33-37°C.[9]
For dermatophytes, examine cultures every 4-6 days for a period of up to 20 days; for others incubate 2-5 days. Plates should be incubated under conditions of increased humidity during prolonged incubation.
The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

Clinical specimen: after incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and sub-culture to appropriate media for further identification tests.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[10]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	20-25°C / ≤ 5 days/ A	good growth, white yeast-like colonies
T. mentagrophytes ATCC 9533	20-25°C / ≤ 5 days/ A	good growth, white colonies with typical morphology
A. brasiliensis ATCC 16404	20-25°C / ≤ 5 days/ A	good growth, white/black colonies with typical morphology
E. coli ATCC 25922	20-25°C / ≤ 5 days/ A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Chloramphenicol may inhibit some pathogenic fungi (e.g, *Actinomyces bovis* and *Nocardia asteroids*).[6,9]
- Sabouraud Dextrose Agar CAF 500 has a poor efficacy in the isolation of *Histoplasma capsulatum* from potentially contaminated clinical specimens.[11]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of yeasts and moulds with lower selectivity such as Sabouraud Dextrose Agar or Potato Dextrose Agar and with different selective compounds such as Dermatophyte Test medium, should be used.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Upon receipt, store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution à l'étude de la trichophytie humaine. Etude clinique, microscopique et bactériologique sur la pluralité des trichophytons de l'homme. Ann Dermatol Syphil 1892; 3:1061-1087.
3. Weidman FD, Spring D. Comparison of ringworm culture ingredients: II and III. Arch Dermatol Syphilol 1928; 18:829–851.
4. Hodges RS. Cultures of ringworm fungi on Sabouraud's proof mediums and on mediums prepared with American peptones and sugars. Arch Dermatol Syphilol 1928; 18:852–856.
5. European Pharmacopoeia, current edition
6. McLean Jr IW, Schwab JL, Hillegas AB, Schlingman AS. Susceptibility of microorganisms to chloramphenicol (chloromycetin). J Clin Invest. 1949; 28(5):953-963.
7. Berkow EL, McGowan KL. Specimen Collection, Transport and Processing: Mycology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
8. Public Health England- UK SMI B 17: tissues and biopsies from deep-seated sites and organs. 05.01.18
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
11. Unis G, da Silva VB, Severo LC. Disseminated histoplasmosis and AIDS. The role of culture medium for the bronchoscopic clinical specimens Rev Soc Bras Med Trop. 2004; 37:234-7

PACKAGING

Product	Type	REF	Pack
Sabouraud Dextrose Agar CAF 500	Dehydrated medium	4020072	500 g (7.6 L)

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SABOURAUD DEXTROSE AGAR +CAF+CYCLOHEXIMIDE

Ready-to-use plates and tubes

Trichophyton mentagrophytes
on Sabouraud Dextrose Agar+ CAF+ Cycloheximide

INTENDED USE

In vitro diagnostic devices. Selective medium for the isolation of pathogenic fungi, especially dermatophytes, from clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	5.00 g
Peptic digest of meat	5.00 g
Glucose	40.00 g
Agar	15.00 g
Chloramphenicol	0.05 g
Cycloheximide	0.50 g
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

By the end of the 1890's, Raymond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for their isolation and classification.[1,2] This formulation was named Sabouraud medium and is the basic routine culture medium used to grow fungi in clinical laboratories. The components of the basal medium Sabouraud Dextrose Agar conform to the recommendations of the current European Pharmacopoeia[3]. The addition of chloramphenicol and cycloheximide is a modification designed to increase the selective properties and to improve the isolation of pathogenic fungi, especially dermatophytes, from specimens contaminated with saprophytic fungi and bacteria.

Following the initial report of Whiffen, *et al.*,[4] cycloheximide has been found to be of value in increasing the number of isolations of pathogenic fungi from clinical materials.[5] Sabouraud Dextrose Agar+CAF+Cycloheximide (SDA CAF-CEX) is particularly useful for the investigation of dermatological specimens for superficial mycosis and the target organisms are dermatophytes and some yeasts.[6]
Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen, carbon and trace elements for microbial growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria; glucose, at high concentration is a carbon and energy source. The selective properties are increased by the presence of chloramphenicol, a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria and cycloheximide that inhibits the faster-growing saprophytic fungi.[7]

PHYSICAL CHARACTERISTICS

Prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Sabouraud Dextrose Agar+CAF+Cycloheximide can be directly inoculated with clinical specimens collected from sites contaminated with saprophytic fungi and bacteria, mainly skin, nail, hair. Consider that cycloheximide may inhibit some opportunistic fungi (see Limitations of the method). Refer to the quoted literature for specimen types, related to specific infections.[6,8] Sabouraud Dextrose Agar+CAF+Cycloheximide is not suitable for direct inoculation of specimens from normally sterile sites. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[6,8]

TEST PROCEDURE

Allow plates or tubes to come to room temperature. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the surface of the medium to obtain well isolated colonies. For cutaneous samples, press specimen lightly into medium. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.
The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.
For dermatophytes detection incubate at 26-30°C and examine cultures every 4-6 days for a period of up to 21 days.[6]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and sub-culture to appropriate media for further identification tests.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	26-28°C / 72 H/ A	inhibited
T. mentagrophytes ATCC 9533	26-28°C / 72 H/ A	good growth, white colonies with typical morphology
E. coli ATCC 25922	26-28°C / 72 H/ A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Cycloheximide may inhibit some important opportunistic fungi such as *Fusarium*, *Scopulariopsis, Pseudallescheria, Trichosporon*, some *Aspergillus* spp., *Talaromyces* (formerly *Penicillium*) *marneffei*, mucoraceous fungi, some dematiaceous fungi, and yeasts such as *Cryptococcus* spp. and some *Candida* species.[7]
- Some rare non-dermatophyte moulds (*N. dimidiatum, N. hyalinum, Hortaea werneckii*) are capable of causing dermatophyte-like lesions but are inhibited by cycloheximide. If the clinician mentions the possibility of infection with those moulds, the sample should be plated on a cycloheximide-free medium.[6]
- Chloramphenicol may inhibit some pathogenic fungi.[10]
- A single medium is only rarely useful to recover all pathogens contained in a specimen, therefore it is necessary to select media both with and without inhibitory agents for the primary inoculation of the specimen.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Ready-to-use plates and tubes
Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution à l'étude de la trichophytie humaine. Etude clinique, microscopique et bactériologique sur la pluralité des trichophytons de l'homme. Ann Dermatol Syphil 1892; 3:1061-1087.
3. European Pharmacopoeia, current edition.
4. Whiffen AJ, Bonoxos N. Emerson RL. The production of an antifungal antibiotic by Streptomyces qriseus. J Bact 1946; 52: 610-611.
5. Stanley A, Rosenthal D. Furnari BA. The use of cycloheximide-chloramphenicol medium in routine culture of fungi. J Invest Dermatol 1957; 28(5):367-71.
6. Public Health England. Investigation of Dermatological Specimens for Superficial Mycoses. UK Standards for Microbiology Investigations. B 39 Issue 3.1, 2016.
7. Lindsley MD. Reagents, stains and media: mycology. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
8. Berkow EL, McGowan KL. Specimen collection, transport and processing: mycology. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
9. Australian Society for Microbiology: Guidelines for assuring quality of medical microbiological culture media. 2nd Ed, July 2012
10. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985

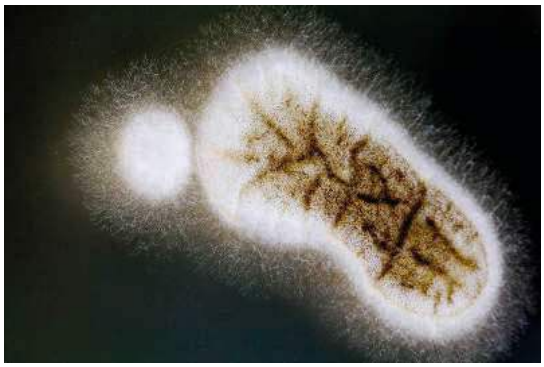
PACKAGING

Product	Type	REF	Pack
Sabouraud Dextrose Agar+CAF+Cycloheximide	Ready-to-use plates	542008	2 x 10 plates ø 90 mm
Sabouraud Dextrose Agar with CAF-CEX	Ready-to-use tubes	552008	20 glass tubes with slanted medium, 17x125 mm,

IFU rev 1, 2021/01

SABOURAUD DEXTROSE AGAR +CAF +GENTAMICIN

Ready-to-use plates



Aspergillus brasiliensis
on Sabouraud Dextrose Agar+CAF+Gentamicin

INTENDED USE

In vitro diagnostic device. Selective medium for the isolation and cultivation of yeasts and moulds in clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	5.00 g
Peptic digest of meat	5.00 g
Glucose	40.00 g
Agar	15.00 g
Chloramphenicol	0.05 g
Gentamicin	0.10 g
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

By the end of the 1890's, Raymond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for their isolation and classification.[1,2]
Numerous experiments were performed to improve Sabouraud's formula with a variety of peptones and carbohydrates by Weidman and Spring[3], but the suitable medium was selected by Hodges[4]. In its final formulation, this medium contained 1% peptone, 4% dextrose, and 1.8% agar, with a final pH of 5.0. This formulation was named Sabouraud medium and is the basic routine culture medium used to grow fungi in clinical laboratories. The components of basal medium Sabouraud Dextrose Agar conform to the recommendations of the current European Pharmacopoeia[5]. The addition of chloramphenicol and gentamicin is a modification designed to increase bacterial inhibition and improve the isolation of opportunistic fungi from contaminated specimens.[6,7]
Sabouraud Dextrose Agar+CAF+Gentamicin is a selective medium for the isolation of yeasts and moulds, cycloheximide sensitive fungi such as *Cryptococcus neoformans* and *Allescheria boydii* and *Candida* spp. in clinical specimens.
Pancreatic digest of casein and peptic digest of animal tissue provide nitrogen, carbon and trace elements for microbial growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria; the selective properties are increased by the presence of chloramphenicol, a broad-spectrum antibiotic, which is inhibitory to a wide range of Gram-negative and Gram-positive bacteria and gentamicin, an aminoglycoside antibiotic that inhibits the growth of Gram-negative bacteria. Glucose, at high concentration, is a carbon and energy source.

PHYSICAL CHARACTERISTICS

Prepared plates appearance	yellow, limpid
Final pH at 20-25°C	5.6 ± 0.2

SPECIMENS

Sabouraud Dextrose Agar +CAF+Gentamicin can be directly inoculated with many clinical specimens collected from various normally non-sterile human sites. Refer to the listed literature for specimen types, related to specific infections.[7] The medium is not suitable for direct inoculation of blood samples or other specimens from normally sterile sites. Media should be carefully selected on the base of specimen type and suspected fungal agents.[7] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[7]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press the specimen lightly into medium.
Inoculate each specimen in duplicate; incubate one set in aerobic condition at 22-25°C, the other at 33-37°C.[8]
For dermatophytes, examine cultures every 4-6 days for a period of up to 20 days; for others incubate 2-5 days. Plates should be incubated under conditions of increased humidity during prolonged incubation.
The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

Clinical specimen: after incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and sub-culture to appropriate media for further identification tests.
Dermatophytes and fungi grow with typical, characteristic colony shapes. For example, *T. mentagrophytes* forms 2-3 cm large white colonies with white spores, *T. rubrum* forms 2-3 cm large, cream-coloured colonies with white spores and pink-coloured underside, *A. brasiliensis* approx. 3-5 cm large colonies with "salt and pepper" appearance which results from darkly pigmented conidia borne in large numbers on conidiophores, and *C. albicans* forms whitish, smooth, round, creamy, slightly domed colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	20-25°C / ≤ 5 days/ A	good growth, white yeast-like colonies
T. mentagrophytes ATCC 9533	20-25°C / ≤ 5 days/ A	good growth, white colonies with typical morphology
A. brasiliensis ATCC 16404	20-25°C / ≤ 5 days/ A	good growth, white/black colonies with typical morphology
E. coli ATCC 25922	25-35°C / ≤ 5 days/ A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Chloramphenicol and gentamicin may inhibit some pathogenic fungi.[8,9]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of yeasts and moulds with lower selectivity such as Sabouraud Dextrose Agar or Potato Dextrose Agar and with higher selectivity such as Dermatophyte Test medium, should be used.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralite des trichophytons de l'homme. Ann Dermatol Syphil1892; 3:1061-1087.
3. Weidman FD, Spring D. Comparison of ringworm culture ingredients: II and III. Arch Dermatol Syphilol 1928; 18:829–851.
4. Hodges RS. Cultures of ringworm fungi on Sabouraud's proof mediums and on mediums prepared with American peptones and sugars. Arch Dermatol Syphilol 1928; 18:852–856.
5. European Pharmacopoeia, current edition
6. Merz WG, Sanford G, Evans L. Clinical Evaluation of the Addition of Gentamicin to Commercially Prepared Mycological Media. J Clin Microbiol 1976; 3: 496-500
7. Lindsley MD. Specimen Collection, Transport and Processing: Mycology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
8. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
9. Dolan CT. Optimal combination and concentration of antibiotics in media for isolation of pathogenic fungi and Nocardia asteroides. Applied Microbiology, 01 Feb 1971, 21(2):195-197

PACKAGING

Product	Type	REF	Pack
Sabouraud Dextrose Agar +CAF +Gentamicin	Ready-to-use plates	542009	2 x 10 plates ø 90 mm

IFU rev 1, 2020/08

SABOURAUD MALTOSE AGAR

Dehydrated culture medium

INTENDED USE

General purposes medium for the isolation and cultivation of yeasts and moulds.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10 g
Maltose	40 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

By the end of the 1890's, Raymond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections and proposed a medium for their isolation and classification.[1,2]
Sabouraud Maltose Agar is a modification of Sabouraud Dextrose Agar with maltose substituted for the dextrose. The medium does not contain selective agents, and the inhibition of bacteria is exclusively due to its acid pH. The medium provides an excellent base for the cultivation of yeasts and moulds. Glucose is replaced by maltose because the latter carbohydrate is especially suitable to fulfil the nutritional requirements of fungi.[3]
The peptones mix Peptocomplex provides nitrogen, carbon and trace elements for microbial growth. The low pH is favourable for the growth of fungi and is slightly inhibitory to contaminating bacteria. Maltose, at high concentration is a carbon and energy source.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 65 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and pour into sterile Petri dishes. Do not exceed the boiling and sterilization times and temperatures.
Alternatively distribute in screw capped tubes before sterilization and solidify in slanted position.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, limpid
Final pH at 20-25 °C	5,6 ± 0.2

SPECIMENS

Sabouraud Maltose Agar can be directly inoculated with many specimens. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE
Allow plates to come to room temperature and to dry the surface of the medium. Inoculate the clinical specimen as soon as possible after collection; streak with a loop over the four quadrants of the plate to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. For cutaneous samples, press specimen lightly into medium.
Inoculate each specimen in duplicate; incubate one set in aerobic condition at 22-25°C, the other at 33-37°C.[10]
For dermatophytes, examine cultures every 4-6 days for a period of up to 20 days; for others incubate 2-5 days. Plates should be incubated under conditions of increased humidity during prolonged incubation.
The user is responsible for choosing the appropriate incubation time and temperature depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies and sub-culture to appropriate media for further identification tests.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 18804	20-25°C / up to 72 h / A	good growth, white yeast-like colonies
T. mentagrophytes ATCC 28185	20-25°C / up to 72 h / A	good growth, white colonies with typical morphology

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Sabouraud Maltose Agar has poor selective properties; a selective medium should be inoculated in parallel for isolation of fungi from potentially contaminated specimens.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed and bottled medium prepared by the user can be stored at +2°C/+8°C for 6 months while the plated media can be stored at +2°C/+8°C for 6-8 weeks.[3]

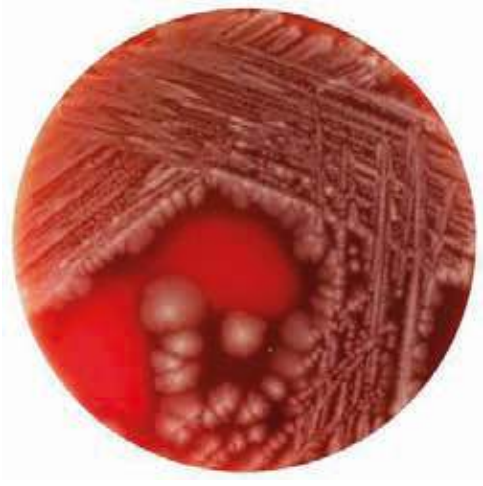
REFERENCES

1. Espinel-Ingroff A. History of medical mycology in the United States. Clin Microbiol Rev 1966; 9:235-272
2. Sabouraud R. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralite des trichophytons de l'homme. Ann Dermatol Syphil1892; 3:1061-1087.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
Sabouraud Maltose Agar	Dehydrated medium	4020102 4020104	500 g (7,7 L) 5 kg (77 L)

IFU rev 1, 2022/05

SCHAEDLER BLOOD AGAR
Ready-to-use plates



Clostridium perfringens on Schaedler Blood Agar

INTENDED USE
In vitro diagnostic device. Non selective medium for the isolation and cultivation of anaerobic bacteria from clinical specimens and other samples.

COMPOSITION - TYPICAL FORMULA *
Pancreatic digest of casein	5.7 g
Enzymatic digest of soya bean	1.0 g
Sodium chloride	1.7 g
Dipotassium hydrogen phosphate	0.8 g
Special peptone	5.0 g
Yeast extract	5.0 g
Glucose	5.8 g
Cysteine HCl	0.4 g
Haemin	0.01 g
Tris Buffer	0.75 g
Agar	13.5 g
Vitamin K1	10.0 mg
Defibrinated sheep blood	50.0 mL
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Schaedler Blood Agar is a modification by Mata, Carillo and Villatoro[1] of the formulation proposed by Schaedler, Dubos and Costello[2]. The modification, evaluated in their studies on anaerobic human faecal microflora, consisted in the substitution of pancreatic digest of casein with 1% Tryptic Soy Broth.

Schaedler Blood Agar has been successfully used for quantitating the faecal human microflora with special attention to criteria for characterizing the culturable aerobic, microaerophilic, and anaerobic bacteria.[2]

Schaedler Blood Agar, used in combination with selective media, is recommended for the detection of Gram-negative anaerobic bacteria, anaerobic cocci and non-spore-forming anaerobic Gram-positive rods.[3-5] Schaedler Blood Agar has been shown to be suitable for the enumeration of Clostridia [6] and has been used for the examination of food and water.[7]

Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride supplies essential electrolytes and maintains the osmotic balance. Yeast extract, haemin, vitamin K1 and sheep blood, enable the growth of the most fastidious obligate and facultative anaerobes. Dextrose provides an energy source and is a reducing agent; cysteine is a reducing agent too and is inhibitory for *E.coli* growth.[8] Dipotassium hydrogen phosphate and tris buffer are used to prevent the pH decreasing, during glucose fermentation.

PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.6 ± 0.2

SPECIMENS

Schaedler Blood Agar can be directly inoculated with clinical specimens such as tissues and biopsies from deep-seated sites and organs, pus and exudates, soft tissue associated with osteomyelitis, orthopaedic implants, fluids from normally sterile sites, aspirates, dental root canal exudates and subgingival plaque.[9, 3-5] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Specimens must be transported to the laboratory under anaerobic conditions and processed within 24 h.[3]

TEST PROCEDURE

Allow plates to come to room temperature. Inoculate the specimen as soon as possible after collection. Streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in anaerobic conditions at 35-37°C for at least 40-48 h or longer (up to 10 days) depending on type of culture being studied or suspected microorganism(s).

The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic haemolytic characteristics of the colonies. Different anaerobic bacteria grow with different colonies morphologies. Confirmatory evidence is required.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[10]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
B. fragilis	ATCC 25285	35-37 °C / 24-48 H / AN	growth
P. anaerobious	ATCC 27337	35-37 °C / 24-48 H / AN	growth

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It is recommended to inoculate together with Schaedler Blood Agar other non-selective and selective media: Columbia Blood Agar incubated in aerobic atmosphere with 5-10% CO_2, on which only the facultative anaerobes will grow, Schaedler Selective CNA Blood Agar incubated in anaerobic conditions, on which Gram-positive obligate anaerobic cocci will grow, and Schaedler Selective Blood Agar (with kanamycin and vancomycin), on which will grow Gram-negative obligate anaerobic bacilli. The comparison of the growths on the four media can help to orient the detection of the isolates.
- The growth rates of strict anaerobes vary considerably: while *Bacteroides fragilis* will grow well after 24 h of incubation, other anaerobes require days or weeks of incubation (e.g. *Actinomycetes* may require 10 days, *Fusobacterium, Peptostreptococcus, Propionibacterium, Prevotella* may require 5-7 days).
- Growth on Schaedler Blood Agar depends on the metabolic requirements of each individual microorganism; some target strains, with specific requirements may not grow on the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Mata LJ, Carrillo C, Villatoro EF. Fecal microflora in healthy persons in a preindustrial region. Appl Microbiol 1969; 17: 596-599
2. Schaedler RW, Dubos R, Castello R. The development of bacterial flora in the gastrointestinal tract of mice. J Exp Med 1965; 122: 59-66.
3. Conrads G, Nagy E, Kononen E. Bacteroides, Porphyromonas, Prevotella, Fusobacterium and other anaerobic Gram negative rods. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
4. Butler-Wu SM, She RC. Actinomyces, Lactobacillus, Cutibacterium and other non-spore-forming Gram-positive rods. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. Veloo ACM, Johnson CN. Peptostreptococcus, Finegoldia, Anaerococcus, Peptoniphilus,, Parvimonas, Murdochiella, Veilonella and other anaerobic cocci. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
6. De Waart J. Pouw H. Studies on the suitability of blood-free media for the enumeration of clostridia. Zbl I Abt 0rig 1970; 214: 551-552.
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
8. Kari C, Nagy Z, Kovacs P and Hernadi F. Mechanism of the growth inhibitory effect of cysteine on Escherichia coli. *J Gen Microbiol* 1971 68. 349-356.
9. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
10. Australian Society for Microbiology: Guidelines for assuring quality of medical microbiological culture media. 2nd Ed, July 2012.

PACKAGING

Product	Type	REF	Pack
Schaedler Blood Agar	Ready-to-use plates	549989	2 x 10 plates ø 90 m

IFU rev 1, 2020/11

SCHAEDLER SELECTIVE BLOOD AGAR

Ready-to-use plates

Bacteroides fragilis on Schaedler Selective Blood Agar

INTENDED USE

In vitro diagnostic device. Selective medium for the isolation of anaerobic Gram-negative bacteria from clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	5.70 g
Enzymatic digest of soya bean	1.00 g
Sodium chloride	1.70 g
Dipotassium hydrogen phosphate	0.80 g
Special peptone	5.00 g
Yeast extract	5.00 g
Glucose	5.80 g
Cysteine HCl	0.40 g
Haemin	0.01 g
Tris Buffer	0.75 g
Agar	13.50 g
Vitamin K1	10.00 mg
Kanamycin	100.00 mg
Vancomycin	7.50 mg
Defibrinated sheep blood	50 mL
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Schaedler Selective Blood Agar is based on the modification by Mata, Carillo and Villatoro[1] of the formulation of basal medium proposed by Schaedler, Dubos and Costello[2]. The modifications, evaluated in their studies on anaerobic human faecal microflora, consisted in the substitution of pancreatic digest of casein with 1% Tryptic Soy Broth.
The use of the aminoglycoside paromomycin 100 mg/L together with vancomycin 7.5 mg/L was first described by Finegold *et al* [3] in 1965 and reported in the NCDC Laboratory Methods in Anaerobic Bacteriology[4]. The current formulation of Schaedler Selective Blood Agar contains kanamycin in substitution of paromomycin.[5]
The kanamycin-vancomycin blood agar plate, used in combination with a non-selective medium, is recommended for the detection of Gram-negative anaerobic bacilli, especially *Bacteroides* and *Prevotella* species, in clinical specimens.[6]
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride supplies essential electrolytes and maintains the osmotic balance. Yeast extract, haemin, vitamin K1 and sheep blood enable the growth of the most fastidious obligate and facultative anaerobes. Dextrose provides an energy source and is a reducing agent; cysteine is a reducing agent too and is inhibitory for *E. coli* growth.[7] Dipotassium hydrogen phosphate and tris buffer are used to prevent the pH decreasing, during glucose fermentation. Kanamycin suppresses the growth of aerobic and facultative anaerobic Gram-negative bacteria while vancomycin is active against Gram-positive bacteria.

PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.6 ± 0.2

SPECIMENS

Schaedler Selective Blood Agar can be directly inoculated with clinical specimens such as specimens such as tissue biopsy specimens, aspirates (e.g. cerebrospinal fluid, joint fluids, and pus), dental root canal exudates and subgingival plaque.[6,] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[8] Specimens must be transported to the laboratory under anaerobic conditions and processed within 24 h.[6]

TEST PROCEDURE

Allow plates to come to room temperature. Inoculate the specimen as soon as possible after collection. Streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate in anaerobic condition at 35-37°C for at least 40-48 h or longer (up to 7 days) depending on type of culture being studied or suspected microorganism(s). An incubation period of 48 h will reveal the presence of rapidly growing strains, such as *Bacteroides* spp., but re-incubation for 5 to 7 days is recommended since some species such as *Bilophila, Desulfovibrio* and *Porphyromonas*, may not be detected with shorter incubation times and require at least 4 to 5 days for growth.[6]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic haemolytic characteristics of the colonies. Different anaerobic bacteria grow with different colony morphologies. Confirmatory evidence is required.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
B. fragilis	ATCC 25285	35-37 °C / 24-48 H / AN	growth
P. mirabilis	ATCC 12453	35-37 °C / 24-48 H / AN	inhibited
E. faecalis	ATCC 29212	35-37 °C / 24-48 H / AN	inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It is recommended to inoculate together with Schaedler Selective Blood Agar other non-selective and selective media: Columbia Blood Agar incubated in aerobic atmosphere with 5-10% CO_2, on which only the facultative anaerobes will grow, Schaedler Selective CNA Blood Agar incubated in anaerobic conditions, on which Gram positive obligate anaerobic cocci will grow and Schaedler Blood Agar, on which all anaerobic bacteria will grow. The comparison of the growths on the four media can help to orient the detection of the isolates.
- The use of solid selective medium together with non-selective medium increases the yield and saves time in term of recognition and isolation of colonies.[6]
- The presence of vancomycin 7.5 mg/L may be inhibitory for some strains of *Porphyromonas* and *Fusobacterium*.[10]
- Plates should not be exposed to air during the first 48 hours of incubation to avoid loss of the more oxygen-sensitive species.[6]
- In many chronic infections, if not in all, several uncultivable anaerobic Gram-negative phylotypes can be present.[6]
- Growth on Schaedler Selective Blood Agar depends on the metabolic requirements of each individual microorganism; some target strains, with specific requirements may not grow on the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Mata LJ, Carrillo C, Villatoro EF. Fecal microflora in healthy persons in a preindustrial region. Appl Microbiol 1969;17: 596-599
2. Schaedler RW, Dubos R, Castello R. The development of bacterial flora in the gastrointestinal tract of mice. J Exp Med 1965;122: 59-66.
3. Finegold SM, AB Miller, Posnick DJ. Further studies on selective media for *Bacteroides* and other anaerobes. Ernährungsforschung 1965; 10:517-528.
4. Dowell, VR, Hawkins TM. Laboratory Methods in Anaerobic bacteriology, NCDC Laboratory Manual. Public Health Service Publication n° 1803, June 1968.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Conrads G, Nagy E, Kononen E. Bacteroides, Porphyromonas, Prevotella, Fusobacterium and other anaerobic Gram negative rods. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
7. Kari C, Nagy Z, Kovacs P and Hernadi F. Mechanism of the growth inhibitory effect of cysteine on Escherichia coli. J Gen Microbiol 1971; 68:349-356.
8. McElvania E, Singh K. Specimen Collection, Transport and Processing: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
9. Australian Society for Microbiology: Guidelines for assuring quality of medical microbiological culture media. 2nd Ed, July 2012.
10. Jousimies-Somer HR. et al. Bacteroides, Porphyromonas, Prevotella, Fusobacterium, and other anaerobic Gram-negative bacteria. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors). Manual of clinical microbiology, 8th ed. Washington, DC: American Society for Microbiology; 2003.

PACKAGING

Product	Type	REF	Pack
Schaedler Selective Blood Agar	Ready-to-use plates	549990	2 x 10 plates ø 90 mm

IFU rev 1, 2020/11

SCHAEDLER SELECTIVE CNA BLOOD AGAR

Ready-to-use plates

Peptostreptococcus anaerobius
on Schaedler Selective CNA Blood Agar

INTENDED USE

In vitro diagnostic device. Moderately selective medium for the isolation of anaerobic Gram-positive bacteria from clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	5.7 g
Enzymatic digest of soya bean	1.0 g
Sodium chloride	1.7 g
Dipotassium hydrogen phosphate	0.8 g
Special peptone	5.0 g
Yeast extract	5.0 g
Glucose	5.8 g
Cysteine HCl	0.4 g
Haemin	0.01 g
Tris Buffer	0.75 g
Agar	13.5 g
Vitamin K1	10 mg
Colistin	0.01 g
Nalidixic acid	0.01 g
Defibrinated sheep blood	50 mL
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Schaedler Selective Blood Agar is based on the modification by Mata, Carillo and Villatoro[1] of the formulation of basal medium proposed by Schaedler, Dubos and Costello[2]. The modifications, evaluated in their studies on anaerobic human faecal microflora, consisted in the substitution of pancreatic digest of casein with 1% Tryptic Soy Broth. The use of colistin and nalidixic acid was first described by Ellner *et al* [3] in 1966 for the growth of many Gram-positive organisms and the inhibition of Gram-negative bacteria such as *Proteus, Klebsiella* and *Pseudomonas* species. The

current formulation of Schaedler Blood Agar supplemented with colistin and nalidixic acid is reported by the reviews of Microbiological Media of Atlas et al.[4,5]
Schaedler Selective CNA Blood Agar plate, used in combination with a non-selective medium, is recommended for the detection of Gram-positive anaerobic cocci, in clinical specimens.[4,6]
Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride supplies essential electrolytes and maintains the osmotic balance. Yeast extract, haemin, vitamin K1 and sheep blood, enable the growth of the most fastidious obligate and facultative anaerobes. Dextrose provides an energy source and is a reducing agent; cysteine is a reducing agent too and is inhibitory for *E. coli* growth.[7] Dipotassium hydrogen phosphate and tris buffer are used to prevent the pH decreasing, during glucose fermentation. Colistin and nalidixic acid are inhibitors of facultative anaerobic Gram-negative bacteria, especially *Enterobacteriaceae*.

PHYSICAL CHARACTERISTICS

Medium appearance	red, opaque
Final pH at 20-25 °C	7.6 ± 0.2

SPECIMENS

Schaedler Selective CNA Blood Agar can be directly inoculated with clinical specimens such as tissue biopsy specimens, aspirates (e.g. cerebrospinal fluid, joint fluids and pus), dental root canal exudates and subgingival plaque.[6,8] Mucosal or cutaneous swabs are not recommended. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Specimens must be transported to the laboratory under anaerobic conditions and processed within 24 h.[6]

TEST PROCEDURE

Allow plates to come to room temperature. Inoculate the specimen as soon as possible after collection. Streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate under anaerobic conditions at 35-37°C for at least 48 hours and up to 7 days before discard the plates as negative.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic haemolytic characteristics of the colonies. Different anaerobic bacteria grow with different colony morphologies. Confirmatory evidence is required.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. anaerobius	ATCC 27337	35-37 °C / 44-48 H / AN	growth
B. fragilis	ATCC 25285	35-37 °C / 44-48 H / AN	growth
P. mirabilis	ATCC 12453	35-37 °C / 44-48 H / AN	inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It is recommended to inoculate together with Schaedler Selective CNA Blood Agar other non-selective and selective media: Columbia Blood Agar incubated in aerobic atmosphere with 5-10% CO_2, on which only the facultative anaerobes will grow, Schaedler Selective Blood Agar (with kanamycin and vancomycin) incubated in anaerobic conditions, on which Gram-negative obligate bacteria will grow and Schaedler Blood Agar, on which all anaerobic bacteria will grow. The comparison of the growths on the four media can help to orient the detection of the isolates.
- The use of solid selective medium together with non-selective medium increases the yield and saves time in term of recognition and isolation of colonies.[8]
- Plates should not be exposed to air during the first 48 hours of incubation to avoid loss of the more oxygen-sensitive species.[8]
- Growth on Schaedler Selective CNA Blood Agar depends on the metabolic requirements of each individual microorganism; some target strains, with specific requirements, may not grow on the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Mata LJ, Carrillo C, Villatoro EF. Fecal microflora in healthy persons in a preindustrial region. Appl Microbiol 1969;17: 596-599
2. Schaedler RW, Dubos R, Castello R. The development of bacterial flora in the gastrointestinal tract of mice. J Exp Med 1965;122: 59-66.
3. Ellner PD, Stoessel CJ, Drakeford E, Vasi, F. A new culture medium for medical bacteriology. Am. J. Clin. Path 1966; 45: 502-504
4. Atlas R. Snyder J. Reagent stains and media: bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
6. Butler-Wu SM, She RC. Actinomyces, Lactobacillus, Cutibacterium and other non-spore-forming Gram-positive rods. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
7. Kari C, Nagy Z, Kovacs P and Hernadi F. Mechanism of the growth inhibitory effect of cysteine on Escherichia coli. J Gen Microbiol 1971; 68:349-356.
8. Conrads G, Nagy E, Kononen E. Bacteroides, Porphyromonas, Prevotella, Fusobacterium and other anaerobic Gram negative rods. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
Schaedler Selective CNA Blood Agar	Ready-to-use plates	549907	2 x 10 plates ø 90 mm

IFU rev 1, 2020/11

SELENITE BROTH

Dehydrated and ready-to-use culture medium

Selenite Broth – from the left: uninoculated tube and *S.* Typhimurium growth

INTENDED USE

In vitro diagnostic. Enrichment liquid medium for the isolation of *Salmonella* spp. in clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE TUBES AND FLASKS

Tryptone	5 g
Lactose	4 g
Sodium phosphate bibasic	10 g
Sodium acid selenite	4 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Selenite broth is based on early works by Klett[1] and Guth[2] who demonstrated the selective inhibitory effects of selenite and used it for the culture of typhoid organisms. Twenty years later, Leifson[3] utilized this information to fully investigate selenite activity, to formulate the liquid medium selenite broth and to promote its wide use as an enrichment medium for the isolation of *Salmonella* spp.
Selenite Broth is a selective enrichment medium intended for the isolation of *Salmonella* spp. from clinical specimens, such as faeces and urine.
Tryptone provides carbon, nitrogen and trace elements for bacterial growth. Sodium acid selenite (synonyms: sodium hydrogen selenite, sodium biselenite), at neutral pH, is inhibitory for coliforms and certain other microbial species, such as faecal streptococci and other Gram-positive bacteria, present in faecal specimens, but not for the majority of *Salmonella* spp. It is believed that, in part, the toxicity of selenite for microorganisms may be attributable to the incorporation of selenium analogues of sulphur-containing amino acids into proteins[4]. The phosphate buffer lessens the toxicity of selenite and tends to minimise the alkalinising effects induced by the reduction of sodium selenite; these alkalinising effects would notably diminish the selective properties of the medium. The acids produced by the microorganisms from lactose also contribute to neutralise alkaline reactions of the medium.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment broth followed by subculture on selective enteric plating media.[5] According to the data of Kelly et al.[6] about 40% of *S. enterica* isolated with an enrichment into Selenite Broth and a subculture onto XLD plates did not grow with a direct inoculation on the primary XLD plates.
Selenite Broth has been demonstrated to be superior to other selective enrichment broths for the isolation of *Salmonella* Typhi from stools.[7]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 23 g in 1000 mL of cold purified water, warm until complete dissolution and distribute into sterile tubes. Do not overheat or autoclave.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Medium appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.1

SPECIMENS

Selenite Broth may be inoculated with human clinical specimens such as faeces or rectal swab and urine. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE

For faeces examination, inoculate test tubes with 1 g of faeces, or 1 mL of faecal suspension obtained suspending 1 g of faeces in 1 mL of saline solution. Rectal swabs received fresh or in transport medium should be rinsed thoroughly in 1 mL of saline.
For urine examination, centrifuge the specimen and inoculate the sediment.
Incubate the inoculated tubes in aerobic atmosphere at 35-37°C for 16-24 hours.

READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by turbidity and often by a colour change of the medium to pink-orange-red.
Sub-culture by streaking a loopful of broth on selective enteric plating media.
The plating media should be chosen as a combination of greater and lesser inhibitory selective agars. For the isolation of S.Typhi, it is advisable to use Bismuth Sulphite Agar or Chromogenic Salmonella Agar as plating medium.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37 °C / 16-24h / A	scanty growth
S. Typhimurium ATCC 14028	35-37 °C / 16-24h / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- After a long storage period of the dehydrated medium, the colour of the prepared broth might change to reddish/red. The microbiological performance however is not affected. Discard the tubes if selenite oxidizes and forms large amounts of a red precipitate.[9]
- Selenite Broth is toxic for *Salmonella* Cholerae-suis and for *Salmonella* Abortus-ovis.[10]

- The value of Selenite Broth as enrichment for *Shigella* spp. has not been clearly established, since some strains of *Shigella*, having similarities with *E. coli*, are inhibited to the same extent as the latter; specimens that might contain organisms inhibited by selective enrichment broth should be plated directly or cultured in a non-selective enrichment broth (e.g. GN Broth).[5]
- Do not incubate the broth over 24 hours. The inhibitory effect diminishes after the first 6-12 hours of incubation.[9]
- The development of *E. coli* and *Proteus* spp. is not indefinitely retarded in Selenite Broth. When the initial proportion of these organisms is high, it is often advantageous to sub-culture onto the solid media after 6 hours as well as after 18 hours.
- After the enrichment in Selenite Broth, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +2°C /+8°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for up to one week.[9]

Ready-to-use medium in tubes and flasks

Store tubes/flasks in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Klett A. (1900) Zeitsch. fÎr Hyg. und Infekt. 33. 137-160.
2. Guth F. (1916) Zbl. Bakt. I. Orig. 77. 487-496.
3. Leifson E. New selenite selective enrichment medium for isolation of typhoid and paratyphoid (salmonella) bacilli. A. J Hyg 1936; 24:423
4. Weiss KF, Ayres JC, Kraft AA. Inhibitory action of selenite on Escherichia coli, Proteus vulgaris, and Salmonella Thompson. J Bacteriol 1995; 90:857
5. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.685.
6. Kelly S, Cormican M, Parke L, Feeney GC, Flynn J. Cost-Effective Methods for Isolation of Salmonella enteric in the Clinical Laboratory. J Clin Microbiol 1999; 37:3369
7. Iveson JB, Kovacs N. Comparative trial of Rappaport enrichment medium for the isolation of Salmonellae from faeces J Clin Path 1967; 20: 290
8. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. Smith HW. The evaluation of culture media for the isolation of salmonellae from faeces. J. Hyg 1952; 50:21-36.

PACKAGING

Product	Type	REF	Pack
Selenite Broth	Dehydrated medium	4020252 4020254	500 g (21,7) 5 kg (217 L)
Selenite Broth	Ready-to-use tubes	552025	20 x 9 mL
Selenite Broth	Ready-to-use flasks	5120252	6 x 100 mL

IFU rev 2, 2022/04

SELENITE BROTH BASE
SODIUM BISELENITE
Dehydrated culture medium and raw material

INTENDED USE

In vitro diagnostic. Enrichment liquid medium for the isolation of *Salmonella* spp. form clinical specimens.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	5 g
Lactose	4 g
Sodium phosphate bibasic	10 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Selenite broth is based on early works by Klett[1] and Guth[2] who demonstrated the selective inhibitory effects of selenite and used it for the culture of typhoid organisms. Twenty years later, Leifson[3] utilized this information to fully investigate selenite activity, to formulate the liquid medium selenite broth and to promote its wide use as an enrichment medium for the isolation of *Salmonella* spp.
Selenite Broth Base, supplemented with sodium biselenite (sodium hydrogen selenite) is a selective enrichment medium intended for the isolation of *Salmonella* spp. from clinical specimens, such as faeces and urine.
Tryptone provides carbon, nitrogen and trace elements for bacterial growth. Sodium hydrogen selenite added to the medium base, at neutral pH, is inhibitory for coliforms and certain other microbial species, such as faecal streptococci and other Gram-positive bacteria, present in faecal specimens, but not for the majority of *Salmonella* spp. It is believed that, in part, the toxicity of selenite for microorganisms may be attributable to the incorporation of selenium analogues of sulphur-containing amino acids into proteins[4]. The phosphate buffer lessens the toxicity of selenite and tends to minimise the alkalinising effects induced by the reduction of sodium selenite; these alkalinising effects would notably diminish the selective properties of the medium. The acids produced by the microorganisms from lactose also contribute to neutralise alkaline reactions of the medium.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment broth followed by subculture on selective enteric plating media.[5] According to the data of Kelly et al.[6] about 40% of *S. enterica* isolated with an enrichment into Selenite Broth and a subculture onto XLD plates did not grow with a direct inoculation on the primary XLD plates.
Selenite broth has been demonstrated to be superior to other selective enrichment broths for the isolation of *Salmonella* Typhi from stools.[7]

DIRECTIONS FOR MEDIUM PREPARATION

Dissolve 4 g of Sodium Biselenite (REF 4123651) in 1 litre of cold purified water and then add 19 g of Selenite Broth Base. Warm until complete dissolution and distribute into sterile tubes. Do not overheat or autoclave.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Sodium biselenite appearance	white powder
Medium appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.1

SPECIMENS

Selenite Broth Base, supplemented with sodium biselenite, may be inoculated with human clinical specimens such as faeces or rectal swab and urine. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE

For faeces examination, inoculate test tubes containing 9-10 mL of Selenite Broth with 1 g of faeces, or 1 mL of faecal suspension obtained suspending 1 g of faeces in 1 mL of saline solution. Rectal swabs received fresh or in transport medium should be rinsed thoroughly in 1 mL of saline.
For urine examination, centrifuge the specimen and inoculate the sediment.
Incubate the inoculated tubes in aerobic atmosphere at 35-37°C for 16-24 hours.

READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by turbidity and often by a colour change of the medium to pink-orange-red.
Sub-culture by streaking a loopful of broth on selective enteric plating media.
The plating media should be chosen as a combination of greater and lesser inhibitory selective agars. For the isolation of S.Typhi, it is advisable to use Bismuth Sulphite Agar or Chromogenic Salmonella Agar as plating medium.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35-37 °C / 16-24h / A	scanty growth
S. Typhimurium ATCC 14028	35-37 °C / 16-24h / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Discard the tubes if selenite oxidizes and forms large amounts of a red precipitate.[9]
- Selenite broth is toxic for *Salmonella* Cholerae-suis and for *Salmonella* Abortus-ovis.[10]
- The value of selenite broth as enrichment for *Shigella* spp. has not been clearly established, since some strains of *Shigella*, having similarities with *E. coli*, are inhibited to the same extent as the latter; specimens that might contain organisms inhibited by selective enrichment broth should be plated directly or cultured in a non-selective enrichment broth (e.g., GN Broth).[5]
- Do not incubate the broth over 24 hours. The inhibitory effect diminishes after the first 6-12 hours of incubation.[9]
- The development of *E. coli* and *Proteus* spp. is not indefinitely retarded in selenite broth. When the initial proportion of these organisms is high, it is often advantageous to sub-culture onto the solid media after 6 hours as well as after 18 hours.
- After the enrichment in selenite broth, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for up to one week.[9]

REFERENCES

1. Klett A. (1900) Zeitsch. fÏr Hyg. und Infekt. 33. 137-160.
2. Guth F. (1916) Zbl. Bakt. I. Orig. 77. 487-496.
3. Leifson E. New selenite selective enrichment medium for isolation of typhoid and paratyphoid (salmonella) bacilli. A. J Hyg 1936; 24:423
4. Weiss KF, Ayres JC, Kraft AA. Inhibitory action of selenite on Escherichia coli, Proteus vulgaris, and Salmonella Thompson. J Bacteriol 1995; 90:857
5. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.685.
6. Kelly S, Cormican M, Parke L, Feeney GC, Flynn J. Cost-Effective Methods for Isolation of Salmonella enteric in the Clinical Laboratory. J Clin Microbiol 1999; 37:3369
7. Iveson JB, Kovacs N. Comparative trial of Rappaport enrichment medium for the isolation of Salmonellae from faeces J Clin Path 1967; 20: 290
8. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. Smith HW. The evaluation of culture media for the isolation of salmonellae from faeces. J. Hyg 1952; 50:21-36.

PACKAGING

Product	Type	REF	Pack
Selenite Broth Base	Dehydrated medium	402025B2	500 g (26.3L)
Sodium Biselenite	Raw material	4123651	100 g (25 L)

IFU rev 2, 2022/04

SELENITE CYSTINE BROTH

Dehydrated and ready-to-use culture medium

INTENDED USE

Selective enrichment liquid medium used in procedures for the detection of *Salmonella* spp. in food and water samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE TUBES AND FLASKS

Tryptone	5.00 g
Lactose	4.00 g
Sodium phosphate bibasic	10.00 g
Sodium acid selenite	4.00 g
L-cystine	0.01 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Selenite Cystine Broth is based on early works by Klett[1] and Guth[2] who demonstrated the selective inhibitory effects of selenite and used it for the culture of typhoid organisms. Twenty years later, Leifson[3] utilized this information to fully investigate selenite activity, to formulate the liquid medium selenite broth and to promote its wide use as an enrichment medium for the isolation of *Salmonella* spp.
Selenite Cystine Broth is based on a modification made in 1953 by North and Bartram[4] of the original Leifson's formula, differing only in the addition of L-cystine which is considered to enhance *Salmonella* growth by reduction of toxicity.[5]
Selenite Cystine Broth is recommended by FDA-BAM[6] and AOAC[7] methods for selective enrichment of guar gum and foods suspected to be contaminated with *Salmonella* Typhi. It is included as selective enrichment in ISO 6579-1 Annex D[8-9] and in ISO 19250[10] for the detection of *Salmonella* Typhi and Paratyphi.
Tryptone provides carbon, nitrogen and trace elements for bacterial growth. Sodium acid selenite (synonyms: sodium hydrogen selenite, sodium biselenite), at neutral pH, is inhibitory for coliforms and certain other microbial species, such as faecal streptococci and other Gram-positive bacteria, but not for the majority of *Salmonella* spp. including *Salmonella* Typhi and *Salmonella* Paratyphi. It is believed that, in part, the toxicity of selenite for microorganisms may be attributable to the incorporation of selenium analogues of sulphur-containing amino acids into proteins[11].
The phosphate buffer lessens the toxicity of selenite and tends to minimise the alkalinising effects induced by the reduction of sodium selenite; these alkalinising effects would notably diminish the selective properties of the medium. The acids produced by the microorganisms from lactose also contribute to neutralise alkaline reactions of the medium. L-cystine enhance *Salmonella* growth by again reducing the toxicity of the culture medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 23 g in 1000 mL of cold purified water, warm to dissolve completely and distribute into sterile tubes or flasks. Do not overheat or autoclave.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Medium appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.1

SPECIMENS

Food, feed, food chain and water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[5-10]

TEST PROCEDURE

The detection of *Salmonella* in foods necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in two selective liquid media, plating out and recognition, confirmation.

Pre-enrichment

Salmonella organisms in foods and water are often present in low numbers and may be sub-lethally injured. Through pre-enrichment *Salmonella* cells grow to a detectable level. US methods[5-7] suggest different pre-enrichment media depending on the sample to be analysed, while ISO Standards[8-10] recommend a sole medium (Buffered Peptone Water).

Selective enrichment

FDA-BAM method[6] for guar gum and foods suspected to be contaminated with serovar Typhi:

- Transfer 1 mL of pre-enrichment broth to 10 mL of Selenite Cystine Broth and another 1 mL in 10 mL of Tetrathionate Broth.
- Incubate 24 ± 2 h at 35°C.

ISO 6579-1 method for the detection of Salmonella Typhi and Paratyphi[8,9]:
10 mL of Selenite Cystine Broth are inoculated with 1 mL of the pre-enrichment culture (in addition to inoculation of RVS broth or MSRV agar and MKTTn broth) and incubated between 34 °C and 38 °C for 24 h and 48 h.

Plating out

US methods[6,7]: vortex the enrichment culture tubes and streak a 10 µL onto Bismuth Sulphite Agar, Hektoen Enteric Agar and XLD Agar and incubate at 35°C for 22-26 hours.
ISO 6578 (Annex D)[8,9]: inoculate by means of a 10 µl loop the surface of an XLD plate so that well-isolated colonies will be obtained. Proceed in the same way with Bismuth Sulphite Agar.
Incubate the plates of both media between 34 °C and 38 °C and examined after 24 h, and again, if necessary, after 48 h.

Confirmation

Perform confirmation tests of colonies obtained on plated media in accordance with the method of analysis in use.

READING AND INTERPRETATION

After incubation, the growth of organisms in Selenite Cystine Broth is indicated by turbidity and often by a colour change of the medium to pink-orange-red.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028 + *E. coli* ATCC 25922 + *E. faecalis* ATCC 29212	34-38 °C / 24 h ± 3 h / A	>10 characteristic colonies on XLD agar or other medium of choice
E. coli ATCC 25922	34-38 °C / 24 h ± 3 h / A	Partial inhibition, ≤100 colonies on TSA
E. *faecalis* ATCC 29212	34-38 °C / 24 h ± 3 h / A	Partial inhibition, <10 colonies on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- After a long storage period of the dehydrated medium, the colour of the prepared broth might change to reddish/red. The microbiological performance however is not affected. Discard the tubes if selenite oxidizes and forms large amounts of a red precipitate.[12]
- Selenite Broth is toxic for *Salmonella* Cholerae-suis and for *Salmonella* Abortus-ovis.[13]
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +12°C /+8°C away from direct light in a dry place.
According to ISO 6579 the self-prepared tubes can be stored at +2°C +8°C in the dark until a red precipitate occurs.[9]

Ready-to-use medium in tubes and flasks

Store in their original pack at 2-8°C away from direct light.

REFERENCES

1. Klett A. Zeitsch fÏr Hyg und Infekt 1900; 33:137-160.
2. Guth F. Zbl Bakt I Orig 1916; 77:487-496.
3. Leifson E. New selenite selective enrichment medium for isolation of typhoid and paratyphoid (salmonella) bacilli. A. J Hyg 1936; 24:423
4. North WR, Bartram MT. The efficiency of selenite broth of different compositions in the isolation of Salmonella. App Microbiol 1953; 1:130-134.
5. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev March 2022.
7. Association of Official Analytical Chemists. Official Methods of Analysis, 19th ed. 2012. AOAC, Arlington, VA
8. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.
9. ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC.
10. ISO 19250:2010 Water quality — Detection of Salmonella spp.
11. Weiss KF, Ayres JC, Kraft AA. Inhibitory action of selenite on Escherichia coli, Proteus vulgaris, and Salmonella Thompson. J Bacteriol 1995; 90:857
12. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
13. Smith HW. The evaluation of culture media for the isolation of salmonellae from faeces. J. Hyg 1952; 50:21-36.

PACKAGING

Product	Type	REF	Pack
Selenite Cystine Broth	Dehydrated medium	4020262 4020264	500 g (21,7) 5 kg (217 L)
Selenite Cystine Broth	Ready-to-use tubes	552026 552026A	20 x 10 mL 20 x 9 mL
Selenite Cystine Broth	Ready-to-use flasks	5120262 512026A2	6 x 100 mL 6 x 90 mL

IFU rev 2, 2022/09

SELENITE CYSTINE BROTH BASE
SODIUM BISELENITE
Dehydrated culture medium and raw material

INTENDED USE

Selective enrichment medium base used in procedures for the detection of *Salmonella* spp. in food and water samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	5.00 g
Lactose	4.00 g
Sodium phosphate bibasic	10.00 g
L-cystine	0.01 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Selenite cystine broth is based on early works by Klett[1] and Guth[2] who demonstrated the selective inhibitory effects of selenite and used it for the culture of typhoid organisms. Twenty years later, Leifson[3] utilized this information to fully investigate selenite activity, to formulate the liquid medium selenite broth and to promote its wide use as an enrichment medium for the isolation of *Salmonella* spp.

Selenite cystine broth is based on a modification made in 1953 by North and Bartram[4] of the original Leifson's formula, differing only in the addition of L-cystine which is considered to enhance *Salmonella* growth by reduction of toxicity.[5]
Selenite cystine broth is recommended by FDA-BAM[6] and AOAC[7] methods for selective enrichment of guar gum and foods suspected to be contaminated with *Salmonella* Typhi. It is included as selective enrichment in ISO 6579-1 Annex D[8-9] and in ISO 19250[10] for the detection of *Salmonella* Typhi and Paratyphi.
To minimise any possible risk of teratogenicity for laboratory operators, sodium acid selenite is not included in the dehydrated medium Selenite Cystine Broth Base, but must be prepared separately as a solution with the supplied raw material REF 4123651 and added to the medium base.
Tryptone provides carbon, nitrogen and trace elements for bacterial growth. Sodium acid selenite (synonyms: sodium hydrogen selenite, sodium biselenite), at neutral pH, is inhibitory for coliforms and certain other microbial species, such as faecal streptococci and other Gram-positive bacteria, but not for the majority of *Salmonella* spp., including *Salmonella* Typhi and *Salmonella* Paratyphi. It is believed that, in part, the toxicity of selenite for microorganisms may be attributable to the incorporation of selenium analogues of sulphur-containing amino acids into proteins[11].
The phosphate buffer lessens the toxicity of selenite and tends to minimise the alkalinising effects induced by the reduction of sodium selenite; these alkalinising effects would notably diminish the selective properties of the medium. The acids produced by the microorganisms from lactose also contribute to neutralise alkaline reactions of the medium. L-cystine enhance *Salmonella* growth by again reducing the toxicity of the culture medium.

Directions for medium preparation

Dissolve 4 g of Sodium Biselenite (REF 4123651) in 1 litre of cold purified water and then add 19 g of Selenite Cystine Broth Base. Warm to dissolve completely and distribute into sterile tubes or flasks. Do not overheat or autoclave.

Physical characteristics

Dehydrated medium base appearance	white, fine, homogeneous, free-flowing powder
Sodium biselenite appearance	white powder
Medium appearance	very pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.1

Specimens

Food, feed, food chain and water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[5-10]

Test procedure

The detection of *Salmonella* in foods necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in two selective liquid media, plating out and recognition, confirmation.

Pre-enrichment

Salmonella organisms in foods and water are often present in low numbers and may be sub-lethally injured. Through pre-enrichment *Salmonella* cells grow to a detectable level. US methods[5-7] suggest different pre-enrichment media depending on the sample to be analysed, while ISO Standards[8-10] recommend a sole medium (Buffered Peptone Water).

Selective enrichment

FDA-BAM method[6] for guar gum and foods suspected to be contaminated with serovar Typhi:

- Transfer 1 mL of pre-enrichment broth to 10 mL of Selenite Cystine Broth and another 1 mL in 10 mL of Tetrathionate Broth.
- Incubate 24 ± 2 h at 35°C.

ISO 6579-1 method for the detection of Salmonella Typhi and Paratyphi[8,9]

10 mL of Selenite Cystine Broth are inoculated with 1 mL of the pre-enrichment culture (in addition to inoculation of RVS broth or MSRV agar and MKTTn broth) and incubated between 34 °C and 38 °C for 24 h and 48 h.

Plating out

US methods[6,7]: vortex the enrichment culture tubes and streak a 10 µL onto Bismuth Sulphite Agar, Hektoen Enteric Agar and XLD Agar and incubate at 35°C for 22-26 hours.

ISO 6578 (Annex D)[8,9]: inoculate by means of a 10 µl loop the surface of an XLD plate so that well-isolated colonies will be obtained. Proceed in the same way with Bismuth Sulphite Agar.

Incubate the plates of both media between 34 °C and 38 °C and examined after 24 h, and again, if necessary, after 48 h.

Confirmation

Perform confirmation tests of colonies obtained on plated media in accordance with the method of analysis in use.

Reading and interpretation

After incubation, the growth of organisms in Selenite Cystine Broth is indicated by turbidity and often by a colour change of the medium to pink-orange-red.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

Control strains	Incubation T°/ t / ATM	Expected results
S. Typhimurium ATCC 14028 + *E. coli* ATCC 25922 + *E. faecalis* ATCC 29212	34-38 °C / 24 h ± 3 h / A	>10 characteristic colonies on XLD agar or other medium of choice
E. coli ATCC 25922	34-38 °C / 24 h ± 3 h / A	Partial inhibition, ≤100 colonies on TSA
E. faecalis ATCC 29212	34-38 °C / 24 h ± 3 h / A	Partial inhibition, <10 colonies on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- After a long storage period of the dehydrated medium, the colour of the prepared broth might change to reddish/red. The microbiological performance however is not affected. Discard the tubes if selenite oxidizes and forms large amounts of a red precipitate.[12]
- Selenite Broth is toxic for *Salmonella* Cholerae-suis and for *Salmonella* Abortus-ovis.[13]
- Colonies of presumptive Salmonella must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place. According to ISO 6579 the self-prepared tubes can be stored at +2°C +8°C in the dark until a red precipitate occurs.[8]

REFERENCES

1. Klett A. Zeitsch fÏr Hyg und Infekt 1900; 33:137-160.
2. Guth F. Zbl Bakt I Orig 1916; 77:487-496.
3. Leifson E. New selenite selective enrichment medium for isolation of typhoid and paratyphoid (salmonella) bacilli. A. J Hyg 1936; 24:423
4. North WR, Bartram MT. The efficiency of selenite broth of different compositions in the isolation of Salmonella. App Microbiol 1953; 1:130-134.
5. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev March 2022.
7. Association of Official Analytical Chemists. Official Methods of Analysis, 19th ed. 2012. AOAC, Arlington, VA
8. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.
9. ISO 6579-1:2017/Amd 1:2020 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. — Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC.
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12. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
13. Smith HW. The evaluation of culture media for the isolation of salmonellae from faeces. J. Hyg 1952; 50:21-36.

PACKAGING

Product	Type	REF	Pack
Selenite Cystine Broth Base	Dehydrated medium	402026B2 402026B4	500 g (21.7) 5 kg (217 L)
Sodium Biselenite	Raw material	4123651	100 g (25 L)

IFU rev 2, 2022/09

ChromArt

SENECA BASE

(**S**imultaneous Enumeration **En**terobacteriaceae **E**.**c**oli **A**gar)

Dehydrated culture medium and supplement

SENECA: *E. coli* blue colonies;
K. pneumoniae and S. Enteritidis (red colonies)

INTENDED USE

For the simultaneous enumeration of *Enterobacteriaceae* and *E. coli* in water, food and other samples of sanitary interest.

COMPOSITION *

SENECA BASE (DEHYDRATED MEDIUM)

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptones	15.00 g
Carbohydrates	2.50 g
Selective compounds	0.50 g
Phosphate buffer	4.30 g
Chromogenic mix	0.12 g
Agar	15.00 g

SENECA EE-EC SUPPLEMENT (VIAL CONTENT FOR 500 ML OF MEDIUM)

Antimicrobial compounds	4.50 mg
Chromogenic substrate	6.25 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

SENECA represents a development of the EE-EC Agar medium, which Biolife designed and proposed, first in the world, for the simultaneous enumeration of *E. coli* and *Enterobacteriaceae*.

Counting of *E. coli* alone is done by determination of the enzyme β-D-glucuronidase on SENECA Base.[1-3] Simultaneous enumeration of *Enterobacteriaceae* and *E. coli* is done by addition of a specific supplement (SENECA EE-EC Supplement) to the base medium that provides the *Enterobacteriaceae* colonies with a pink-red coloration.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 18.7 g of SENECA Base in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 min. Cool to 47-50°C.

For *E. coli* enumeration: use as is.

For simultaneous enumeration of *E. coli* and *Enterobacteriaceae*: dissolve the contents of one vial of SENECA EE-EC Supplement (REF 4240023) with 1 mL of ethanol, mix and then add 1 mL of sterile purified water; add to 500 mL of autoclaved and cooled to 47-50° SENECA Base.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared plates appearance	dark yellow, limpid
Freeze-dried supplement	pink pellet; pale yellow and clear solution with a slight precipitate after reconstitution
Final pH of complete medium (at 20-25°C)	7.3 ± 0.2

SPECIMENS

Water, food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable international standards.

TEST PROCEDURE

Membrane filtration method

Filter 100 mL (or other volumes, e.g., 250 mL for bottled water) of the sample using a membrane filter usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm and, preferentially, with grid lines. The minimum volume for filtration is 10 mL of sample or dilutions thereof to ensure even distribution of the bacteria on the membrane filter.
After filtration place the membrane filter on the medium, ensuring that no air is trapped underneath

Pour-plate method

Pour 1 mL of the initial suspension and decimal dilutions of the sample into the plates. Add about 15 mL of pre-cooled medium. Mix well the inoculum with the medium.

Surface plating technique

1. Dry the prepared plates before the use.
2. Using a sterile pipette, transfer 0.1 mL of the test sample, if the product is liquid, or of the initial suspension in the case of other products, to the centre of a plate.
3. Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
4. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.

Incubation

Incubate for 24 ± 2 h at 37°± 1 C. In case of slight growth, poor pigmentation or no growth, re-incubate for additional 24 h.

READING AND INTERPRETATION

After incubation, observe bacterial growth, record each specific colour and morphological characteristic of the colonies.
Enumeration of *E. coli* + *Enterobacteriaceae* (SENECA Base 405582S + SENECA EE-EC Supplement 4240023).
Count as *Enterobacteriaceae* all red and blue colonies.
Count as *E. coli* β-D glucuronidase positive all blue colonies.
Enumeration of *E. coli* (SENECA Base 405582S)**.**
Count as *E. coli* β-D glucuronidase positive all blue colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Productivity control - *E. coli* ATCC 25922: growth, blue colonies
Specificity control - *E. aerogenes* ATCC 13048: growth, red colonies
Selectivity control - *S. aureus* ATCC 25923: inhibited; *P. aeruginosa* ATCC 14207: inhibited

LIMITATIONS OF THE METHOD

- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[4] Consequently, some strains of *E. coli*, including pathogenic ones, will not be detected on SENECA Base and will be recognized as *Enterobacteriaceae* on SENECA EE-EC.
- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia, Enterobacter* and *Yersinia* strains (1-5%).[5-6]
- If heavy contamination is suspected, in the pour plate technique, after inoculation of the base layer and its solidification, add a surface layer of about 5 mL of the same medium.

STORAGE CONDITIONS

Dehydrated medium

Store at +2°C /+8°C away from direct light in a dry place.

Freeze-dried supplement

Store the product in the original package at +2°C /+8°C away from direct light.

REFERENCES

1. Bascomb S. Enzyme tests in bacterial identification. Methods Microbiol 1987; 19:105-160.
2. Manafi M, Kneifel W, Bascomb S Fluorogenic and chromogenic substrates used in bacterial diagnostics. Micr Rev, Sept. 1991, p. 335-348
3. Watson RR. Substrate specificities of aminopeptidases:a specific method for microbial differentiation, p. 1-14. In J. R.Norris (ed.), Methods in microbiology, vol. 9. 1976, Academic Press (London), Ltd., London.
4. Feng P, Lampel KA, Karch H, Whittam TS. Genotypic and phenotypic changes in the emergence of Escherichia coli O157:H7. J. Infect. Dis. 177: 1750–1753.
5. Kilian M. & Bulow P. Rapid diagnosis of Enterobacteriaceae. Detection of bacterial glycosidases. Acta Pathol Microbiol Scand Sect B. 1976, 84: 245–251.
6. Le Minor, Buissière J, Novel G, Novel M. Relation entre le sérotype et l'activité β-glucuronidasique chez les Salmonella. Ann Microbiol (Paris) 1978; 129B (2) :155–165.

PACKAGING

Product	Type	REF	Pack
SENECA Base	Dehydrated medium	405582S2	500 g (13.36 L)
SENECA EE-EC Supplement	Freeze-dried supplement	4240023	10 vials, each for 500 mL of medium

IFU rev 2, 2023/02

SERUM TELLURITE AGAR

Ready-to-use plates

C. diphtheriae on Serum Tellurite Agar

INTENDED USE

In vitro diagnostic device. Selective and differential medium for the isolation and detection of *Corynebacterium diphtheriae* from clinical specimens.

COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	15 g
Papaic digest of soy bean meal	5 g
Sodium chloride	5 g
Agar	15 g
Horse serum	50 mL
Potassium tellurite, 1% solution	10 mL
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Serum Tellurite Agar is a modification of Hoyle Medium[1], described in the Manual of Bacteriological Techniques of the Regional Committee for the Organization of Pathology Services[2]. Serum Tellurite Agar is a selective and differential medium for the isolation and detection of *Corynebacterium diphtheriae*, in clinical specimens.[2] The medium contains two peptones as sources of nitrogen and carbon, necessary for microbial growth. Sodium chloride provides essential electrolytes and contributes to the osmotic balance. Horse serum stimulates the growth of corynebacteria and potassium tellurite inhibits the growth of most normal Gram-negative bacteria of the upper respiratory tract[3]; it is reduced by corynebacteria and other microorganisms with the formation of grey or black colonies.

PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.1

SPECIMENS

Serum Tellurite Agar plates can be directly inoculated with clinical specimens or with culture obtained on Loeffler's medium. In case of respiratory diphtheria, material for culture should be obtained on a swab (either cotton or polyester tipped swab) from the inflamed area of nasopharynx; if membranes are present and can be removed, they should also be sent to the laboratory.[4] Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information. Collect specimens before antimicrobial therapy where possible.

TEST PROCEDURE

Allow plates to come to room temperature. Roll the swab with the specimen or with the sub-culture from Loeffler medium on a restricted area of the Serum Tellurite Agar plate, then streak with a loop on four quadrants, to disperse the inoculum and obtain isolated colonies. Incubate aerobically at 37°C for 24-48 hours, but discarding the plates as negative after 4 days of incubation. Observe daily for the development of typical colonies.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. After 24-48 hours of incubation *C. diphtheriae* cultivates with grey-black colonies with light halo and jagged edges, 1 to 5 mm in diameter, with often rough and sometimes smooth surfaces (forms RS). The greyish colour of the colonies intensifies with the prolongation of the incubation until it reaches grey-black in the 4th day.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. diphtheriae ATCC 11913	35-37°C / 24-48 H / A	growth, grey colonies
E. coli ATCC 25922	35-37°C / 24-48 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Tellurite inhibits the growth of many non-coryneform bacteria but even a few *C. diphtheriae* strains are sensitive to potassium tellurite and therefore do not grow on Serum Tellurite Agar.[4]
- The growth on Serum Tellurite Agar and the reduction of tellurite are not specific for *C. diphtheriae* since many other coryneforms and other Gram-positive bacteria may also produce black colonies.[4]
- The medium is not inhibitory to Gram-positive bacteria: pseudodiphtheria, staphylococci, streptococci, micrococci, listeriae can grow with white-grey-black colonies. *Candida* grows with small greyish-white colonies.[2]
- It is advisable to inoculate, together with Serum Tellurite Agar, other plated or tubed media such as Blood Agar and Loeffler's Medium.[2,3,4]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Hoyle L. A tellurite blood-agar medium for the rapid diagnosis of diphtheria. Lancet, 1941;1:175
2. Comitato Regionale per l'Ordinamento dei Servizi di Patologia (1977) Manuale di Tecniche Batteriologiche. Giunta Regionale della Lombardia Ass. Sanità.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Bernard KA. Coryneform Gram-positive rods. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING
Product	Type	REF	Pack
Serum Tellurite Agar	Ready-to-use plates	549998	2 x 10 plates ø 90 mm

IFU rev 1, 2020/12

SHIGELLA BROTH BASE
NOVOBIOCIN ANTIMICROBIC SUPPLEMENT
Dehydrated culture medium and supplement

INTENDED USE
With the addition of novobiocin, Shigella Broth Base is used as a selective enrichment broth for the determination (presence or absence) of *Shigella* in samples of the food chain according to ISO 21567 and FDA-BAM.

COMPOSITION *
SHIGELLA BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER*

Enzymatic digest of casein	20.0 g
Potassium hydrogen phosphate (anhydrous)	2.0 g
Potassium dihydrogen phosphate (anhydrous)	2.0 g
Sodium chloride	5.0 g
Glucose	1.0 g
Tween® 80	1.5 mL

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT - VIAL CONTENT

Novobiocin	10 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Shigella species are Gram-negative, nonmotile, facultatively anaerobic, non-sporeforming, nonmotile rods, belonging to the family of *Enterobacteriaceae*, closely related to *E. coli*. The genus is named after its discovery by Kiyoshi Shiga in 1897. The current classification divides the genus into four species based on serological typing: *S. dysenteriae, S. boydii, S. flexneri* and *S. sonnei*. *Shigella* spp. cause dysentery (shigellosis) in primates, but not in other mammals.[1] Their pathogenic action is due to the marked invasiveness towards the intestinal epithelium of the ileum and the colon and to endotoxins and exotoxins production.[2] Yearly, about 80–165 million cases of *Shigella* diseases are recorded in the world, which lead to between 74,000 and 600,000 states of death, especially in the developing countries.[3]
Shigella Broth is based on the formula developed by Mehlman, Romero and Wentz[4] and is recommended by ISO 21567[5] and FDA-BAM[6] as a selective enrichment broth for the determination of *Shigella* in samples of the food chain.
Essential growth factors are provided by enzymatic digest of casein which is a source of nitrogen, carbon and minerals. Glucose is a source of carbon and energy; sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Phosphates are used as buffering agents to control the pH in the medium. Polysorbate 80 neutralizes preservatives in food products, allowing bacteria to grow. Novobiocin is active mostly against Gram-positive bacteria but also against a few Gram-negative bacteria. Its concentration in the medium recommended by ISO 21567[5] is 0.5 mg/L with anaerobic incubation at 41.5°C, while FDA-BAM[6] recommends 0.5 mg/L for the detection of *S. sonnei* with anaerobic incubation at 44.0°C, and 3 mg/L for the enrichment of other *Shigella* species, with anaerobic incubation at 42°C.

DIRECTIONS FOR MEDIA PREPARATION
Suspend 31.5 g in 1000 mL of cold, purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 225 mL in bottles and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature.
Dissolve the content of one vial of Novobiocin Antimicrobic Supplement (REF 4240045) with 4 mL of sterile purified water (novobiocin concentration: 2.5 mg/mL). Add a volume of novobiocin solution to the basic medium to obtain the required antibiotic concentration:
Shigella Broth according to ISO 21567 and FDA-BAM for *S. sonnei*: add 50 µL of solution to 225 mL of Shigella Broth Base (final concentration of 0,5 µg/mL broth after 25 g or 25 mL of sample is added).
Shigella Broth according to FDA-BAM for other *Shigella* species: add 300 µL of solution to 225 mL of Shigella Broth Base (final concentration 3 µg/mL broth after 25 g or 25 mL of sample is added).
The remaining novobiocin solution can be stored at 2-8 ° C for one month.[5]

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	greyish, fine, homogeneous, free-flowing powder
Prepared flasks appearance	yellow, slightly opalescent
Freeze-dried selective supplements	low, dense, white tablets; colourless limpid solution after reconstitution
Final pH of complete media (at 20-25°C)	7.0 ± 0.2

SPECIMENS
Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[5,6]

TEST PROCEDURE

Enrichment of *Shigella* species according to ISO 21567[5] (or *S. sonnei* according to FDA-BAM[6])

The following method is a summary of the technique recommended by

1. In general, to prepare the initial suspension, add a test portion of 25 g or 25 mL to 225 g or 225 mL of Shigella Broth containing 0.5 µg/mL of novobiocin, to obtain a tenfold dilution, and homogenize.
2. Incubate under anaerobic conditions with caps and closures loose, or with equipment giving an equivalent effect, so that gas exchange can readily occur without contamination, at 41.5 ± 1 °C (44 °C: FDA-BAM).
3. Transfer a loopful of growth on plates of MacConkey Agar REF 401670 (low selectivity), XLD Agar ISO Formulation REF 402208 (moderate selectivity), and Hektoen Enteric Agar REF 401541 (greatest selectivity). FDA-BAM: streak on a MacConkey Agar plate.
4. Incubate the plating-out media at 37 °C for 20 h to 24 h. If no typical colonies are seen and the growth of other microorganisms is weak (particularly on the more selective agar), re-incubate the plates for a further 24 h. Examine them again for typical *Shigella* colonies.

Enrichment of other *Shigella* species according to FDA-BAM.[6]

Proceed as above, but use novobiocin at 3.0 µg/mL and incubate anaerobically at 42 °C.

READING AND INTERPRETATION

After incubation of Shigella Broth, growth is evident by the appearance of turbidity.

After subculture on the plating out media and incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Consult ISO 21567[5], FDA-BAM[6] and the instructions for use of the plated media for a description of *Shigella* colonies on the different selective agars used.

Perform the biochemical and serological confirmation tests on the typical or suspect colonies as reported in the International Standards.[5,6]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Shigella boydii ATCC 9207	41,5°C ± 1°C / 18h ± 2h / AN	Good growth
Shigella flexneri ATCC 12022	41,5°C ± 1°C / 18h ± 2h / AN	Good growth
Shigella sonnei ATCC 9290	41,5°C ± 1°C / 18h ± 2h / AN	Good growth
Staphylococcus aureus ATCC 25923	41,5°C ± 1°C / 18h ± 2h / AN	Inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- *Shigella* species can form a minority proportion of the total microbial flora when contaminating a food sample or after enrichment. In these circumstances, the direct streaking of the enrichment broth onto one plate per selective agar may fail to allow the detection of *Shigella* colonies. It may therefore be appropriate in some circumstances (e.g., the investigation of foods implicated in illness) to consider the inoculation of either two 90 mm dishes or one large (140 mm) Petri dish to increase the possibility of detection.[5]
- The colonies of some *Enterobacteriaceae* strains are very similar in appearance to those of *Shigella*. Any typical or suspect colonies shall be confirmed as *Shigella* species or not. Also, in some circumstances (e.g., foods implicated in food poisoning), it may be appropriate to investigate more than five colonies from a plate to increase confidence in the absence of *Shigella* in the food sample tested.[5]

STORAGE CONDITIONS

Dehydrated medium

Store at +10/+30°C away from direct light in a dry place.

Freeze-dried supplement

Store the product in the original package at +2/+8°C away from direct light

According to ISO 21567, Shigella Broth prepared in flasks and the remaining novobiocin solution may be stored at +2/+8°C for up to 1 month.[5]

REFERENCES

1. Bakera S, Chung H. Thea Recent insights into Shigella: a major contributor to the global diarrhoeal disease burden. Curr Opin Infect Dis. 2018 Oct; 31(5): 449–454.
2. Ryan, Kenneth James; Ray, C. George, eds. (2004). Sherris medical microbiology: an introduction to infectious diseases (4th ed.). McGraw-Hill Professional Med/Tech.
3. Bowen A. Chapter 3: Infectious Diseases Related to Travel". The Yellow Book: Health Information for International Travel. Retrieved 22 June 2016.
4. Mehlman IJ, Romero A, Wentz BA. Improved enrichment for recovery of Shigella sonnei from foods. J Assoc Off Anal Chem 1985 May-Jun; 68(3): 552-5.
5. ISO 21567:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 6: Shigella. Rev. February 2013.

PACKAGING

Product	Type	REF	Pack
Shigella Broth Base	Dehydrated medium	4020402	500 g (15.8 L)
Novobiocin Antimicrobic Supplement	Freeze-dried supplement	4240045	10 vials (10 mg/vial)

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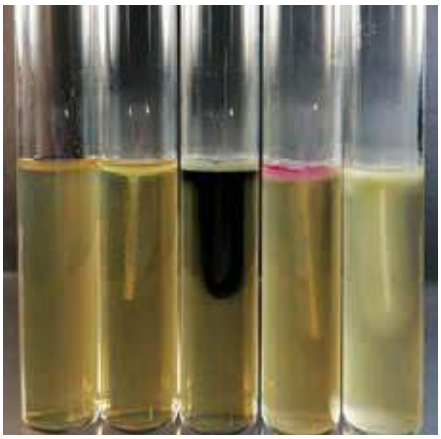
IFU rev 1, 2022/08

SIM BIOS MEDIUM

Dehydrated culture medium



SIM Bios Medium: from left: uninoculated tube, *K. pneumoniae* (not motile), *S. arizonae* H_2S+, *E. coli* (motile, indole+), *E aerogenes* (motile)

INTENDED USE

In vitro diagnostic. Differential medium used as an aid in identifying Gram negative *Enterobacteriaceae,* especially *Salmonella* and *Shigella*, by ability to produce indole and hydrogen sulphide and exhibit motility.

COMPOSITION - TYPICAL FORMULA* (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	20.0 g
Peptone	6.1 g
Ferric ammonium citrate	0.2 g
Sodium thiosulphate	0.2 g
Agar	3.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

SIM (Sulfide Indole Motility) Bios Medium is used for the differentiation of *Enterobacteriaceae*, especially *Salmonella* and *Shigella*, based on the production of H_2S, indole and the mobility test.[1,2]
Peptones provide carbon, nitrogen and trace elements for bacterial growth. Ferric ammonium citrate is as an indicator of the formation of hydrogen sulphide. H_2S positive strains produce thiosulphate reductase that cause the release of a sulfide molecule from sodium thiosulfate present in the medium; this sulfide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric ammonium citrate, forming ferrous sulphide, resulting in a black precipitate. Tryptone is rich in tryptophan, that is hydrolysed by tryptophanase to produce three possible end products indole, pyruvate and ammonia. Indole production is detected by Kovac's reagent which contains 4 (p)-dimethylamino benzaldehyde: this reacts with indole to produce a red coloured compound.
The detection of bacterial motility is favoured by the low concentration of agar: in the semi-solid medium, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye.
The medium does not contain carbohydrates since they are inhibitory for tryptophanase[3] and for the production of iron sulphide[4].

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute in tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool in upright position.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

SIM Bios Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

TEST PROCEDURE

With inoculating needle stab two-thirds the depth of medium in the centre of tube. Incubate tubes with loosened caps, aerobically, at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

Determine motility and H_2S production before the addition of the reagent for determination of indole production.
Motility positive: diffuse growth outward away from stab line or turbidity of the medium; a negative motility test is indicated by growth confined to the stab line.
H_2S production: blackening along stab line or extensive blackening of medium; negative test: no blackening
Indole production: add 3-4 drops of Kovacs' reagent. Positive test: red colour in upper position of medium; negative test: yellow colour in upper position of medium.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	H_2S	MOTILITY	INDOLE
E. coli ATCC 25922	35-37°C / 18-24H /A	-	+	+
S. Typhimurium ATCC 14028	35-37°C / 18-24H /A	+	+	-
S. sonnei ATCC 9290	35-37°C / 18-24H /A	-	-	-

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The tests that can be performed with the SIM Bios Medium are not sufficient to identify *Enterobacteriaceae* at the species level.
- Do not take inoculums from liquid or broth suspension.
- It is necessary to inoculate the medium taking care to remove the needle along the same stabbing line.
- Hydrogen sulfide reactions are intensified by motile cultures.[1]

- Studies of Edmondson and Sanford showed that non-motile mucoid *Klebsiella* strains may give false positive motility reaction; this is due to mucoid strains spilling between medium and tube giving a cloudy appearance which is often confused with motility.[5]
- Temperature-dependent mobility is observed for many strains of *Yersinia enterocolitica*; the ambiguous results that are reported for this microorganism do not recommend adopting the mobility test in the identification schemes for *Y. enterocolitica*.[6]
- Motile bacteria but with damaged flagella can give false negative results.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification.

Storage conditions

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks.[1]

References

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Atlas D, Snyder J. Media Reagents and Stains. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.345.
3. Freundlich M, Lichstein HC. Inhibitory effect of glucose on thryptophanase. J Bacteriol 1960; 80:633-638.
4. Bulmash JM, Fulton M. Discrepant tests for hydrogen sulfide J. Bact 1964;88:1813.
5. Edmondson EB, Sanford JP. The Klebsiella-Enterobacter (Aerobacter)-Serratia group. Medicine 1967; 46(4): 323.
6. D'Amato RF, Tomfohrde KM. Influence of Media on Temperature-Dependent Motility Test for Yersinia enterocolitica. J Clin Microbiol 1981; 14:347-348.

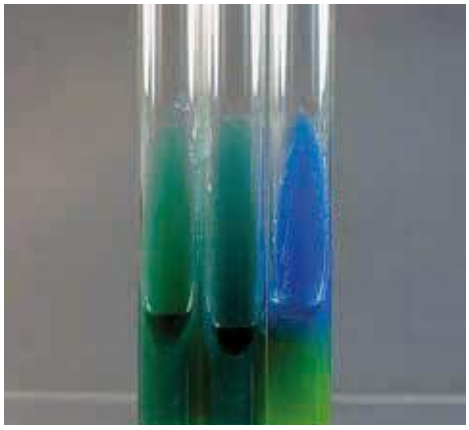
Packaging

Product	Type	REF	Pack
SIM Bios Medium	Dehydrated medium	4020362	500 g (16.6 L)

IFU rev 2, 2022/03

SIMMONS CITRATE AGAR

Dehydrated culture medium



Simmons Citrate Agar-from left:
uninoculated tube, *E. coli*, *E. aerogenes*

Intended use

In vitro diagnostic device. Medium for the differentiation of *Enterobacteriaceae* based on the utilization of citrate as the sole source of carbon.

Composition -typical formula * (after reconstitution with 1 L of water)

Ammonium dihydrogen phosphate	0.20 g
Sodium ammonium phosphate	0.80 g
Sodium chloride	5.00 g
Sodium citrate	2.00 g
Magnesium sulphate	0.20 g
Bromothymol blue	0.08 g
Agar	15.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Simmons Citrate Agar is a modification developed by Simmons[1] of the liquid medium of Koser[2], with the addition of 1.5% agar and bromothymol blue as pH indicator.[3]
In Simmons Citrate Agar the sole source of nitrogen is sodium ammonium phosphate and the sole source of carbon is sodium citrate. The metabolism of citrate is based on the enzyme citrate-permease, which requires for its activation the presence of divalent cations, given in the medium by magnesium sulphate. The result of citrate metabolism is the formation, in an acid environment, of acetate, lactate, acetoin, carbonates and bicarbonates. The degradation of inorganic ammonium salts results in the formation of ammonia. Bacteria that utilize citrate as the sole source of carbon, utilize ammonium salt as the sole source of nitrogen and grow with alkalinisation of the medium, indicated by the colour change of bromothymol blue from green to blue.
Simmons Citrate Agar may be used to differentiate *E. coli* (citrate negative) from *E. aerogenes* (citrate positive), and citrate-positive Salmonella Enteritidis and members of *Salmonella* subgenera II, III and IV from Salmonella Typhi, Salmonella Paratyphi A, Salmonella Pullorum and Salmonella Gallinarum (citrate negative).
Citrate test is recommended by ISO 10273 for differentiating *Y. enterocolitica* (citrate negative) from other species of the genus *Yersinia* such as *Y. intermedia, Y. frederiksenii, Y. rohdei, Y. aldovae* (citrate positive or variable).[4]

Directions for medium preparation

Suspend 23.2 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute into tubes and autoclave at 121°C for 15 minutes. Cool in slanted position (long slant/short butt). All glassware must be chemically clean and alkali free. The medium can be used for citrate test in Petri dishes too.

Physical characteristics

Dehydrated medium appearance	dark yellow, fine, homogeneous, free-flowing powder
Solution and tubed medium appearance	green, limpid
Final pH at 20-25 °C	7.0 ± 0.2

Specimens
This medium is not intended to be used for primary isolation; it must be inoculated with pure cultures of organisms isolated from clinical and non-clinical specimens.

Test procedure
The medium may be used either as slopes in test tubes or as a plate medium in Petri dishes. In both cases the surface of the medium must be lightly inoculated by streaking and, where slopes are used, the butt of medium is inoculated by stabbing. Incubate in aerobic atmosphere with the loose caps at 35-37°C and record the results after 24-48 hours. Some citrate positive bacteria require more than 48 hours to develop the reaction: in case of a negative result, incubate up to 4 days.

Reading and interpretation
After incubation, observe the growth and the colour change of the medium.
Citrate positive: growth with intense blue colour on the slant (alkalinity).
Citrate negative: no growth with no change in colour.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.
Citrate positive test-strain: *E. aerogenes* ATCC 13048
Citrate negative test-strain: *E. coli* ATCC 25922
Incubation in aerobic atmosphere at 35-37°C for 18-24 hours.

ATCC is a trademark of American Type Culture Collection

Limitations of the method
- If a large inoculum is used to streak, the slant may give a false positive result.[3]
- Be careful not to remove traces of culture medium together with the colonies to be tested as this can lead to false positives. Some authors recommend diluting the inoculums in saline prior to inoculation of the medium to avoid a carry-over of other carbon sources.[3]
- Some citrate positive organisms require 48 h or longer incubation for a pH change to occur (up to 4 days).[3]
- If results are equivocal (± e.g., with *Providencia*) re-incubate a new slant and incubate at room temperature (22-25°C) for 7 days.[3]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks.[3]

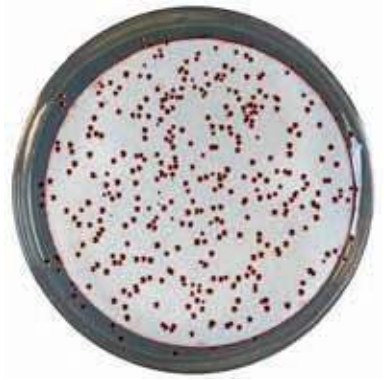
References

1. Simmons JS. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. J Infect Dis 1926; 39:209
2. Koser SA 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J Bacteriol 1923; 8:493
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. ISO 10273:2017. Microbiology of the food chain — Horizontal method for the detection of pathogenic Yersinia enterocolitica

Packaging
Product	Type	REF	Pack
Simmons Citrate Agar	Dehydrated medium	4020452	500 g (21.7L)

IFU rev 2, 2022/04

SLANETZ BARTLEY AGAR
Dehydrated and ready-to-use culture medium



Slanetz Bartley Agar: colonies of *Enterococcus faecalis*

Intended use
Selective and differential medium for the enumeration of enterococci in water and in other materials by the membrane filtration technique.

Composition - typical formula * (after reconstitution with 1 L of water)
Dehydrated medium and ready-to-use plates

Tryptose	20.00 g
Yeast extract	5.00 g
Glucose	2.00 g
Potassium phosphate bibasic	4.00 g
Sodium azide	0.40 g
Triphenyl tetrazolium chloride (TTC)	0.10 g
Agar	10.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure
Slanetz Bartley Agar is a selective and differential medium prepared according to the formulation devised by Slanetz, Bent and Bartley[1] and later modified by Slanetz and Bartley[2] with the introduction of triphenyl tetrazolium chloride (TTC). Slanetz Bartley Agar, also called mEnteroccous agar or m-Azide Agar, meets the requirements of ISO 7899-2[3] and APHA[4] for the enumeration of intestinal enterococci in water using the membrane filtration technique. Burkwall and Hartman[5] demonstrated that the addition of 0.5 mL of Tween 80 and 20 mL of a 10% sodium carbonate or bicarbonate solution to each litre of the medium was valuable when investigating enterococci in frozen foods. The method described in ISO 7899-2 involves enumeration of intestinal enterococci with membrane filters on Slanetz Bartley Agar medium, followed by confirmation on Bile Aesculin Azide Agar.

Tryptose and yeast extract provide nitrogen, carbon, vitamins, amino acids and trace elements for microbial growth. Glucose is a source of carbon and energy; dipotassium phosphate buffers the medium and sodium azide is the selective agent to suppress the growth of Gram-negative bacteria. TTC acts as an indicator: enterococci reduce it to insoluble formazan inside the bacterial cells and grow with red/brown/pink colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g of in 1000 ml of cold purified water. Heat to boiling with frequent agitation. Do not exceed heating time and temperature, do not autoclave. Cool to approximately 47-50 °C, mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Prepared medium appearance	yellow with pink hues, clear or slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.1

SPECIMENS

The method of analysis described here, taken from ISO 7899-2, is suitable for the examination of drinking water, water from swimming pools and other clean or disinfected water. However, the method can be applied to all types of water with the exception of water with a high amount of suspended matter or considerable load of interfering microorganisms. The application of the method appears particularly appropriate for the analysis of large quantities of water containing low number of intestinal enterococci. Refer to the cited Standard[3,4] and other applicable Standards for operational sampling details.

TEST PROCEDURE,

Membrane filter technique[3]

1. Filter a suitable volume of sample through a 0.45 µm membrane filter.
2. Place the membrane on a Slanetz Bartley Agar plate and incubate at 36 ± 2°C for 44 ± 4 hours.
3. After incubation, consider as typical all colonies showing red, brown or pink colour.
4. If typical colonies are observed, transfer the membrane to the surface of a Bile Aesculin Azide Agar ISO Formulation plate (REF 401018) and incubate at 44 ± 0.5°C for 2 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth, recording each specific morphological and colour characteristic of the colonies.
Count as intestinal enterococci all colonies red-brown or pink on Slanetz Bartley Agar and which grow with a brown to black halo on Bile Aesculin Azide Agar.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 29212	35-37°C /44-48 H-A	good growth, red colonies
E. faecium ATCC 6057	35-37°C /44-48 H-A	good growth, red colonies
E. coli ATCC 25922	35-37°C /44-48 H-A	totally inhibited
S. aureus ATCC 25923	35-37°C /44-48 H-A	totally inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- ISO 7899-2 describes a method for the isolation and enumeration of intestinal enterococci, mainly belonging to the species *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae*. In addition, other species referable to the genus *Enterococcus* and some species referable to the genus *Streptococcus* (i.e., *S. bovis* and *S. equinus*) may occasionally be detected. These *Streptococcus* species do not survive long in water and it is likely that a quantitative assessment is not possible. For the purposes of water testing, enterococci may be considered as indicators of faecal pollution. However, it should be noted that some enterococci found in water may also occasionally originate from different habitats.[3]
- In the confirmation test performed with filter membrane transfer, an uneven distribution of bacterial colonies or the presence of high microbial loads may interfere with the differentiation of positive colonies due to the spread of colour to adjacent colonies.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 7889-2, prepared plates can be stored in the dark and protected against evaporation for up to 2 weeks at 5 °C ± 3 °C.

Ready-to-use plates

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Slanetz LW, Bent DF, Bartley CH. Use of the membrane filter technique to enumerate enterococci in water. Public Health Rep (1896),1955;70:67-72.
2. Slanetz LW, Bartley CH. Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. J Bacteriol 1957; 74:591-5.
3. ISO 7899-2:2000 Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method.
4. APHA Standard Methods for the Examination of Water and Wastewater. 23th ed. American Public Health Association, Washington, D.C. 2017.
5. Burkwall MK, Hartman PA. Comparison of direct plating media for the isolation and enumeration of enterococci in certain frozen foods. Appl Microbiol. 1964; 12:18-23.

PACKAGING

Product	Type	REF	Pack
Slanetz Bartley Agar	Dehydrated medium	4020462 4020464	500 g (12.1 L) 5 kg (121 L)
Slanetz Bartley Agar	Ready-to-use plates	542046	2 x 10 plates ø 90 mm
Slanetz Bartley Agar	Ready-to-use plates	492046	3 x 10 plates ø 55 mm

IFU rev 4, 2022/06

SLANETZ BATLEY AGAR W/O TTC

Dehydrated culture medium and supplement

INTENDED USE

Selective and differential medium to be supplemented with TTC for the enumeration of enterococci in water and in other materials by the membrane filtration technique.

COMPOSITION*

SLANETZ BARTLEY AGAR W/O TTC, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	20.00 g
Yeast extract	5.00 g
Glucose	2.00 g
Potassium phosphate bibasic	4.00 g
Sodium azide	0.40 g
Agar	10.00 g

TTC 1% SOLUTION (BOTTLE CONTENT)

Triphenyl tetrazolium chloride	0.3 g
Purified water	30 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Slanetz Bartley Agar completed with TTC is a selective and differential medium prepared according to the formulation devised by Slanetz, Bent and Bartley[1] and later modified by Slanetz and Bartley[2] with the introduction of triphenyl tetrazolium chloride (TTC). Slanetz Bartley Agar completed with TTC, also called mEnteroccous agar or m-Azide Agar, meets the requirements of ISO 7899-2[3] and APHA[4] for the enumeration of intestinal enterococci in water using the membrane filtration technique. Burkwall and Hartman[5] demonstrated that the addition of 0.5 mL of Tween 80 and 20 mL of a 10% sodium carbonate or bicarbonate solution to each litre of the medium was valuable when investigating enterococci in frozen foods. The method described in ISO 7899-2 involves enumeration of intestinal enterococci with membrane filters on Slanetz Bartley Agar medium, followed by confirmation on Bile Aesculin Azide Agar.
Slanetz Bartley Agar without TTC is proposed for industrial preparation of poured plates, where big volumes cause the medium (with TTC included in the powder) to become light red.
Tryptose and yeast extract provide nitrogen, carbon, vitamins, amino acids and trace elements for microbial growth; glucose is a source of carbon and energy, dipotassium phosphate buffers the medium, sodium azide is the selective agent to suppress the growth of Gram-negative bacteria; TTC acts as an indicator: enterococci reduce it to insoluble formazan inside the bacterial cells and grow with red/brown/pink colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.4 g of in 1000 ml of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to approximately 47-50 °C and ad 10 mL of TTC 1% solution (REF 42111801). Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution appearance	yellow, clear or slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.1

SPECIMENS

The method of analysis described here, taken from ISO 7899-2, is suitable for the examination of drinking water, water from swimming pools and other clean or disinfected water. However, the method can be applied to all types of water with the exception of water with a high amount of suspended matter or considerable load of interfering microorganisms. The application of the method appears particularly appropriate for the analysis of large quantities of water containing low number of intestinal enterococci. Refer to the cited Standard[3,4] and other applicable Standards for operational sampling details.

TEST PROCEDURE,

Membrane filter technique[3]

1. Filter a suitable volume of sample through a 0.45 µm membrane filter.
2. Place the membrane on a Slanetz Bartley Agar plate and incubate at 36 ± 2°C for 44 ± 4 hours.
3. After incubation, consider as typical all colonies showing red, brown or pink colour.
4. If typical colonies are observed, transfer the membrane to the surface of a Bile Aesculin Azide Agar ISO Formulation plate (REF 401018) and incubate at 44 ± 0.5°C for 2 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth, recording each specific morphological and colour characteristic of the colonies.
Count as intestinal enterococci all colonies red-brown or pink on Slanetz Bartley Agar and which grow with a brown to black halo on Bile Aesculin Azide Agar.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis ATCC 29212	35-37°C /44-48 H-A	good growth, red colonies
E. faecium ATCC 6057	35-37°C /44-48 H-A	good growth, red colonies
E. coli ATCC 25922	35-37°C /44-48 H-A	totally inhibited
S. aureus ATCC 25923	35-37°C /44-48 H-A	totally inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- ISO 7899-2 describes a method for the isolation and enumeration of intestinal enterococci, mainly belonging to the species *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae*. In addition, other species referable to the genus *Enterococcus* and some species referable to the genus *Streptococcus* (i.e., *S. bovis* and *S. equinus*) may occasionally be detected. These *Streptococcus* species do not survive long in water and it is

likely that a quantitative assessment is not possible. For the purposes of water testing, enterococci may be considered as indicators of faecal pollution. However, it should be noted that some enterococci found in water may also occasionally originate from different habitats.[3]

- In the confirmation test performed with filter membrane transfer, an uneven distribution of bacterial colonies or the presence of high microbial loads may interfere with the differentiation of positive colonies due to the spread of colour to adjacent colonies.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 7889-2, prepared plates can be stored in the dark and protected against evaporation for up to 2 weeks at 5 °C ± 3 °C.

REFERENCES

1. Slanetz LW, Bent DF, Bartley CH. Use of the membrane filter technique to enumerate enterococci in water. Public Health Rep (1896),1955;70:67-72.
2. Slanetz LW, Bartley CH. Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. J Bacteriol 1957; 74:591-5.
3. ISO 7899-2:2000 Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method.
4. APHA Standard Methods for the Examination of Water and Wastewater. 23th ed. American Public Health Association, Washington, D.C. 2017.
5. Burkwall MK, Hartman PA. Comparison of direct plating media for the isolation and enumeration of enterococci in certain frozen foods. Appl Microbiol. 1964; 12:18-23.

PACKAGING

Product	Type	REF	Pack
Slanetz Bartley Agar w/o TTC	Dehydrated medium	4020472 4020464	500 g (12.1 L) 5 kg (121 L)
TTC 1% Solution	Liquid supplement	42111801	30 mL

IFU rev 4, 2022/06

SPORULATION AGAR AK
SPORULATION BROTH

Dehydrated culture media

INTENDED USE

For the preparation of spore suspensions used to detect antibiotic residues in milk and dairy products.

COMPOSITION - TYPICAL FORMULAS (AFTER RECONSTITUTION WITH 1 L OF WATER)

SPORULATION AGAR AK	
Gelatin peptone	6.0 g
Yeast extract	3.0 g
Tryptone	4.0 g
Beef extract	1.5 g
Glucose	1.0 g
Manganous sulphate	0.3 g
Agar	15.0 g

SPORULATION BROTH	
Gelatin peptone	6.0 g
Yeast extract	3.0 g
Tryptone	4.0 g
Beef extract	1.5 g
Glucose	1.0 g
Manganous sulphate	0.3 g

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Sporulation, unique to two bacterial species, *Clostridium* and *Bacillus*, is a process induced by reduced levels of nutrients in the environment or in culture.[1] A specific sporulation medium is required for producing spores from specific group of spore-forming bacteria.
Sporulation Agar AK, also known as AK 2 Agar or USP Antibiotic Medium 32, is a modification of Antibiotic Assay Medium No.1, described by Arret and Kirshbaum[2] for the production of *Bacillus subtilis* spores for rapid disc assay method for detecting penicillin in milk. The medium was also found useful for spore production in *Bacillus megaterium* ATCC 9885.[3,4]
Gelatin peptone, beef extract and tryptone provide nitrogen, amino acids and trace elements for microbial growth. The yeast extract is a source of vitamins, particularly of the B-group. Glucose is a fermentable carbohydrate and a source of energy. Manganese ions are necessary for the activity of the enzyme phosphoglycerate phosphomutase involved in the sporification process.[5]
Sporulation Broth is prepared with the same formulation of Sporulation Agar AK with the omission of agar. It can be used for sporification in a liquid culture medium or added to agar for growth on the medium surface.

SPORULATION AGAR AK: DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30.8 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and autoclave at 121° C for 15 minutes.

SPORULATION BROTH: DIRECTIONS FOR MEDIUM PREPARATION

Suspend 15.8 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and autoclave at 121° C for 15 minutes. If required, add 15 g/L of agar (REF 411030) before autoclaving.

PHYSICAL CHARACTERISTICS

Dehydrated media appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	6.6 ± 0.2

SPECIMENS

Pure culture of spore-forming bacteria.

TEST PROCEDURE

Preparation of *B. subtilis* spores with Sporulation Agar AK [6]

1. Transfer monthly *B. subtilis* ATCC 6633 culture to fresh slant of Antibiotic Seed Agar A1 (REF 401075)
2. Wash the growth from the slant with sterile physiological saline onto the surface of a Roux bottle containing 300 mL of solidified Sporulation Agar AK.

3. Incubate the bottle aerobically at 35°C for 5 days with caps loosened.
4. Aseptically wash off the resulting growth into 50 mL of sterile physiological saline with the aid of sterile crystal pearls if necessary.
5. Centrifuge and aseptically decant and discard the supernatant.
6. Resuspend the sediment in few millilitres of sterile saline and heat to 70°C for 30 minutes.
7. The spore suspension can be stored for several months.[6]

Preparation of *B. subtilis* spores with Sporulation Broth[7]

1. For sporulation in the liquid medium, 25 mL of an overnight bacterial culture is added into a 2 L flask containing 250 mL of fresh Sporulation Broth and incubated at 37 °C with vigorous shaking at 200 rpm for at least 3 days, until the fraction of spores/vegetative cells came to a maximum level.
2. Centrifuge, resuspend the sediment and heat the culture as described above.

Consult the references for the test procedures utilizing *B. subtilis*[2] or *B. megaterium*[4] spore suspensions.

READING AND INTERPRETATION

The resulting growth on the medium surface is compact and the extent of sporulation must be assessed by microscopic observation.
The resulting growth on the liquid medium is indicated by a varying degree of turbidity, specks and flocculation in the medium. The extent of sporulation must be assessed by microscopic observation.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
B. subtilis ATCC 6633	35°/ 5 days/A	Good growth; spores present

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Driks A. Overview: development in bacteria: spore formation in Bacillus subtilis. Cell Mol Life Sci 2002; 59: 389-391.
2. Arret B, Kirshbaum A. A rapid disc assay method for detecting penicillin in milk. J Milk Food Tec. 1959; 22:329.
3. Verma N, Singh NA, Kumar N, Raghu HV. Screening of different media for sporulation of Bacillus megaterium. Int J Microbiol Res and Rev 2013;1: 68-73.
4. APHA Standard Methods for the Examination of Dairy Products. American Public Health Association. Washington, D.C. 15th ed.1985
5. Vasantha N, Freese E. The role of manganese in growth and sporulation of Bacillus subtilis. J Gen Microbiol 1979; 112:329-36.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
7. Li L, Jin J, Hu H, Deveau IF, Foley SL, Chen H. Optimization of sporulation and purification methods for sporicidal efficacy assessment on Bacillus spores. J Ind Microbiol and Biotechnol 2022; 49:1-10.

PACKAGING

Product	Type	REF	Pack
Sporulation Agar AK	Dehydrated medium	4020702	500 g (16.2 L)
Sporulation Broth	Dehydrated medium	4020712	500 g (31.6 L)

IFU rev 1, 2022/12

SPS AGAR: see SULPHITE POLYMIXIN SULFADIAZINE (SPS) AGAR

SS AGAR

Dehydrated and ready-to-use culture medium

SS Agar:
Salmonella arizonae colonies with large black centre

INTENDED USE

In vitro diagnostics. Selective and differential medium for the isolation of Gram-negative enteric pathogens, especially *Salmonella* from clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Beef extract	5.000 g
Peptocomplex	5.000 g
Lactose	10.000 g
Bile salts n°3	8.500 g
Sodium thiosulphate	8.500 g
Sodium citrate	8.500 g
Ferric citrate	1.000 g
Neutral red	0.025 g
Agar	13.500 g
Brilliant green	0.330 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In the first half of the twentieth century, several culture media were developed and proposed for the isolation of enteric pathogens from faeces and other materials. SS (Salmonella Shigella) Agar is a modification of deoxycholate medium described by Leifson[1] in 1935, and successfully tested by Catherine Mayfield and Maud Gober[2] in 1941 for the isolation of *Shigella dysenteriae* and *Salmonella* from stool. Several years later,

this medium was discovered to be overly selective and some strains of *Shigella* were missed.[3,4] For the isolation of *Shigella* the recommended plating media are Hektoen Enteric Agar or XLD Agar.[5]
SS Agar is a selective and differential medium intended for the isolation of Gram-negative enteric pathogens, especially *Salmonella* from clinical specimens.[5,6]
Peptones provide carbon, nitrogen and trace elements for bacterial growth; the high concentration of bile salts n° 3, sodium citrate and brilliant green inhibit Gram-positive organisms and most of the non-pathogenic coliform flora of the intestinal tract. Since the enteric pathogen *Salmonella* can tolerate these inhibitory substances, they generally grow faster and larger than the coliforms. Lactose is fermented by coliforms, that are able to grow in the presence of the bile salts, with production of acids. The acid condition causes the neutral red indicator to change to a pink-red colour and to bile salts to precipitate over the medium appearing as a hazy zone around the colonies. Ferric citrate is as an indicator of the formation of hydrogen sulphide. *Salmonella* spp. produce thiosulphate reductase that causes the release of a sulphide molecule from the sodium thiosulfate present in the medium. This sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric ammonium citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 60 g in 1000 mL of cold purified water. Heat to boiling stirring constantly. Do not overheat, do not autoclave. Cool to 47-50°C and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-orange, limpid or slightly opalescent
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

SS Agar is intended for the bacteriological processing of clinical specimens such as faeces and rectal swabs[5,6] Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[7]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium. Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using an enrichment step in Selenite Broth, followed by sub-culture to SS Agar and to a second less selective plating medium.[5,7]
Incubate inoculated SS Agar plates with the specimen or with specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Do not examine areas of confluent growth as false negative fermentation reactions may occur.
Smooth, opaque colourless colonies with black centres: no fermentation present, H_2S production present: suspect *Salmonella*.
Smooth, opaque colourless colonies without black centre: no fermentation present, H_2S production absent: suspect H_2S negative *Salmonella* or *Shigella* strains that have by-passed the selective system of the medium.
Pink-red colonies: fermentation of lactose: not likely to be *Salmonella*
E. coli grows slightly with red colonies, with intercolonial precipitate, *E. aerogenes* may appear as large, mucoid, opaque pink to cream coloured colonies.
Since H_2S positive *Proteus* spp. may grow with colourless colonies with black or grey-black centre and if *Proteus* colonies are mixed with H_2S positive *Salmonella* colonies, it could be difficult to choose the colonies for further biochemical and serological identification.
It is advised to screen the colonies by flooding the plate with one drop of MUCAP Test reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of the C_8 esterase enzyme, typical of *Salmonella* spp.[8]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[9]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, colourless colonies with black centre
S. flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, colourless colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, red colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Be aware that *Proteus* spp. may or may not be inhibited and colonies may resemble *Salmonella*.[6] Rapid differentiation between very similar colonies may be performed with MUCAP Test.[8]
- Some lactose fermenting *Shigella* and *Salmonella* strains may resemble coliforms and are not recognized on SS Agar.
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, for the isolation of *Salmonella,* additional media with lower selectivity, such as Mac Conkey Agar, should be used. For the isolation of *Shigella* spp. the recommended media are Hektoen Enteric Agar or XLD Agar and a second medium with lower selectivity such as Mac Conkey Agar. Other media for the isolation of other enteric pathogens should be inoculated with the specimen.[5]
- Over time and during the shelf-life, bile salts in SS Agar plates may crystallize and form a precipitate in the medium. This does not affect the performance of the medium.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the plated medium prepared by the user can be stored at +2°C +8°C for up to 6-8 weeks.[6]

Ready-to-use plates
Store plates in their original pack at +2°C/+8°C away from direct light.

References

1. Leifson, E. 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. 40:581–599.
2. Mayfield, C. R., and M. Gober. 1941. Comparative efficiency of plating media for the isolation of Shigella dysenteriae. Am. J. Public Health 31:363–368.
3. King, S., and W. I. Metzger. 1968. A new plating medium for the isolation of enteric pathogens: II. Comparison of Hektoen Enteric Agar with S S and E M B Agar. Appl. Microbiol. 16: 579–581.
4. Taylor, W. I. 1965. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. Am. J. Clin. Pathol. 44:471–475.
5. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.685.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.270.
8. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526.
9. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

Packaging
Product	Type	REF	Pack
SS Agar	Dehydrated medium	4020752 4020754	500 g (8,3 L) 5 kg (83 L)
SS Agar	Ready-to-use plates	542075	2 x 10 plates ø 90 mm

IFU rev 2, 2022/01

STAA AGAR BASE
STAA SELECTIVE SUPPLEMENT
Dehydrated culture medium and selective supplement

Intended use
For the enumeration of *Brochothrix spp.* in food samples, especially meat and meat products.

Composition*
STAA Agar Base
Typical formula (after reconstitution with 1 L of water)

Peptone	20.00 g
Yeast extract	2.00 g
Dipotassium hydrogen phosphate	1.00 g
Magnesium sulphate	0.49 g^
Cycloheximide	0.05 g
Bromocresol purple	0.02 g
Agar	11.50 g

STAA Selective Supplement
(Vial content for 500 mL of medium)

Streptomycin sulphate	250 mg
Thallous acetate	25 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.
^ Corresponding to 1 g of magnesium sulphate heptahydrate

Principle of the method and explanation of the procedure
The genus *Brochothrix* includes 2 species, *B. thermosphacta* and *B. campestris*.[1] *B. thermosphacta* is a Gram-positive, rod-shaped, nonmotile, non-spore-forming, facultative anaerobic, and psychrotrophic organism frequently involved in the spoilage of prepacked and vacuum-packaged meat or meat products.[2] *B. campestris* has so far only been identified in samples from soil and grass.[1]
Streptomycin thallous acetate actidione (STAA) agar is based on the formulation described by Gardner[2] for the enumeration of *Brochothrix* spp. in foods, especially meat and meat products. The complete medium is prepared with the basic medium to which glycerol and the selective supplement consisting of streptomycin and thallium acetate are added.
Peptone provides nitrogen and minerals for microbial growth; yeast extract is a source of B-vitamins complex for growth stimulation. Dipotassium phosphate is used as buffering agent to control the pH in the medium while magnesium sulphate enhances the microbial growth. Streptomycin sulphate suppressed the growth of some Gram-positive and most Gram-negative bacteria but has no effect on the development of *Brochothrix* spp. Thallous acetate inhibits most yeasts as well as many aerobic and facultatively anaerobic bacteria. Cycloheximide is included as antifungal agent

Directions for medium preparation
Suspend 17.5 g in 500 mL of cold purified water and add 7.5 g of glycerol (REF 421015). Heat to boiling with frequent agitation to dissolve the medium completely and sterilize by autoclaving at 115°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of STAA Selective Supplement (REF 4240052), reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.
WARNING: the powder medium STAA Agar Base contains cycloheximide. Handle with care, avoiding contact with skin and eyes. The selective supplement contains thallous acetate. Don't inhale, avoid contact with skin and eyes. Handle the product wearing gloves and using eye protective gear. Read the Material Safety Data Sheets.

Physical characteristics
STAA Agar Base ISO

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, limpid
Final pH at 20-25 °C	7.0 ± 0.2

STAA Selective Supplement

Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid

Specimens
Products intended for human consumption and animal feeding especially meat and meat products, environmental samples in the area of food and feed production, handling, and samples from the primary production stage. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

Test procedure
1. Transfer, by means of a sterile pipette 0.1 ml of the test sample if the product is liquid, or of the initial suspension in the case of other products, to the STAA Agar plate.
2. Repeat the procedure using further decimal dilutions, if necessary.
3. Spread the inoculum as quickly as possible over the surface of the agar plate without touching the sides of the dish with the spreader. Leave the plates with the lids on for about 15 min at room temperature for the liquid to be absorbed into the agar.
4. Invert the prepared plates and incubate at 22°C / 25 °C for 48 h ± 4.

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
On STAA Agar, *Brochothrix* usually produce shiny, round or circular colonies of diameter 1 mm or larger with off-white colour.
Count the characteristic colonies on each dish containing 10 to 150 colonies.
Perform oxidase and catalase tests: *Brochothrix* is oxidase-negative and catalase positive.

User quality control
All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

Control strains	Incubation T°/ t / ATM	Expected results
B. thermosphacta ATCC 11509	22-25°C/ 44-52 H / A	good growth
E. faecalis ATCC 19433	22-25°C/ 44-52 H / A	totally or partially inhibited
L. sakei ATCC 15521	22-25°C/ 44-52 H / A	totally or partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- *Pseudomonads* may grow on STAA medium. They shall be differentiated from *Brochothrix* spp. by performing the oxidase test.[1]
- Certain lactic acid bacteria may produce characteristic colonies on STAA medium. Lactic acid bacteria are differentiated from *Brochothrix* spp. by performing the catalase test.[1]

Storage conditions
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at 2-8°C away from direct light.
According to Baird RM et al. and ISO 13722, the self-prepared plates may be stored at between 2 °C and 8°C °C for up to 1 week and the bottled basal medium for 1 month at room temperature.[1,3]

References

1. ISO 13722:2017. Microbiology of the food chain — Enumeration of Brochothrix spp. — Colony-count technique.
2. Gardner GA. A selective medium for the enumeration of Microbacterium thermosphactum in meat and meat products. J Appl Bacteriol. 1966; 29:455-60.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:270-1.

Packaging
Product	Type	REF	Pack
STAA Agar Base	Dehydrated medium	4020792	500 g (28.9 L)
STAA Selective Supplement	Freeze-dried supplement	4240052	10 vials, each for 500 mL of medium

IFU rev 1, 2022/09

STAPHYLOCOCCI 110 MEDIUM

Dehydrated culture medium

Staphylococci 110 Medium plate flooded with ammonium sulphate solution: gelatinase positive *S. aureus* colonies

Intended use
Selective medium for the isolation and differentiation of staphylococci.

Composition - typical formula * (after reconstitution with 1 L of water)
Tryptone	10.0 g
Yeast extract	2.5 g
Gelatin	30.0 g
Lactose	2.0 g
Mannitol	10.0 g
Sodium chloride	75.0 g
Dipotassium hydrogen phosphate	5.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Staphylococci 110 Medium, also known as Stone Gelatin Agar, is prepared on the basis of the formula described by Chapman[1] in 1946, following Stone's discovery in 1935[2] of the positivity to gelatinase test by food-poisoning staphylococci.
Staphylococci 110 Medium is a selective medium for the isolation and differentiation of staphylococci, based on the tolerance to high concentrations of sodium chloride, pigmentation of colonies, fermentation of mannitol and liquefaction of gelatin.[3] The medium complies with the formulations described by APHA[4] and AOAC[5].
The medium is suitable for isolation and differentiation of staphylococci for studies of food-poisoning outbreaks.[3]
Tryptone and yeast extract provide nitrogen, carbon, minerals and vitamins for microbial growth. Potassium phosphate prevents pH changes. The selectivity of the medium is due to the presence of a high NaCl content, which allows a good growth of staphylococci and a partial to total inhibition of Gram-negative bacteria and enterococci. Mannitol is included as a fermentable carbohydrate, lactose is an additional source of carbon; *S. aureus* ferments mannitol producing the acidification around the colonies; its fermentation can be detected by adding a few drops of bromocresol purple on the area of removed colonies from the agar plate, resulting in the production of a yellow colour. Gelatin serves as a substrate for gelatinase activity: gelatin hydrolysis is observed as clear zones around colonies after the addition of saturated aqueous solution of ammonium sulphate (Stone reaction).

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 149 g in 1000 ml of cold purified water; heat to boiling with frequent agitation and autoclave at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	beige, hazy
Prepared plates appearance	beige, opalescent
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Staphylococci 110 Medium is intended for the bacteriological processing of food samples. For samples collection and preparation, refer to the applicable international standards.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the sample with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Routinely incubate in aerobic atmosphere at 35°C for 43 hours. Alternatively incubate at 30°C for 48 hours. The incubation at 30°C produces a deeper pigmentation with no interference with Stone reaction or acid production from mannitol fermentation[3].

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
The presumptive differentiation between *S. aureus* and *S. epidermidis* is performed by reading the mannitol fermentation, gelatinase test and colony pigmentation.
S. aureus colony pigmentation: usually bright golden yellow-orange colour; *S. epidermidis, S. saprophyticus* colony pigmentation: white to cream coloured, basically non pigmented.
Mannitol fermentation: determine after reading colony pigmentation. Add a drop of 0,04% solution of bromocresol purple or bromothymol blue to the area where the colony was removed: a positive reaction results in an acid pH with resultant yellow colour.
Gelatinase activity: determine after recording mannitol fermentation. Flood the plate with 5 mL of saturated aqueous solution of ammonium sulphate pre-warmed at 35°C; incubate for 10 minutes at 35°C; a positive reaction is indicated by the formation of clear zones in an opaque white background, around pigmented colonies or areas of removed colonies.
Yellow-orange, gelatinase positive and mannitol positive colonies are presumptively identified as *S. aureus*.
White colonies, gelatinase positive and mannitol negative are probably *S. epidermidis*.
Confirm a possible *S. aureus* by testing for coagulase production that must be performed after the colony subculture in Nutrient Broth or BHI Broth or on a blood agar plate and incubation at 35°C for 18-24 hours; do not perform coagulase test directly with colonies grown on Staphylococci 110 Medium as salt content may interfere with coagulase results.
Emulsify 0.5 mL of broth culture or a colony from blood agar with 0.5 mL of rabbit plasma (Coagulase Plasma EDTA cat. no. 429937).
Incubate at 35-37°C and observe every 60 minutes in the first 4 hours of incubation for clotting by gently slanting the tube. Do not shake.
If no clot is observed by 4 hours, the tube should be read again after 18-24 h of incubation at 35-37°C.
This is because a small proportion of strains require longer than 4 hours for clot formation.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	30-35°C / 44-48 H / A	growth, yellow colonies with a clear zone, mannitol positive
S. epidermidis ATCC 12228	30-35°C / 44-48 H / A	growth, white colonies with a clear zone, mannitol negative
E. coli ATCC 25922	30-35°C / 44-48 H / A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- *E. faecalis* and other enterococci may exhibit growth and slight mannitol fermentation; however, the colonies are tiny and are easily differentiated from staphylococci by Gram staining and catalase test (*E. faecalis*: catalase negative, cocci in chains; staphylococci: catalase positive, cocci in clusters).[3]
  On primary isolation from the sample *S. aureus* can produce a bright golden yellow-orange pigmentation of the colonies; however, they may also be white or even colourless. The optimal temperature for the production of pigments is slightly lower (25-30°C) than that required for growth. However, colony pigmentation is not a reliable criterion for the differentiation of species of the genus *Staphylococcus*.[3]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the plated medium prepared by the user can be stored at +2°C +8°C for up to 6-8 weeks.

REFERENCES

1. Chapman GH. J. Bacteriol. 1946; 51:409.
2. Stone RV. Proc. Soc. Exper. Biol. & Med. 1935; 33. 185-187.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. American Public Health Association (1978) Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.
5. Association of Official Analytical Chemists (1992) Bacteriological Analytical Manual. 7th Edn. AOAC. Washington DC.

PACKAGING
Product	Type	REF	Pack
Staphylococci 110 Medium	Dehydrated medium	4020852	500 g (3.3 L)

IFU rev 2, 2022/05

STREPTOCOCCUS SELECTIVE AGAR
STREPTOCOCCUS SELECTIVE BROTH
Dehydrated culture media

INTENDED USE
For the selective enrichment and isolation streptococci.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Streptococcus Selective Agar

Tryptone	15.0 g
Soy peptone	5.0 g
Sodium chloride	4.0 g
L-cystine	0.2 g
Sodium Sulphite	0.2 g
Glucose	5.0 g
Sodium azide	0.2 g
Agar	15.0 g
Sodium citrate	1.0 g
Crystal violet	0.2 mg

Streptococcus Selective Broth

Tryptone	15.0 g
Soy peptone	5.0 g
Sodium chloride	4.0 g
L-cystine	0.2 g
Sodium sulphite	0.2 g
Glucose	5.0 g
Sodium azide	0.2 g
Sodium citrate	1.0 g
Crystal violet	0.2 mg

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Streptococcus Selective Agar and Broth are prepared according to the formulation devised by Pike[1,2] The media are known also as Pike Streptococcus Agar/Broth or Streptococcus Enrichment Broth.
They have been proposed for the selective enrichment and isolation of streptococci from various materials, especially those which are heavily contaminated with accompanying microbial flora.[3] They favour the growth of beta-haemolytic streptococci.[3,4]
Tryptone and soy peptone provide nitrogen and minerals for microbial growth, glucose is a source of carbon and energy, sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Sodium azide and sodium sulphite inhibit Gram-negative bacteria while crystal violet suppresses the growth of staphylococci. Beta-haemolytic streptococci are not affected at the low concentration of crystal violet.

DIRECTIONS FOR MEDIA PREPARATION
Streptococcus Selective Agar
Suspend 45.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 118º C for 15 minutes. Do not overheat.
Streptococcus Selective Broth
Suspend 30.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 118º C for 15 minutes. Do not overheat

PHYSICAL CHARACTERISTICS
Streptococcus Selective Agar and Streptococcus Selective Broth

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes/plates appearance	pale yellow, limpid
Final pH at 20-25°C	7.4 ± 0.2

SPECIMENS
Various materials, especially those which are heavily contaminated with accompanying microbial flora.

TEST PROCEDURE
Inoculate the specimen as soon as possible after collection. For isolation procedure, streak with a loop over the four quadrants of the plate of Streptococcus Selective Agar to obtain well isolated colonies. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
For the enrichment procedure put the swab directly into Streptococcus Selective Broth and discharge the material by rotating the swab.
Incubate aerobically at 35-37°C for 18-24 hours

READING AND INTERPRETATION
After incubation, observe the bacterial growth (turbidity) on the broth and record the specific morphological and chromatic characteristics of the colonies on the agar. Subculture to appropriate plate and biochemical media for identification.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
S. pyogenes ATCC 19615	35-37°C / 18-24 h / A	good growth
E. coli ATCC 25922	35-37°C / 18-24 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the plated medium prepared by the user can be stored at +2°C +8°C for up to 6-8 weeks.[3]

REFERENCES

1. Pike RM. Enrichment broth for isolating hemolytic streptococci from throat swabs. Proc Soc Exp Biol Med 1944; 57:186.
2. Pike RM. Isolation of hemolytic streptococci from throat swabs; experiments with sodium azide and crystal violet in enrichment broth. Am J Hyg 1945; 41:211.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Welch DF, Hensel D, Pickett D, Johnson S. Comparative evaluation of selective and nonselective culture techniques for isolation of group A beta-haemolytic streptococci. Am J.Clin.Pathol 1991; 95:587.

PACKAGING

Product	Type	REF	Pack
Streptococcus Selective Agar	Dehydrated medium	4020872	500 g (11 L)
Streptococcus Selective Broth	Dehydrated medium	4020882	500 g (16.3 L)

IFU rev 1, 2022/05

STUART TRANSPORT MEDIUM

Dehydrated culture medium

INTENDED USE

Medium for collecting, transporting and preserving specimens for microbiological analysis.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Sodium glycerophosphate	10.000 g
Sodium thioglycolate	1.000 g
Calcium chloride	0.100 g
Agar	3.400 g
Methylene blue	0.002 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Stuart Transport Medium is prepared in accordance with Ringertz's modification[1] of the medium described by Stuart.[2]
It is a chemically defined, semi-solid, non-nutritive, reductive medium used for the transport and preservation of specimens for microbiological analysis. The medium is particularly suitable for routine transport of gonococcal and other fastidious organisms (e.g., Shigella, *Bordetella*, other respiratory tract pathogens and anaerobes)[3]. The medium maintains viable microorganisms within 24 hours of storage without their significant multiplication.[3]
Sodium thioglycolate, by lowering the redox potential of the medium, allows better preservation of anaerobic bacteria; sodium glycerophosphate and calcium chloride act as a buffer system; methylene blue is an oxidation indicator.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 14.4 g in 1000 mL of cold purified water. Bring to the boil under agitation, dispense into screw-cap tubes, filling them almost completely, so that the height of the medium is about 7 cm. Autoclave with the cap loose for 10 minutes at 121°C; after sterilisation close the tubes tightly and cool rapidly in upright position.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	grey-blue, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, slightly opalescent; light blue on top of tubes.
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Stuart Transport Medium is suitable for the transport and storage of specimens for the isolation of aerobic and anaerobic microorganisms. Good laboratory practices for collection, transport and storage of specimens should be applied.

TEST PROCEDURE

- Insert the swab into the medium to one-third of the medium depth.
- Cut or break the swab stick if longer than the tube.
- Screw the cap firmly.
- Transport to the laboratory as soon as possible or preferably within 6 hours (maximum up to 24 hours).
- Transfer to appropriate isolation media depending on specimen source.
- Incubate plated media using proper microbiological procedures for cultivation of the suspected pathogens.

READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of colonies of varying morphology and size on the isolation media. The characteristics of the growths are closely related to the type or types of cultivated microorganisms.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes ATCC 19615	20-25°C / 24h / A	good recovery after subculture to Blood Agar
N. gonorrhoeae ATCC 19424	20-25°C / 24h / CO_2	good recovery after subculture to Chocolate Agar

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- After 24 hours there is a gradual diminution of viable cells.[3]
- Tubes of Stuart Transport medium will undergo a slight degree of oxidation at mouth of tube; this oxidation is indicated by a blue colour at the upper periphery of the medium. However, if tube exhibits a distinct blue colour throughout the medium, discard.
- Anaerobiosis of the medium may be restored by reliquefying the medium prior to its use.
- Sodium glycerophosphate is a buffer; however, some organisms metabolize it with resultant promotion of bacterial growth.[4]
- According to the data of Barry et al.[5], survival of anaerobes is best if the sample is collected with a cotton swab rather than on calcium alginate swabs.
- The survival of bacteria in a transport medium depends on many factors, including the type and concentration of bacteria in the sample and the temperature during the transport. Optimal growth and typical morphology can only be predicted following direct inoculation of the specimen and the use of an adequate isolation medium. Stuart Transport medium, however, provides an adequate level of microbial survival in specimens that cannot be immediately forwarded to the laboratory.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months

REFERENCES

1. Ringertz O.A modified Stuart Medium for the transport of gonococcal specimens. Acta Pathol. Microbiol.Scand 1960; 48:105
2. Stuart RD, Toshach Sheila R, Patsula TM. The problem of transport of specimens for the culture of gonococci. Acta Pathol Microbiol Scand 1954; 74: 371-374.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. Cary SG, Blair EB. New transport medium for shipment of clinical specimens. J Bacteriol 1964; 88:96-98
5. Barry AL, Fay GH, Sauer RL, (1972) Efficiency of a transport medium for the recovery of aerobic and anaerobic bacteria in various transport media. Appl Microbiol 1972; 24(1): 31.

PACKAGING

Product	Type	REF	Pack
Stuart Transport Medium	Dehydrated medium	4020912	500 g (34.7 L)

IFU rev 1, 2022/05

SUGAR FREE AGAR BASE
PENICILLIN G 500 IU SELECTIVE SUPPLEMENT
SUGAR FREE AGAR

Dehydrated and ready-to-use culture medium, selective supplement

INTENDED USE

For the enumeration of microorganisms not related to dairy-products production process.

COMPOSITION *

SUGAR FREE AGAR BASE – DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Gelatin peptone	7.5 g
Tryptone	7.5 g
Sodium chloride	5.0 g
Agar	13.0 g

SUGAR FREE AGAR – READY-TO-USE PLATES
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Sugar Free Agar Base	33 g
Penicillin G sodium salt	5000 IU

PENICILLIN G 500 IU SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 100 mL OF MEDIUM)

Penicillin G sodium salt	500 IU

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The microbiology of butter and other dairy products reflects the microflora present in milk and water, the sanitary conditions of process equipment, manufacturing environment, and conditions under which the product is stored. A large number of microorganisms may contaminate the dairy products: psychrophilic/psychrotrophic bacteria, mesophilic bacteria, sporeformers, yeasts and moulds. Th estimation of the number of “infective organisms” which are not directly involved in the production process, taken at various stages of processing, is useful in tracing the source of contamination. Sugar Free Agar Base is based on the formulation of Ritter and Eschmann[1], is free of fermentable carbohydrates, has low nutritive characteristics and is primary used for the enumeration of contaminant microorganisms not directly involved in the production process of butter and dairy products.[2]

Supplemented with penicillin G, Sugar Free Agar can be used for the enumeration of mesophilic aerobic Gram-negative bacteria such as *Pseudomonas, Flavobacterium, Alkaligenes, Aeromonas, Xantomonas, Acinetobacter, Enterobacteriaceae*.[3]
The medium contains peptones with a little nutritive value which provide the growth factors for microbial growth, substantially reducing the growth of microorganisms related to production process, in particular lactobacilli, thus favouring the growth of spoiling microorganisms. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Agar is the solidifying agent.

DIRECTIONS FOR MEDIA PREPARATION

Sugar Free Agar
Suspend 33 g in 1000 mL of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes.
Sugar Free Penicillin Agar
If a Sugar Free Penicillin Agar is required, subdivide the medium after boiling in 100 mL aliquots and autoclave at 121°C for 15 minutes. Cool to approximately 45°C and add the contents of one vial of Penicillin G 500 IU Selective Supplement (REF 4240050) reconstituted with 2 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Freeze dried supplement	short, dense, white pellet; clear colourless solution after reconstitution
Final pH at 20-25 °C	7.6 ± 0.2

SPECIMENS

Butter and other dairy products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

1. Prepare the initial suspension of the sample and the decimal dilutions with the suitable diluent.
2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of Sugar Free Agar Base or Sugar Free Agar Base supplemented with penicillin G, cooled to approximately 47°C, into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.
5. Incubate at the temperature required by the analysis for detecting psychrophilic/psychrotrophic /mesophilic bacteria (e.g., 30°C for 72 hours, or 48 hours at 35 °C, followed by 48 hours at 20 °C).

READING AND INTERPRETATION

Enumerate the number of colonies per plate and calculate the microbial count. Do not count pin-point colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 25922	30°/ 24 H-A	good growth
P. aeruginosa ATCC 14207	30°/ 24 H-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplement and ready-to-use plates
Store the product in the original package at 2-8°C away from direct light.

REFERENCES

1. Ritter P., Eschmann KH. 1966, Alimenta, 5 (2): 433.
2. International Dairy Federation: Methods of sampling milk and milk products. International Standard, FIL/IDF 50 B, 1985.
3. Manuel Suisse des Denrées Alimentaires. 5° edition, deuxième volume, Chap. 56. 1988.

PACKAGING

Product	Type	REF	Pack
Sugar Free Agar Base	Dehydrated medium	4020982	500 g (15.1 L)
Penicillin G 500 IU Selective Supplement	Freeze-dried supplement	4240050	10 vials, each for 100 mL of medium
Sugar Free Agar	Ready-to-use plates	542098	2 x 10 plates ø 90 mm

IFU rev 2, 2022/08

SULFITE POLYMYXIN SULFADIAZINE (SPS) AGAR

Dehydrated culture medium

INTENDED USE

For the isolation and enumeration of *Clostridium perfringens* and other clostridia in foodstuffs.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptic digest of casein	15.00
Yeast extract	10.00
Sodium sulphite	0.50
Ferric citrate	0.50
Polymyxin B sulphate	0.01
Sulphadiazine	0.12
Agar	13.50

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Food poisoning caused by *Clostridium perfringens* may occur when foods such as raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices are cooked and held without maintaining adequate heating or refrigeration before serving.[1] The enumeration of *C. perfringens* in food samples plays a key role in the epidemiological investigation of food-borne disease outbreaks.
In the 1950s and 1960s, several studies were carried out to develop a suitable culture medium that would allow the isolation and counting of *C. perfringens.* Mossel *et al.*[2] and Mossel,[3] reported on an iron-sulphite agar medium containing 0.05 % sodium sulphite and 10 ppm polymyxin B sulphate, which yielded quantitative recovery of pure cultures of several species of clostridia. Angelotti et al.[4] modified Mossel's formulation by adding sulphadiazine for suppressing the growth of *Enterobacteriaceae* and proposed Sulfite Polymyxin Sulfadiazine (SPS) Agar. The low sulphite content in the medium permits adequate blackening of colonies and at the same times allows sulphite-sensitive clostridia to grow.[5] According to MacFaddin[5] the medium can be used for the isolation and enumeration of *C. perfringens* and *Clostridium botulinum* from foodstuffs.
The medium is moderately selective: polymyxin B and sulfadiazine are inhibitors to most organisms other than *Clostridium* spp. The selectivity may be increased by the addition of neomycin 20 mg/L for the inhibition of *Clostridium bifermentans*.[4] Essential growth factors are provided by casein peptone while the yeast extract is a source of vitamins, particularly of the B-group. Ferric citrate and sodium sulphite are indicators of sulphite reduction by *C. perfringens* and other clostridia which produces black colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 39.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely the medium and sterilise by autoclaving at 118°C for 15 minutes. Cool to 47°C-50°C mix well and pour into sterile Petri dishes. If required, dispense before sterilisation 20 mL in 20x200 mm tubes and autoclave at 118°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	beige-grey, limpid
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.

1. Pipette 1 mL of decimal dilutions of the homogenised foodstuff into sterile plates and add 15-20 mL of SPS Agar.
2. Tilt the plate to mix the inoculum with the agar and allow to solidify.
3. Alternatively inoculate by stabbing deep tubes with the initial suspension of the specimen and its dilutions.
4. Incubate the plates or the tubes at 35-37ºC for 24 hours in an anaerobic atmosphere.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Most clostridia including *C. perfringens* and *C. botulinum* reduce sulphite to sulphide and causes colonies to turn black.

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	46°C/ 18-24 H / AN	growth with black colonies
E. coli ATCC 25922	46°C/ 18-24 H / AN	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATION OF THE METHOD

- SPS Agar is not sufficient for identification. Organisms other than clostridia may grow and all black colonies should be checked for presence of spores.[5]
- Black colonies should be confirmed as *C. perfringens* by appropriate tests: motility (-), nitrate reduction (+), acid and gas from lactose (+), gelatin liquefaction (+).[6]

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C /+8°C for 6 months.[5]

References

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. Mossel DAA, Debruin S, Van Diepen HMJ, Vendring CMA, Zoutewelle G. The enumeration of anaerobic bacteria, and of Clostridium species in particular, in foods. J Appl Bacteriol 1956; 19:142-154.
3. Mossel DAA. Enumeration of sulphite reducing clostridia occurring in foods. J Sci Food Agr 1959; 10:662-669
4. Angelotti R, Hall HE, Foster MJ, Lewis KH. Quantitation of Clostridium perfringens in foods. Appl Microbiol 1962 May;10(3):193-9.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. ISO 7937:2004. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of Clostridium perfringens - Colony-count technique

Packaging

Product	Type	REF	Pack
Sulfite Polymyxin Sulfadiazine (SPS) Agar	Dehydrated medium	4020942	500 g (12.6 L)

IFU rev 2, 2022/08

SUPPLEMENTED BRUCELLA AGAR

Ready-to-use plates

Intended use

In vitro diagnostic device. Culture medium for quantitative determination of susceptibility of anaerobes to antimicrobial agents by gradient-based strips.

Composition - typical formula *

Tryptone	10.0 g
Peptone	10.0 g
Yeast extract	2.0 g
Glucose	1.0 g
Sodium chloride	5.0 g
Sodium bisulphite	0.1 g
Agar	15.0 g
Vitamin K1	1.0 mg
Haemin	5.0 mg
Lysed horse blood	50.0 mL
Purified water	1000 mL

* The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

The reference tests for antimicrobial susceptibility testing of anaerobic bacteria are the agar dilution and the broth microdilution methods devised by the Clinical and Laboratory Standards Institute (CLSI).[1]
According to the international guidelines, the susceptibility testing of anaerobic bacteria is very expensive, time consuming and requires experienced laboratory staff.[2] To overcome these limitations some methods based on gradient strip testing have been developed and are commercially available for the laboratory. Such tests are based on the diffusion of a stable concentration gradient of an antimicrobial agent from a plastic or paper strip onto an agar medium.
The medium recommended for the detection of MICs of anaerobes with gradient based strips, is Brucella Agar supplemented with vitamin K1, haemin and lysed horse or sheep blood.[3] Supplemented Brucella Agar derives from the formulation recommended by CLSI for the broth microdilution assays[1] and contains lysed horse blood.
In Supplemented Brucella Agar, peptones and yeast extract, together with glucose, supply nitrogen, carbon and vitamins for microbial growth. Lysed horse blood provides additional nutrients. Sodium bisulphite lowers the redox potential to values suitable for strict anaerobes. Both haemin and vitamin K1 increase growth of certain anaerobic bacteria. Comparative studies of the gradient strip method performances against CLSI agar dilution method have shown generally high categorical agreement across many anaerobic species with a variety of antibiotics.[4-8] Gradient strip method can be also used for the detection of hetero-resistance (e.g., in the case of imipenem resistance of *B. fragilis* or in the case of metronidazole resistance of *C. difficile* and *B. fragilis*).[2,9,10]

Physical characteristics

Medium appearance	red, transparent
Final pH at 20-25 °C	7.2 ± 0.2

Specimens

Supplemented Brucella Agar must be used with pure culture of anaerobes isolated from clinical specimens. Supplemented Brucella Agar is not intended for microbial isolation directly from clinical specimens.

Test procedure

Allow plates and antimicrobial gradient strips to equilibrate to room temperature. The surface of the agar should be dry before use.
Guidelines for inoculums preparation:
Prepare a bacterial suspension in Brucella Broth or Mueller Hinton Broth with a turbidity equivalent to 1 McFarland.
Dip a sterile cotton swab into the suspension and remove excess fluid by pressing and turning the swab against the inside of the tube.
Plates can be inoculated either by swabbing in three directions or by using an automatic plate rotator. Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks.
As soon as the inoculum has been absorbed and the agar surface is dry, apply the gradient-strips. Make sure that the strips are in complete contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from strips is very rapid. The strips should be placed on the agar plate in a manner which does not result in overlapping zones of inhibition.
Guidelines for incubation:
Incubate at 35 ± 2°C for 24-48-72 hours (depending on the species) in anaerobic atmosphere (80-85% N_2/10% CO_2/10% H_2).
For the details of inoculation and incubation procedures consult the gradient strips manufacturer's package insert.

Reading and interpretation

After incubation, read the plates from the front with the lid removed and with reflected light.

A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light and the test must be repeated.
The growth should be evenly distributed over the agar surface to achieve a uniformly inhibition ellipse.
Check that inhibition zones for quality control strain are within acceptable range.
Determination of the MIC is at the point at which the lower part of the bacterial growth ellipse intersects with the corresponding number on the test strip.
For specific reading and interpretation instructions consult the gradient strips manufacturer's package insert.

User quality control

All manufactured lots of Supplemented Brucella Agar plates are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. For QC organisms testing, details are described in the strips manufacturer's package insert. At a minimum, at least one QC strain should be tested to ensure proper product functionality.[4]
A strain and the gradient strip useful for the quality control is: *B. fragilis* ATCC 25285 / metronidazole.
Refer to interpretation guidelines provided by the strips manufacturer's package insert.

Limitations of the method

- Incorrect inoculum concentration, improper storage of antimicrobial strips, improper storage of the plates resulting in an agar depth and pH out of the specifications, excessive moisture, improper measurement of endpoints, may produce incorrect results.
- The inoculation, incubation and reading methods here described are to be considered as guidelines; strict adherence to the protocol suggested by the gradient strips manufacturer is required to ensure reliable results.
- Pre-reduction of test plates in an anaerobic environment overnight prior to use decreases the likelihood of false resistance.[4]
- Reading and interpretation require expertise and close adherence to the gradient strips manufacturer's instructions; Occasionally, certain antibiotic/bacterium combinations may give unusual results. In these cases, judgement of the MIC endpoint may be difficult for inexperienced personnel. [3]

Storage conditions

Store plates in their original pack at 2-8°C away from direct light.

References

1. Clinical and Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, 8th ed. CLSI document M11-A9,. Clinical and Laboratory Standards Institute, Wayne, Pa. 2012.
2. Gajdács M, Spengler G, Urbán E. Identification and Antimicrobial Susceptibility Testing of Anaerobic Bacteria: Rubik's Cube of Clinical Microbiology. Antibiotics (Basel) 2017;6(4):25.
3. Etest Application Guide-16273B - en - 2012/07 /Etest IFU 9392553C en 2012/01
4. Schuetz A, Carpenter DE. Susceptibility test method: anaerobic bacteria *In*: Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. Bolmström A, Karlsson A, Engelhardt A, Phion Ho P, Petersen PJ, Bradford PA, Hal Jones C. Validation and reproducibility assessment of tigecycline MIC determinations by Etest. J Clin Microbiol. 2007; 45(8):2474-9.
6. Citron DM, Ostovari MI, Karlsson A, Goldstein EJ. Evaluation of the Etest for susceptibility testing of anaerobic bacteria. J Clin Microbiol 1991; 29(10):2197-203.
7. Rosenblatt JE, Gustafson DR. Evaluation of the Etest for susceptibility testing of anaerobic bacteria. Diagn Microbiol Infect Dis. 1995; 22(3):279-84.
8. Poulet P, Duffaut D, Lodter JP. Evaluation of the Etest for determining the in-vitro susceptibilities of Prevotella intermedia isolates to metronidazole J Antimicrob Chemother 1999;43(4):610-1.
9. El-Halfawy OM. Valvano MA. Antimicrobial hetero-resistance: An emerging field in need of clarity. Clin. Microbiol. Rev. 2015; 28: 191–207.
10. Huang H, Weintraub A, Fang H, Wu S, Zhang Y, Nord CE. Antimicrobial susceptibility and hetero-resistance in Chinese Clostridium difficile strains. Anaerobe 2010; 16: 633–635.

Packaging

Product	Type	REF	Pack
Supplemented Brucella Agar	Ready-to-use plates	549850	2 x 10 plates ø 90 mm

IFU, rev 1, 2021/01

Supplemented Brucella Agar:
Bacteroides fragilis and cefoxitin gradient strip

TAT BROTH BASE

Dehydrated culture medium

INTENDED USE

Supplemented with polysorbate 20, TAT Broth Base is used for the dilution of pharmaceutical and cosmetic samples intended for total microbial count and for testing the presence of microorganisms in viscous products such as salves, ointments and other cosmetic products.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	20 g
Soy lecithin	5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Preservatives are generally present in cosmetics to reduce the risk of microbial contamination and to ensure that the product remains suitable and safe during the period of storage and consumer use. The constant development of the cosmetics industry has generated the need for microbiological analysis of raw materials and final products in order to obtain cosmetics of good microbiological quality.
TAT (Tryptone-Azolecithin-Tween) Broth Base, supplemented with polysorbate 20, is used for the dilution of pharmaceutical and cosmetic samples intended for total microbial count and for testing the presence of microorganisms in viscous products such salves, ointments and other products.[1,2]
TAT Broth meets the requirements of neutralizing diluent “Fluid casein digest–soy lecithin–polysorbate 20 medium” (SCDLP 20 broth) described by ISO 21149.[2]
Casein peptone provides nitrogen and carbon for microbial growth and regeneration of damaged cells. The combination of soy lecithin and polysorbate 20 inactivates many antimicrobial compounds present in the sample such as phenolic compounds, cetrimide, chlorhexidine, benzoic acid, quaternary ammonium salts.

DIRECTIONS FOR MEDIUM PREPARATION

Dissolve 40 mL of Tween® 20 (REF 42120501) in 960 mL of purified water by mixing while heating in a water bath at 49 °C ± 2 °C. Add 25 g of TAT Broth. Heat for about 30 min with occasional agitation to obtain solution. Mix and dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid to slightly opalescent, may have a slight precipitate.
Final pH at 20-25 °C	7.2 ± 0.1

SPECIMENS

Pharmaceutical and cosmetics samples, viscous products such as salves, ointments.
Good laboratory practices for collection, transport and storage of specimens should be applied; consult appropriate applicable Standards.

TEST PROCEDURE

Add 10 g of sample to 90 mL of complete TAT Broth and shake to obtain a homogeneous suspension.
If needed, additional serial dilutions (e.g., 1:10 dilutions) may be performed from the initial suspension using the same diluent, according to the expected contamination level of the product.
Use the initial suspension and the dilutions for plate count by poured plate method or by membrane filtration
Alternatively, incubate the initial suspension at 35 ± 2°C for 18-48 hours.
Apply analytical procedures in accordance with the chosen reference Standard or norm.

READING AND INTERPRETATION

After incubation, the presence of microbial growth is indicated by the formation of turbidity in the culture broth.
Perform a sub-culture from the positive containers for the isolation procedure and identification tests.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 8739	35-37°C / 18-24 h -A	good growth
S. aureus ATCC 6538	35-37°C / 18-24 h -A	good growth
P. aeruginosa ATCC 9027	35-37°C / 18-24 h -A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Food and Drug Administration (1969) Procedure for Examination of Topical Drugs and Cosmetics. FDA, Rockville, MD.
2. ISO 21149:2017. Cosmetics -Microbiology -- Enumeration and detection of aerobic mesophilic bacteria.

PACKAGING

Product	Type	REF	Pack
TAT Broth Base	Dehydrated medium	4021002	500 g (20 L)

IFU rev 1, 2022/09

TAYLOR LYSINE DECARBOXYLASE BROTH

Dehydrated culture medium

Taylor Lysine Decarboxylase Broth – from the left: uninoculated tube, *P. vulgaris* Lys -, *S.* Enteritidis Lys +

INTENDED USE

The medium is used, together with other biochemical tests, for the confirmation of *Salmonella* colonies, isolated from samples of the food chain.

COMPOSITION TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Yeast extract	3 g
Glucose	1 g
L-lysine	5 g
Bromocresol purple	15 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The amino acid decarboxylation test was developed by Moeller[1] in 1955 for the determination of lysine and ornithine decarboxylase and arginine dehydrolase. Falkow[2] in 1958 modified Moeller's formula for differentiating *Salmonella* and *Shigella*. Taylor[3] in 1961 modified the Falkow medium by omitting the peptone from the formulation, because it is the origin of false positive results given by *Citrobacter* and other bacteria, due to its oxidation and deamination with the formation of an alkaline environment. The good results obtained by Taylor have been confirmed by Bonev[4] in a comparative study of the three media with 2764 strains of *Enterobacteriaceae*.
Taylor Lysine Decarboxylase Broth corresponds to Lysine decarboxylation medium (LDC), recommended by ISO 6579[5] as a confirmatory test for *Salmonella* colonies cultivated on selective medium, together with TSI and urease test. Yeast extract provide nitrogen, carbon, vitamins and trace elements for bacterial growth. Glucose is the fermentable carbohydrate and bromocresol purple is the pH indicator. The amino acid L-lysine is included to detect the production of the specific enzyme lysine decarboxylase that removes COOH group from the lysine to produce CO_2 and cadaverine, an alkaline polyamine. Facultatively fermenting bacteria ferment glucose resulting in the production of acid, which lower the pH of the medium and activate the enzyme lysine decarboxylase. When the medium containing lysine is inoculated with a glucose-fermenting and decarboxylase positive strain, it turns first to yellow for the production of acids then to purple for the production of alkalinity, based on the formation of amines. The positive test for lysine decarboxylase is therefore indicated by the formation of a purple colour, the negative test by the presence of a yellow colour.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 9 g in 1000 mL of cold purified water. Heat with frequent agitation to dissolve the medium completely. Distribute 2-5 mL in screw-capped tubes, and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Prepared medium appearance	violet, limpid or slightly opalescent
Final pH at 20-25 °C	6.8 ± 0.2

SPECIMENS

The specimens consist of bacteria strains isolated from food sample on *Salmonella* primary isolation agar and purified on appropriate medium (e.g., Nutrient Agar).

TEST PROCEDURE

With an inoculating needle or loop, transfer one colony into the tube just below the surface of the medium.
Incubate the tubes, with the caps tightened, at 37°C for 24 ± 3 hours.

READING AND INTERPRETATION

After incubation, observe the presence of growth (turbidity) and the colour change of the medium.
Turbidity and a purple colour after incubation indicate a positive reaction (decarboxylation of lysine).
A yellow colour indicates a negative reaction (fermentation of glucose).
The majority of typical *Salmonella* cultures show a positive reaction.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
P. vulgaris ATCC 9484	37° / 24 H /A	negative test (yellow colour)
S. Enteritidis ATCC 13076	37° / 24 H /A	positive test (purple)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- With the tubes held vertically during incubation, the decarboxylase test may show two layers of different colour; shake the tube gently before attempting to make an interpretation.[6]
- Taylor medium with lysine is indicated to differentiate *Citrobacter* (generally negative) from *Salmonella* (generally positive); however, *Salmonella* Paratyphi A gives negative reactions (yellow test tube).[6]
- Lysine decarboxylation is one of the tests necessary for the identification of *Salmonella*. The result of the decarboxylation test must be interpreted together with other tests for a correct identification of the strains.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579 the self-prepared tubes may be stored at 2-8 for up to three months.[5]

References

1. Moeller V. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta Pathol Microbiol Scand 1955;36(2):158-72.
2. Falkow S. Activity of lysine decarboxylase as an aid identification of *Salmonella* and *Shigella*. Amer J Clin Path1958; 29: 589-600.
3. Taylor, WI. Isolation of Salmonellae from Food Supplies. V. Determination of the Method of Choice for Enumeration of *Salmonella*. Appl Microbiol 1961; 9:487-490.
4. Bonev SI, Zakhariev Z, Gentchev P. Comparative Study of Media for Determination of Lysine Decarboxylase Activity. App Microbiol 1974; 27: 464-468.
5. ISO 6579:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella —Part 1: Detection of Salmonella spp.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

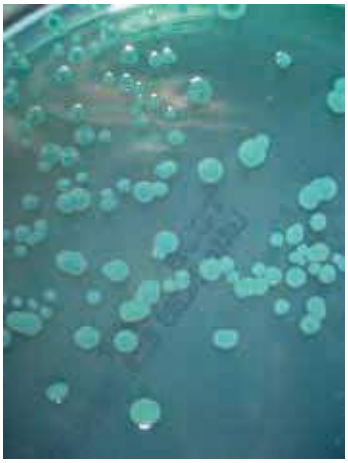
Packaging

Product	Type	REF	Pack
Taylor Lysine Decarboxylase Broth	Dehydrated medium	401367L2	500 g (55.5 L)

IFU rev 2, 2022/09

TCBS KOBAYASHI AGAR

Dehydrated and ready-to-use culture medium



V. parahaemolyticus on TCBS Agar

Intended use

In vitro diagnostics. Selective and differential medium for the isolation of *Vibrio* spp. from clinical specimens and other materials.

Composition - typical formula * (after reconstitution with 1 L of water)

Dehydrated medium and ready-to-use plates

Peptone	10.00 g
Yeast extract	5.00 g
Sodium thiosulphate	10.00 g
Sodium citrate	10.00 g
Sodium chloride	10.00 g
Oxgall	8.00 g
Sucrose	20.00 g
Ferric citrate	1.00 g
Thymol blue	0.04 g
Bromothymol blue	0.04 g
Agar	16.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Members of the genus *Vibrio* are Gram-negative, asporogenous rods, straight or with a single, rigid curve. They are motile; most have a single polar flagellum, when grown in liquid medium. Most produce oxidase and catalase, and ferment glucose without producing gas. *Vibrio* spp. are natural inhabitants of brackish and salt water. Several species are pathogenic to humans and are usually associated with the ingestion of contaminated water or seafood. The species that most frequently cause diarrhoea are *Vibrio cholerae* (the causative agent of cholera), *Vibrio parahaemolyticus, Vibrio fluvialis* and *Vibrio mimicus*. *Vibrio vulnificus* does not cause diarrhoea, but has been isolated from extra-intestinal sites of septic patients.[1]

TCBS (Thiosulphate-Citrate-Bile-Sucrose) agar was developed by Kobayashi, Enomoto, Sakazaki and Kuwahara in 1963,[2] who modified the selective isolation medium of Nakanishi[2]. TCBS Kobayashi Agar is used for the selective and differential isolation of *Vibrio* spp. from clinical specimens, environmental samples and foodstuffs.[4] TCBS Agar is recommended by ISO 21872[5] and by FDA-BAM[6] for the isolation of *Vibrio* spp. from foodstuffs.

Peptone and yeast extract provide nitrogen, carbon, vitamin B complex and other essential growth nutrients for bacterial growth. Inhibition of Gram-positive bacteria and coliforms is achieved by the strong alkalinity and by the incorporation of bile salts (oxgall), sodium thiosulphate and sodium citrate. Sodium chloride is incorporated to provide optimum growth of halophilic *Vibrio* spp. Sucrose is a fermentable carbohydrate for the metabolism of vibrios: sucrose fermenting bacteria produce acid end-products that makes the pH indicators (bromothymol blue and thymol blue) turn yellow. Inclusion of sucrose allows preliminary differentiation of *Vibrio* spp., with *V. cholerae, V. fluvialis* and *V. alginolyticus* producing yellow colonies, while *V. parahaemolyticus, V. mimicus* and most strains of *V. vulnificus* produce green colonies (sucrose non fermented). Using thiosulfate as a sulphur source, the production of hydrogen sulphide is visualized in the presence of ferric citrate. The alkaline pH of the medium improves the recovery of *V. cholerae*. Enteric bacteria are strongly inhibited on TCBS Agar; the rare colonies of some strains of *Proteus* and enterococci are easily distinguished by their reduced size and absence of colour.

Directions for medium preparation

Suspend 90 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Cool to 47-50ºC, mix well and pour into sterile Petri dishes. Do not sterilise in the autoclave.

Physical characteristics

Dehydrated medium appearance	grey-green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	dark green, limpid
Final pH at 20-25 °C	8.6 ± 0.2

Specimens

TCBS Kobayashi Agar is used for the bacteriological processing of clinical specimens such as faeces, rectal swab, vomitus[1,7] and non-clinical samples such as environmental samples, seafoods, foodstuffs.[4,5,6] Collect clinical specimens before antimicrobial therapy where possible. Stool specimens for detecting *Vibrio* spp. should be inoculated within 2-4 hours; for more prolonged storage use transport media such as Cary Blair, because *Vibrio* spp. are particularly susceptible to drying.[7] Special methods for the collection and processing of extra-intestinal specimens (blood, wounds etc.) are not required as vibrios, as a rule, are isolated in pure culture from these sites.[7]

Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[1,7] Consult appropriate standard methods for details of collection and preparation of non-clinical samples.[5,6]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Faeces may be diluted 1:4 in appropriate diluent prior to inoculation of culture medium. It has been shown that dilution significantly reduces the amount of competing flora without compromising isolation of low numbers of pathogens.[1]
In acute diarrhoeal disease, stool enrichment is generally not required; however, when enrichment is necessary, Alkaline Peptone Water is the most commonly used enrichment broth for human specimens. It should be incubated at 35-37°C and sub-cultured at 18 hours on TCBS Agar.
Incubate inoculated TCBS Agar plates with the specimen or with a specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours.
According to ISO 21872[5] method, the detection of potentially enteropathogenic *Vibrio* spp. *(V. parahaemolyticus, V. cholerae* and *V. vulnificus)* in products intended for human consumption and the feeding of animals and environmental samples in the area of food production and food handling, requires four successive phases:

1. selective enrichment in Alkaline Peptone Water with incubation at 41.5 °C for 6 h and/or 37°C for 6 h.;
2. secondary enrichment in a selective liquid medium (Alkaline Peptone Water) with incubation at 41.5 °C for 18 h and/or 37°C for 18 h;
3. inoculation of two solid selective media: TCBS Kobayashi Agar incubated at 37°C for 24 hours and another appropriate solid selective medium, left to the choice of the laboratory;
4. presumptive colonies of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* are sub-cultured and confirmed by means of appropriate biochemical and/or polymerase chain reaction (PCR) tests.

Recovery of certain *Vibrio* spp. from foodstuffs may be improved by the use of different incubation temperatures depending upon the target species or state of the food matrix.[5]
Consult appropriate references for the details of the procedures for the detection of *Vibrio* spp. in food.[5,6]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Interpretation of colonies' colours:

V. cholerae	Yellowish-brown colonies surrounded by yellow zones in medium
V. alginolyticus	Large yellow colonies
V. fluvialis, *V. furnissii*	Yellow or translucent colonies.
V. metschnikovii	Yellow (reduced growth).
V. parahaemolyticus	Colourless or green colonies with blue-green centre; the medium does not turn or turns slightly blue.
V. mimicus	Green colonies
V. vulnificus	Green (85%) or yellow (15%).
V. hollisae	Green (poor growth).
Proteus /Enterococci	Partial to complete inhibition. If growth, small, yellow to translucent colonies.
Pseudomonas / *P. shigelloides*	Partial to complete inhibition. If growth, blue colonies.
A. hydrophila	Partial to complete inhibition. If growth some strains produce yellow colonies
E. coli	Partial to complete inhibition. If growth, translucent colonies

After subculture on Nutrient Agar, Tryptic Soy Agar or Blood Agar the suspected colonies are submitted to oxidase test and to the agglutination tests with specific antisera.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[8]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
V. parahaemolyticus	ATCC	17802	36-38°C / 18-24H / A	good growth, green colonies
V. furnissii	NCTC	11218	36-38°C / 18-24H / A	good growth, yellow colonies
P. mirabilis	ATCC	12453	36-38°C / 18-24H / A	partially inhibited, yellow-green colonies with black centre
E. coli	ATCC	25922	36-38°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Cultures

LIMITATIONS OF THE METHOD

- On initial isolation, *V. parahaemolyticus* may be confused with *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Pseudomonas* species.[4]
- A few strains of *V. cholerae* may appear green or colourless on TCBS due to delayed sucrose fermentation.[4]
- Some strains of *Proteu*s and enterococci may exhibit growth and form small colourless colonies; however, these organisms are easily distinguished. Any coliforms that may grow do not metabolize sucrose.[4]
- Oxidase and agglutination tests are unreliable when performed directly on colonies growing on this medium. Growth from a non-sugar containing medium, such as blood agar or nutrient agar should be used for oxidase and agglutination testing.[4,7]
- It should be noted that yellow colonies may convert to green if plates are examined after more than 24 hours or are refrigerated after incubation.[7]
- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, for the isolation of *Vibrio* spp. additional media such us chromogenic media should be used. For enhanced recovery of *V. vulnificus*, a medium containing derivative of cellobiose-polymyxin B-colistin has been shown to be effective.[5]
- Inoculate extra-intestinal specimens also on non-selective blood agar plates and on other selective plates to identify other pathogens possibly involved in the infection.
- Some strains of *V. vulnificus* produce better recovery at 30°C.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 6-8 weeks.[4]
Ready to use plates
Store plates in their original pack at +2°C/+8°C away from direct light.

References

1. Public Health England. Investigations of Faecal Specimens for Enteric Pathogens. UK Standards for Microbiology Investigations. 2014, B 30 Issue 8.1.
2. Kobayashi T, Enomoto S, Sakasaki R, Kuwahara S. A new selective isolation medium for the *Vibrio* group; on a modified Nakanishi's Medium (TCBS Agar Medium). Jap J Bacteriol 1963; 18:392-397
3. Nakanishi Y. An isolation agar medium for cholerae and enteropathogenic halophilic vibrios. Modern Media 1963; 9:246.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
5. ISO 21872:2017. Microbiology of the food chain -Horizontal method for the determination of Vibrio spp. - Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 9: Vibrio. Content current as of:12/16/2019
7. Tarr CI, Bopp CI, Farmer III JJ. Vibrio and related organisms. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
8. The Australian Society for Microbiology. Guidelines for Assuring Quality of Medical Microbiological Culture Media. 2nd ed. 2012.

Packaging

Product	Type	REF	Pack
TCBS Kobayashi Agar	Dehydrated medium	4021062 4021064	500 g (5.6L) 5 kg (56 L)
TCBS Kobayashi Agar	Ready-to-use plates	542106	2 x 10 plates ø 90 mm

IFU rev 2, 2022/03

TETRATHIONATE BROTH BASE

Dehydrated culture medium and supplements

Intended use

Selective liquid medium for the enrichment of *Salmonella* from food animal feeding stuffs and other samples of sanitary importance.

Composition - typical formula*

(after reconstitution with 1 L of water)

Tetrathionate Broth Base, dehydrated medium

Peptocomplex	5 g
Bile salts	1 g
Calcium carbonate	10 g
Sodium thiosulphate	30 g

Brilliant Green 0.1% Solution, flask content (50 mL)

Brilliant green	50 mg
Purified water	50 ml

Iodine Solution, flask content (50 mL)

Iodine	10.0 g
Potassium iodide	12.5 g
Purified water	50 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Mueller[1] first described the use of Tetrathionate Broth for the isolation of salmonellae. The modification of the original formula, developed by Kauffmann[2], with the addition of bile salts and brilliant green to suppress bacteria such as *Proteus* spp., notably increased the percentage of positive results in the isolation of *Salmonella* spp.

The detection of *Salmonella* in foods and other samples of sanitary interest. necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in one or two selective liquid media, plating out and recognition, confirmation.

Tetrathionate Broth Base with the addition of iodine-iodide and brilliant green solutions is used for the selective enrichment of *Salmonella* from food animal feeding stuffs and other samples of sanitary importance and conforms to FDA-BAM formulation.[3]

Peptocomplex provide carbon, nitrogen, vitamins and minerals for microbial growth; the selective agents of the medium are ox bile, brilliant green and sodium tetrathionate which is formed from sodium thiosulfate when the iodine-potassium iodide solution is added to the medium; calcium carbonate neutralizes the sulfuric acid that is produced by the reduction of tetrathionate during the growth of salmonellae, keeping the pH at neutral values. The complete medium allows the development of salmonellae and is inhibitory for Gram-positive bacteria and for a large part of Gram-negative bacteria of enteric origin.

Directions for medium preparation

Suspend 46 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Avoid overheating, do not autoclave. Cool to 42-45° and aseptically add 20 mL of iodine-iodide solution and 10 mL of brilliant green 0.1% Solution (REF 421505). Mix well and aseptically distribute 10 mL into sterile tubes. Preparation of iodine-iodide solution according to FDA BAM[4]: dissolve 5 g of potassium iodide in 5 mL sterile purified water; add 6 g of iodine and stir to dissolve; dilute to 20 mL with sterile purified water.

Physical characteristics

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder.
Solution and prepared tubes appearance	colourless to light yellow supernatant over a heavy white precipitate
Final pH of the complete medium at 20-25°C	8.4 ± 0.2

Specimens

Food, feed, food chain sample and other samples of sanitary. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[3]

Test procedure

The following method is a summary taken from the FDA-BAM.[3] In the microbiological examination of foods, FDA-BAM recommends the use of Tetrathionate (TT Broth) together with Rappaport Vassiliadis (RV) Broth or Selenite Cystine Broth (SCB) (for guar gum).

TT Broth is used as the second selective enrichment broth, incubated at 43°C for the analysis of high microbial load foods and at 35°C for the analysis of low microbial load foods.

1. Transfer 0.1 mL of non-selective pre-enrichment culture to 10 mL of RV Broth or to 10 mL of SCB and another 1 mL aliquot to 10 mL of TT Broth.
2. Mix well and incubate selective enrichment media as follows:
   - Foods with a high microbial load: incubate RV Broth 24 ± 2 h at 42 ± 0.2°C. Incubate TT Broth 24 ± 2 h at 43 ± 0.2°C
   - Foods with a low microbial load (except guar gum and foods suspected to be contaminated with S. Typhi): incubate RV Broth 24 ± 2 h at 42 ± 0.2°C. Incubate TT Broth 24 ± 2 h at 35 ± 2.0°C.

- Guar gum and foods suspected to be contaminated with S. Typhi: incubate SC and TT broths 24 ± 2 h at 35°C.

3. Mix the selective enrichment broths and streak 10 µl incubated TT broth on Bismuth Sulfite Agar, XLD Agar and Hektoen Enteric Agar.
4. Repeat with 3 mm loopful (10 µl) of RV Broth (for samples of high and low microbial load foods) and of SC broth (for guar gum).
5. Incubate plates 24 ± 2 h at 35°C. Examine plates for presence of colonies that may be *Salmonella*.

READING AND INTERPRETATION

After incubation, growth of organisms in Tetrathionate Broth is indicated by turbidity.
Refer to the instructions for use of the plated media for the description of *Salmonella* colony characteristics.
Mark suspect colonies on each plate. Select suspect colonies for subculture and confirmation. For the choice of biochemical tests for the identification of *Salmonella* and the criteria for the evaluation of positive and negative reactions, refer to Chapter 5 of the FDA-BAM.[3]
Biochemical confirmation may be substituted with the rapid MUCAP Test (REF 191500). All the colonies MUCAP Test positive must be serologically confirmed.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of medium.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium ATCC 14028	35-37°C / 21-24h A	good growth after subculture on TSA
E. coli ATCC 25922	35-37°C / 21-24h A	partially inhibited after subculture on TSA

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

After the enrichment in Tetrathionate Broth, the isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to FDA-BAM iodine and brilliant green solutions should be added to medium base on day of use.[4] According to MacFaddin, Tetrathionate Broth Base without the addition of iodine solution may be stored at +2°C /+8°C in tightly closed containers for up to 2 weeks.[5]

REFERENCES

1. Muller L. A nouveau milieu d'enrichissement pour la recherche du bacille typhique e des paratyphiques. C.R. Soc. Biol. (Paris) 1923; 89:434-443
2. Kauffmann F. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren für Salmonellabacillen. Z Hyg Infektionskr. 1935; 117: 26-32
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 03/2022.
4. U.S. Food and Drug Administration. BAM Media M145: Tetrathionate (TT) Broth.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Tetrathionate Broth Base	Dehydrated culture medium	4021252	500 g (10.9 L)
Brilliant Green 0.1% Solution	Liquid supplement	4240047	50 mL
Iodine Solution	Liquid supplement	421501	50 mL

IFU rev 1, 2023/02

THIOGLYCOLLATE MEDIUM

Dehydrated and ready-to-use culture medium

Thioglycollate Medium. From left: un-inoculated tube, facultative anaerobe (*S. aureus*), anaerobe (*B. fragilis*), strict aerobe (*P. aeruginosa*)

INTENDED USE

In vitro diagnostics. General purpose liquid medium for the cultivation of aerobic, anaerobic, microaerophilic bacteria from clinical specimens and other materials. Suitable for the bacterial sterility test according to the harmonized method EP, USP, JP.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE TUBES AND FLASKS

Tryptone	15.000 g
Glucose	5.500 g
Yeast extract	5.000 g
Sodium chloride	2.500 g
L-cystine	0.500 g
Sodium thioglycollate	0.500 g
Agar	0.750 g
Resazurin	0.001 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Thioglycollate Medium, also known as Fluid Thioglycollate Medium, is a liquid medium formulated by Brewer in 1940[1], subsequently to previous studies by Quastel and Stephenson in 1926[2] and by Falk, Bucca and Simmons[3] in 1939, focused on formulations that allowed microbial growth starting from low inocula and the growth of anaerobic bacteria in liquid media containing a low concentration of agar and reducing compounds.
Thioglycollate Medium is prepared according to the formula specified in EP, USP, JP harmonized method[4] for sterility test of pharmaceutical products.
Cystine and sodium thioglycollate, at a concentration with a low toxicity for microorganisms, act as reducing substances by reacting with and removing molecular oxygen from the medium and preventing accumulation of peroxides, which may be lethal to some aerobic and anaerobic

microorganisms.[5] Sulfhydryl groups (SH) of the two compounds inactivate arsenic, mercury and other heavy metal compounds, maintaining a low redox potential and ensuring anaerobic conditions.[5]
Agar, included at a concentration of 0.75%, aids in initialization of the growth of anaerobes and allows their growth from low inocula; it also retards the dispersion of CO_2, diffusion of oxygen and reducing substances; small concentration reduces convection currents within the medium to enhance condition in lower portion of tubed medium.[5] Resazurin is an oxidation-reduction indicator, being pink when oxidized and colourless when reduced, replacing the methylene blue present in Brewer's original formula. Casein peptone and yeast extract are sources of nitrogen, carbon, vitamins and minerals for microbial growth, glucose is a source of carbon and energy, sodium chloride maintains osmotic equilibrium. Thioglycollate Medium can be enriched with various compounds, including Vitamin K to stimulate the growth of some strains of *Bacteroides* and *Prevotella* and hemin, as a source of factor X, to increase microbial growth.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water, heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121 °C for 15 minutes. Cool rapidly and store in the dark at room temperature. If necessary, heat in a water bath before use.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear when hot, slightly turbid at room temperature, with a pink ring indicating medium oxidation on top
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

Thioglycollate Medium may be used for the bacteriological processing of clinical specimens such as tissues, purulent exudates, wounds and abscess,[5,6] Collect clinical specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied. For samples collection and handling intended for sterility test consult the appropriate reference.[4]

TEST PROCEDURE

Allow the tubes to come to room temperature.
Check the upper portion of each tubed medium prior to inoculation. If greater than one third of fluid column is oxidized indicated by a pink colour, discard the tube. If one-third or less is pink loosen the caps and boil in a water bath, without agitation, 5 minutes to drive off absorbed oxygen; tighten caps immediately after removing from heat and cool to room temperature before use. Do not boil more than once.
For general use, inoculate specimens directly into the medium and incubate tubes for up to 7 days at 35 ± 2 °C.
For specific applications, incubate at the temperature and for the time provided by Laboratory procedures and according to the cultivated microorganisms. For sterility testing, recommendations of EP[4] should be followed.

READING AND INTERPRETATION

After incubation, the presence of bacterial growth is evidenced by the presence of turbidity compared to an un-inoculated control.
Obligate aerobes tend to grow on the upper portion of the broth in the oxidized pink layer, while anaerobes grow only in the lower, oxygen - deficient and pink-free portion of tubed broth. Microaerobes grow in the middle portion of the broth.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
B. fragilis **	ATCC	25285	35-37°C / 48h - A	good growth
*S. aureus***	ATCC	25293	35-37°C / 48 h -A	good growth
C. sporogenes *	ATCC	19404	35-37°C / 72h - A	good growth
P. aeruginosa *	ATCC	9027	35-37°C / 72h - A	good growth
S. aureus *	ATCC	6538	35-37°C / 72h - A	good growth
*B. subtilis**	ATCC	6633	35-37°C / 72h - A	good growth
C. perfringens ***	ATCC	13124	35-37°C / 18-24 h - A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection
*: EP[4]: ** CLSI[8]; *** ISO 11133:2014/Amd 1:2018[9]

LIMITATIONS OF THE METHOD

- Fast-growing facultative anaerobic bacteria can grow in excess and mask the growth of strict anaerobes.
- Some anaerobes can be inhibited by the metabolic products or acids formed during the growth of fast-growing facultative anaerobic bacteria.
- Rapid death of bacteria may occur in Thioglycollate Medium especially with Gram-negative cocci, *S. pneumoniae*, *C. perfringens* and other acid-sensitive organisms; if the subculture from tubes to plated media does not reveal microbial growth, perform a Gram staining from the broth culture.
- If the tubes of Thioglycollate Medium are used for the preservation of bacteria, add approximately 0.1 g of calcium carbonate to each tube which neutralizes the acids produced during microbial growth, promotes bacterial vitality and the formation of spores, thus improving the shelf life of acid-sensitive bacteria.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months unless greater than one-third of upper fluid column becomes oxidized.[5]
Ready-to-use medium in tubes and flasks
Store in their original pack at 10-30°C away from direct light.

REFERENCES

1. Brewer JH. Clear liquid medium for the “aerobe” cultivation of anaerobes. J Am Med Assoc 1940; 115:598-600
2. Falk Bucca and Simmons. J Bacteriol 1939; 37:121
3. Quastel and Stephenson. J Biochem 1926; 20:1125
4. European Pharmacopoeia, current edition.

5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Baron EJ, Specimen Collection, Transport and Processing:Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015.
7. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve:World Health Organization
8. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
9. ISO 11133:2014/Amd.1:2018 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media. Amendment 1.

PACKAGING

Product	Type	REF	Pack
Thioglycollate Medium	Dehydrated medium	4021372 4021374	500 g (16.7 L) 5 kg (167 L)
Thioglycollate Medium	Ready-to-use tubes	552137	20 x 10 mL
Thioglycollate Medium	Ready-to-use flasks	5121372 5121373	6 x 100 mL 6 x 200 mL

IFU rev 3, 2022/03

THIOGLYCOLLATE MEDIUM ALTERNATIVE

Dehydrated culture medium

INTENDED USE

General purpose liquid medium for the cultivation of aerobic, anaerobic, microaerophilic bacteria. Suitable for the bacterial sterility test of turbid and viscous products.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	15.0 g
Yeast extract	5.0 g
Glucose	5.5 g
Sodium chloride	2.5 g
L-cystine	0.5 g
Sodium thioglycollate	0.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Brewer in 1940[1] formulated Fluid Thioglycollate Medium, subsequently to previous studies by Quastel and Stephenson in 1926[2] and by Falk, Bucca and Simmons[3] in 1939, focused on formulations that allowed microbial growth starting from low inocula and the growth of anaerobic bacteria in liquid media containing a low concentration of agar and reducing compounds.
Thioglycollate Medium Alternative, also known as NIH Thioglycollate Broth and USP Alternate Thioglycollate Medium, is an alternative formulation prepared without agar and resazurin. This formula conforms to the specifications given in the U.S. Pharmacopeia and the National Formulary[4,5].
The medium is suitable for testing the sterility of turbid and viscous products and for washing articles on which sterility of the lumen is tested, when the lumen is very small. The medium may be used for the cultivation of aerobic and facultative anaerobic bacteria and, when incubated under anaerobic conditions, for the cultivation of strict anaerobes.
Cystine and sodium thioglycollate, at a concentration with a low toxicity for microorganisms, act as reducing substances by reacting with and removing molecular oxygen from the medium and preventing accumulation of peroxides, which may be lethal to some aerobic and anaerobic microorganisms.[6] Sulfhydryl groups (SH) of the two compounds inactivate arsenic, mercury and other heavy metal compounds, maintaining a low redox potential and ensuring anaerobic conditions.[6] A ratio of 15 mL of liquid medium and 3 mL of inoculum is sufficient to inactivate the mercurial preservatives present at a concentration not greater than 0.08% in the sample. For the neutralisation of other preservatives, different from mercurial compounds, adequate dilutions of the sample are required.
Tryptone and yeast extract are sources of nitrogen, carbon, vitamins and minerals for microbial growth, glucose is a source of carbon and energy, sodium chloride maintains osmotic equilibrium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 29 g in 1000 mL of cold purified water, heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121 °C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pale yellow, clear
Final pH at 20-25 °C	7.1 ± 0.2

SPECIMENS

For collection and handling of samples intended for sterility test consult the appropriate reference.[5]

TEST PROCEDURE

For general use, inoculate specimens directly into the medium and incubate tubes for up to 7 days at 35 ± 2 °C.
For specific applications, incubate at the temperature and for the time provided by Laboratory procedures and according to the cultivated microorganisms. To perform the sterility test with Thioglycollate Medium Alternative, USP recommendations should be followed.[5]

READING AND INTERPRETATION

After incubation, the presence of bacterial growth is evidenced by the presence of turbidity compared to an un-inoculated control.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus	ATCC	25293	35°C / 72h /A	good growth
P. aeruginosa	ATCC	9027	35°C / 72h / A	good growth
B. subtilis	ATCC	6633	35°C / 72h / A	good growth
C. perfringens	ATCC	13124	35°C / 72 h / AN	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It is recommended that medium be boiled prior to its use to enhance recovery rate. However, do not heat tubes more than once to drive off absorbed oxygen.[6]
- Fast-growing facultative anaerobic bacteria can grow in excess and mask the growth of strict anaerobes.
- Some anaerobes can be inhibited by the metabolic products or acids formed during the growth of fast-growing facultative anaerobic bacteria.
- Rapid death of bacteria may occur especially with Gram-negative cocci, *S. pneumoniae*, *C. perfringens* and other acid-sensitive organisms; if the subculture from tubes to plated media does not reveal microbial growth, perform a Gram staining from the broth culture.[6]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Brewer JH. Clear liquid medium for the “aerobe” cultivation of anaerobes. J Am Med Assoc 1940; 115:598-600
2. Falk CR, Bucca HB, Simmons MP. A comparative study of the use of varying concentrations of agar in the test medium used to detect contaminants in biologic products. J Bacteriol 1939; 37:121-131.
3. Quastel JH, Stephenson M. Experiments on "strict" anaerobes: the relationship of B. sporogenes to oxygen. J Biochem 1926; 20:1125-1137.
4. N.I.H. Memorandum, Culture Media for Sterility Tests, 4th Revision (1955)
5. The United States Pharmacopeia/National Formulary, current edition
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Thioglycollate Medium Alternative	Dehydrated medium	4021352	500 g (17.2 L)

IFU rev 1, 2022/12

TODD HEWITT BROTH

Dehydrated and ready-to-use culture medium

Todd Hewitt Broth
from left: uninoculated tube, *S. pyogenes*

INTENDED USE

In vitro diagnostics. General purpose medium primarily used for the cultivation of β-haemolytic streptococci especially for serological studies.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE TUBES

Meat extract	10.0 g
Tryptone	20.0 g
Glucose	2.0 g
Sodium chloride	2.0 g
Sodium carbonate	2.0 g
Disodium hydrogen phosphate	0.4 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Todd Hewitt Broth is a medium developed by Todd and Hewitt[1] in 1932 for the production of streptococcal haemolysin and later modified by Updyke and Nickle[2] in 1954 for the growth of β-haemolytic streptococci for use in fluorescent antibody test procedures.
Todd Hewitt Broth enhances the growth of β-haemolytic streptococci and the production of antigenic streptococcal haemolysin; it favours the production of type-specific M protein and it is used in the procedures for extracting group antigens.[3]
Todd Hewitt Broth is also used as general all-purpose medium for the cultivation of most pathogenic microorganisms.[3]
Todd Hewitt Broth has a high concentration of peptones which promotes excellent microbial growth and prevents the formation of proteases; glucose stimulates haemolysin production; sodium chloride maintains the osmotic balance; sodium carbonate and disodium hydrogen phosphate neutralize the acidity that is formed during microbial growth and glucose fermentation protecting produced haemolysin from destruction.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 36.4 g in 1000 mL of cold purified water, heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 115 °C for 20 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25°C	7.8 ± 0.2

SPECIMENS

Todd Hewitt Broth may be inoculated with any type of clinical specimens from which it is intended to isolate streptococci (e.g., throat swabs). Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE

Remove the cap aseptically from the container and place the swab in the Todd Hewitt Broth, break off the swab stick and replace the cap. Caps should be kept loose during incubation.
Incubate the inoculated tubes in ambient air or 5% CO_2 for 2-5 hours prior to use in fluorescent antibody procedures for the identification of group A streptococci.
Continue incubation for 18-24 hours for antigen extraction procedures prior to serotyping and subculture on isolation plates of suitable selective or non-selective blood agar medium.
Todd Hewitt Broth may be inoculated with pure culture of streptococci for the preparation of extracts for serological typing.
Consult appropriate references for further instructions.[4]

READING AND INTERPRETATION

After incubation, growth of organisms is indicated by turbidity of inoculated tubes.
Perform serotyping according to the IFU of manufacturer.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes ATCC 12834	35-37°/ 18-24H / A	good growth
S. pneumoniae ATCC 6303	35-37°/ 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Todd Hewitt Broth is an enrichment broth: sub-culture on selective or non-selective media is necessary for pathogen isolation and identification.
- Todd Hewitt Broth cannot be used for bile solubility testing.[3]
- After the enrichment in Todd Hewitt Broth, even if the microbial colonies on the isolation plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months.[3]
Ready-to-use medium in tubes
Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Todd EW, Hewitt LF. A new culture medium for the production of antigenic streptococcal hemolysin. J Pathol Bacteriol 1932; 35:973
2. Updyke EL, Nickle MI. A dehydrated medium for the preparation of type specific extracts of group A streptococci. Appl Microbiol 1954; 2:117
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
4. Spellenberg B, Brandt B, Sendi P. Streptococcus. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
Todd Hewitt Broth	Dehydrated culture medium	4021342	500 g (13.7L)
Todd Hewitt Broth	Ready-to-use tubes	552134	20 x 9 mL

IFU rev1, 2022/03

TODD HEWITT CNA BROTH

Ready-to-use tubes

Todd Hewitt CNA Broth – from left: uninoculated tube, *S. agalactiae*

INTENDED USE

In vitro diagnostic device. Liquid medium for the selective enrichment of Group B streptococci (*S. agalactiae*) from clinical specimens.

COMPOSITION -TYPICAL FORMULA *

Beef heart infusion from	500.0 g
Peptones	20.0 g
Glucose	2.0 g
Sodium chloride	2.0 g
Sodium bicarbonate	2.0 g
Disodium hydrogen phosphate	0.4 g
Yeast extract	10 g
Colistin	10 mg
Nalidixic acid	15 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Group B Streptococci (GBS) or *Streptococcus agalactiae* are facultatively anaerobic, oxidase-negative, catalase-negative, Gram-positive cocci that cause postpartum infections (neonatal sepsis, pneumonia, respiratory diseases) within seven days from birth, but also within the first 24 hours of life.[1] Approximately 50% of women who are colonized with GBS will transmit the bacteria to their new-borns. Vertical transmission usually occurs during labour or after rupture of membranes. In European countries and the United States, the incidence of the disease is around 0.5-1 per thousand live births.[1,2]

Todd Hewitt CNA Broth, also known as Lim Broth, is based on a modification of the formulation of Lim *et al*.[3,4] who proposed a selective enrichment broth and the slide co-agglutination test for the rapid screening of these obstetric patients.

At least 50% of women with GBS give false negatives results if the culture is performed by inoculating the specimens directly into primary isolation medium rather than by performing a preliminary step in the selective enrichment broth.[5] The use of Todd Hewitt broth with colistin and nalidixic acid, followed by plate isolation, is recommended to maximize the recovery of GBS.[5-7]

Todd Hewitt CNA Broth has a high concentration of peptones which promotes excellent microbial growth and prevents the formation of proteases; glucose stimulates haemolysin production; sodium chloride maintains the osmotic balance; sodium carbonate and disodium hydrogen phosphate neutralize the acidity that is formed during microbial growth and glucose fermentation. Colistin and nalidixic acid inhibit Gram negative bacteria, promoting the growth of streptococci.

PHYSICAL CHARACTERISTICS

Medium appearance	yellow, limpid
Final pH at 20-25°C	7.8 ± 0.2

SPECIMENS

Todd Hewitt CNA Broth may be inoculated with maternal low vaginal and anorectal swabs; maternal high vaginal swabs should not be collected as these have a lower sensitivity.[6] A lower vaginal and rectal swab should be obtained with either one or two different swabs.[7] Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature. GBS isolates can remain viable in transport media (Amies or Stuart with or without charcoal) for several days at room temperature. However, the recovery of isolates declines over 1-4 days, especially at elevated temperatures, which can lead to false-negative results.[6,7]

Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE

Remove the cap aseptically from the container and place the swab(s) in the Todd Hewitt CNA Broth, break off the swab stick(s) and replace the cap. Caps should be kept loose during incubation.

Incubate the inoculated tubes in ambient air or 5% CO_2 for 18-24 hours.[5-7]

Sub-culture with a sterile loop and inoculate appropriate plating media (Blood Agar and/or Columbia CNA Blood Agar and/or Chromogenic Strep B Agar). Optimum detection of GBS may require the use of more than one culture medium.[6]

READING AND INTERPRETATION

After incubation, growth of organisms is indicated by turbidity of inoculated tubes.

After an overnight incubation of the isolation media, observe plates for suggestive GBS colonies and identify them. If negative after overnight incubation, re-incubate an additional 24 hours before reporting a negative result. On blood agar, suggestive colonies of GBS are grey, translucent, with a surrounding zone of beta-haemolysis (or no haemolysis: very rare).[6]

Perform serological identification on the colonies by latex agglutination test with a group B antiserum.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. agalactiae ATCC 12386	35-37 °C / 16-24h / A or CO_2	good growth
E. coli ATCC 25922	35-37 °C / 16-24h / A or CO_2	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The isolation rate of GBS from clinical specimens depends on several factors; studies have shown that detection of GBS colonisation can be improved by attention to the timing of cultures, the sites swabbed and the microbiological method used for culture of microorganisms.[6]
- Todd Hewitt CNA Broth is an enrichment broth: sub-culture on selective or non-selective media is necessary for pathogen isolation and identification.
- After the enrichment in Todd Hewitt CNA Broth, even if the microbial colonies on the isolation plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Società Italiana di Neonatologia Prevenzione della malattia perinatale da streptococco di gruppo B [2010]
2. Istituto Superiore di Sanità. Infezioni neonatali precoci e tardive da streptococco di gruppo B in Italia [2011]
3. Lim DV, Kanarek KS, Peterson ME. Magnitude of colonization and sepsis by group B streptococci in newborn infants. Curr Microbiol 1982; 7:99-101.
4. Jones DE, Friedl EM, Kanarek KS, Williams JK, Lim DV. Rapid identification of pregnant women heavily colonized with group B streptococci. J Clin Microbiol. 1983;18(3):558-60.
5. Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. Recommendations and Reports, November 19, 2010 / 59(RR10);1-32.
6. Public Health England- UK Standards for microbiology investigations (UK SMI): Detection of Carriage of Group B Streptococci(Streptococcus agalactiae) B58, 2018
7. Spellenberg B, Brandt B, Sendi P. Streptococcus. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

PACKAGING

Product	Type	REF	Pack
Todd Hewitt CNA Broth	Ready-to-use tubes	552134B	20 x 9 mL

IFU rev 1, 2021/01

TRIPLE SUGAR IRON AGAR ISO

Dehydrated and ready-to-use culture medium

INTENDED USE

For the differentiation of *Enterobacteriaceae*, especially *Salmonella*, based on carbohydrate fermentation and production of hydrogen sulphide.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE TUBES

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Lactose	10.0 g
Sucrose	10.0 g
Glucose	1.0 g
Iron (III) ammonium citrate	0.3 g
Sodium chloride	5.0 g
Sodium thiosulphate	0.3 g
Agar	12.5 g
Phenol red	24.00 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The formulation of Triple Sugar Iron Agar medium is based on several microbiologists' attempts to develop a medium to aid in the identification of intestinal Gram-negative bacilli: Russel[1], Kliger,[2] Krunweide and Kohn[3]. In 1940, Sulkin and Willet[4] modified the triple sugar medium of Krunweide and Kohn by the addition of H_2S indicators. The current formulation of triple sugar iron medium is essentially a modification of Haja[5] to Sulkin and Willet triple sugar ferrous sulphate medium.
Triple Sugar Iron (TSI) Agar ISO is intended for the differentiation of *Enterobacteriaceae*, especially *Salmonella* spp., grown on primary isolation media, based on the fermentation of glucose, lactose and sucrose, with production of acids and gas, and the production of hydrogen sulphide.[6,7]
The culture medium is prepared according to the formula included in ISO 6579[7] and differs from the classical TSI formulation (REF 402141) by the additional presence of beef extract and yeast extract and slightly different concentrations of sodium thiosulphate and phenol red.
The fermentation of the three carbohydrates can take place both on the surface of the slant and in the butt with or without the presence of gas (CO_2 + H_2) and 3 reaction models can be registered:
1-fermentation of glucose; 2-fermentation of glucose, lactose and/or sucrose; 3-no fermentation.[8]
In the first case, after 18-24 hours of incubation, an alkaline reaction on the slant and an acid reaction in the butt is observed. The complete consumption of glucose, present at a concentration of 0.1%, on the surface, where aerobic conditions exist, after 18-24 hours induces the oxidative degradation of peptones, with production of ammonia, alkalinity and a red colour change of phenol red (reversal of the acid-alkaline reaction). However, in the anaerobic butt the bacteria metabolize the glucose producing ATP and pyruvate, which is converted into stable acid end-products with a colour change of the indicator to yellow (acid pH).
In the second case, the microorganisms ferment glucose and one or both lactose and sucrose: after 18-24 hours of incubation an acid reaction is recorded on the slant and in the butt. This is due to the high concentration of lactose and sucrose: after 18-24 hours their degradation is not exhausted on the surface and therefore there is no utilisation of peptones and therefore no reversal of the reaction.
In the third model an alkaline reaction is recorded both on the slant and in the butt. This behaviour is not typical of *Enterobacteriaceae* but of some non-enteric non fermenting Gram-negative bacteria that can utilise the peptones for growing (*Alcaligenes faecalis, Acinetobacter, Pseudomonas*).
If the degradation of the peptones is anaerobic the indicator will turn to red (alkaline pH) both on the surface and in the butt, if the degradation is aerobic, there is no colour change of phenol red in the butt.

Ferrous ammonium sulphate is an indicator of the formation of hydrogen sulphide. Thiosulphate reductase producing organisms cause the release of a sulphide molecule from the sodium thiosulfate. The hydrogen sulphide will react with ferric ions in the medium to produce iron sulphide, a black insoluble precipitate.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 65 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute into tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool in a slanted position to obtain deep butts and short slopes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	red-orange, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Pure colonies from a culture on solid media.

TEST PROCEDURE

With an inoculating needle, pick the centre of a well-isolated pure colony, inoculate the slant by first stabbing the butt to the bottom; withdraw the needle, and then streak the surface of the slant. Loosen the cap of the tube before incubating.
Incubate aerobically at 37°C for 24 h ± 3 hours.

READING AND INTERPRETATION

Interpret the changes in the medium as follows:
a) butt
- yellow: glucose positive (glucose fermentation);
- red or unchanged: glucose negative (no fermentation of glucose);
- black: formation of hydrogen sulphide;
- bubbles or cracks: gas formation from glucose;

b) slant surface
- yellow: lactose and/or sucrose positive (lactose and/or sucrose fermentation);
- red or unchanged: lactose and sucrose negative (no fermentation of lactose or sucrose).

The majority of the typical *Salmonell*a cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90 % of the cases) formation of hydrogen sulphide (blackening of the agar).
When a lactose-positive *Salmonella* is isolated, the TSI slant is yellow.

Interpretation of TSI reactions with *Salmonella* spp.[7]

	Acid from glucose	Gas from glucose	Acid from lactose	Acid from sucrose	H2S production
S. Typhi	+	-	-	-	-
S. Paratyphi A	+	+	-	-	-
S. Paratyphi B	+	+	-	-	+
S. Paratyphi C	+	+	-	-	+
S. Gallinarum biovar gallinarum	+	+	-	-	V
S. Gallinarum biovar pullorum	+	+	-	-	V
Other *Salmonella* spp.	+	+	-	-	+

Notes
Not all isolates of *Salmonella* serovars show the reactions marked + or −. Reactions may also vary between and within serovars.
Salmonella Typhi is anaerogenic.
V = Variable results.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

S. Typhimurium ATCC 14028:	growth, red slant, yellow butt, gas +, H_2S +
S. flexneri ATCC 12022:	growth, red slant, yellow butt, gas -, H_2S -
P. aeruginosa ATCC 10145:	growth, red or unchanged slant, red or unchanged slant, gas -, H_2S -

Aerobic incubation at 37°C for 24 h.

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It is necessary to inoculate the medium with a microbiological needle without breaking the agar (do not use loops).
- Perform the reading between 18 and 24 hours of incubation; early readings can induce false acidity results of the A/A type or there is not enough time for the sugar fermentation with consequent colour change of the indicator; delayed readings can give false K/K results due to the use of peptones and alkaline change of the medium.[9]
- H_2S production can mask the acid reaction in the butt, however the production of H_2S requires acidic conditions therefore the butt must be considered acid when there is blackening.
- Hydrogen sulphide production may be evident on KIA but negative on TSI. Studies by Bulmash and Fulton[10] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader[11] found that not all H_2S-positive *Salmonella* are positive on TSI.
- An H_2S producing organism may exhibit blackening on SIM medium (positive) but none on TSI medium.[9]

- The medium does not contain inhibitors therefore a large variety of microorganisms can grow on it; for this reason, before inoculation, make sure that the organisms are catalase positive, Gram-negative bacilli.
- The addition of sucrose allows the earlier detection of coliform bacteria that ferment sucrose more rapidly than lactose. Adding sucrose also aids the identification of certain Gram-negative bacteria that could ferment sucrose but not lactose.[8]
- A pure culture is essential when inoculating the medium. If the culture is not pure, irregular results may be obtained.
- Some organisms such as the *Klebsiella-Enterobacter* group produce such an abundance of gas that the medium may be completely displaced by gas resulting in the medium being blown up into the cap. If this occurs, handle the culture with caution when sub-culturing to avoid contaminations.
- Make sure that the caps are loosened during incubation since for a correct medium performance a free exchange of air is necessary. If the caps are too closed, an acid reaction occurs only on the slant even in the presence of glucose fermentation.[9]
- Preliminary confirmation of *Salmonella* cultures shall not be based on the results of the TSI agar test only; further suitable tests are needed for a complete identification of the colonies.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to ISO 6579, the self-prepared tubes can be stored at +2°C +8°C for up to 4 weeks.[4]

Ready-to-use medium in tubes

Store tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Russell FF. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J Med Res 1911; 25:21
2. Kliger IJ. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am J Public Health 1917; 7:1042-1044
3. Krumwiede C, Kohn L. A triple sugar modification of the Russell Double Sugar medium. J Med Res1917; 37:225.
4. Sulkin SE, Willet JC. A triple sugar ferrous sulphate medium for use in identification of enteric organisms. J Lab Clin Med 1940; 25:649.
5. Hajna AA. Triple sugar iron agar medium for the identification of intestinal group of bacteria. J Bacteriol 1945; 49:516.
6. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition CRC Press,1997
7. ISO 6579:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.
8. Lehman D. Triple sugar iron agar protocols. 30 September 2005. American Society for Microbiology © 2016.
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. Bulmash JM, Fulton MD. Discrepant tests for hydrogen sulfide. J Bacteriol 1964; 88(2):1813
11. Padron AP, Dockstader WB. Selective medium for hydrogen sulfide production Appl Microbiol 1972; 23:1107

PACKAGING

Product	Type	REF	Pack
Triple Sugar Iron Agar ISO	Dehydrated culture medium	402141S2	500 g (7.7 L)
Triple Sugar Iron Agar ISO	Ready-to-use tubes	552141S	20 slanted tubes

IFU rev 1, 2020/09

TRIPLE SUGAR IRON AGAR

Dehydrated and ready-to-use culture medium

Triple Sugar Iron Agar – from left: uninoculated tube, S. Typhimurium, *E. coli.*

INTENDED USE

In vitro diagnostics. For the differentiation of *Enterobacteriaceae*, especially *Salmonella*, based on carbohydrate fermentation and production of hydrogen sulphide.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE TUBES

Peptocomplex	20.000 g
Lactose	10.000 g
Sucrose	10.000 g
Glucose	1.000 g
Ferrous Ammonium Sulphate	0.200 g
Sodium Chloride	5.000 g
Sodium Thiosulphate	0.200 g
Agar	14.000 g
Phenol Red	0.025 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The formulation of Triple Sugar Iron Agar medium is based on several microbiologists' attempts to develop a medium to aid in the identification of intestinal gram-negative bacilli: Russel[1], Kliger,[2] Krunweide and Kohn[3]. In 1940, Sulkin and Willet[4] modified the triple sugar medium of Krunweide and Kohn by the addition of H_2S indicators. The current formulation of triple sugar iron medium is essentially a modification of Haja[5] to Sulkin and Willet triple sugar ferrous sulphate medium.

Triple Sugar Iron (TSI) Agar is intended for the differentiation of *Enterobacteriaceae*, especially *Salmonella* spp., grown on primary isolation media, based on the fermentation of glucose, lactose and sucrose, with production of acids and gas, and the production of hydrogen sulphide.[6] The medium is included in the FDA-BAM[7] procedures for the identification of *Salmonella* from food, together with other biochemical tests. TSI Agar proposed by the ISO Standard 6579 for *Salmonella* identification has a different formulation and corresponds to Biolife medium Triple Sugar Iron Agar ISO Formulation (REF 402141S).

The fermentation of the three carbohydrates can take place both on the surface of the slant and in the butt with or without the presence of gas (CO_2 + H_2) and 3 reaction models can be registered:

1-fermentation of glucose; 2-fermentation of glucose, lactose and/or sucrose; 3-no fermentation.

In the first case, after 18-24 hours of incubation, an alkaline reaction on the slant and an acid reaction in the butt is observed. The complete consumption of glucose, present at a concentration of 0.1%, on the surface, where aerobic conditions exist, after 18-24 hours induces the oxidative

degradation of peptones, with production of ammonia, alkalinity and a red colour change of phenol red (reversal of the acid-alkaline reaction). However, in the anaerobic butt the bacteria metabolize the glucose producing ATP and pyruvate, which is converted into stable acid end-products with a colour change of the indicator to yellow (acid pH).
In the second case, the microorganisms ferment glucose and one or both lactose and sucrose: after 18-24 hours of incubation an acid reaction is recorded on the slant and in the butt. This is due to the high concentration of lactose and sucrose: after 18-24 hours their degradation is not exhausted on the surface and therefore there is no utilisation of peptones and therefore no reversal of the reaction.
In the third model an alkaline reaction is recorded both on the slant and in the butt. This behaviour is not typical of *Enterobacteriaceae* but of some non-enteric non fermenting Gram-negative bacteria that can utilise the peptones for growing (*Alcaligenes faecalis, Acinetobacter, Pseudomonas*).
If the degradation of the peptones is anaerobic the indicator will turn to red (alkaline pH) both on the surface and in the butt, if the degradation is aerobic, there is no colour change of phenol red in the butt.
Ferrous ammonium sulphate is an indicator of the formation of hydrogen sulphide. Thiosulphate reductase producing organisms cause the release of a sulphide molecule from the sodium thiosulfate. The hydrogen sulphide will react with ferric ions in the medium to produce iron sulphide, a black insoluble precipitate.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 60.4 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Distribute into tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool in a slanted position to obtain deep butts and short slopes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pink, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	red-orange, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Triple Sugar Iron Agar Medium is not intended for primary isolation from clinical specimens; it is inoculated with pure colonies from a culture on solid media, isolated from clinical specimens or other materials.

TEST PROCEDURE

With an inoculating needle, pick the centre of a single pure colony, inoculate the slant by first stabbing the butt to the bottom; withdraw the needle, and then streak the surface of the slant. Loosen the cap of the tube before incubating.
Incubate aerobically at 35-37°C for 18 to 24 hours.

READING AND INTERPRETATION

Three kinds of data may be obtained from the reactions.[8]

Sugar fermentations

Acid (yellow) butt, alkaline (red) slant: glucose fermented, sucrose or lactose not fermented.
Acid (yellow) butt, acid (yellow) slant: glucose, lactose and/or sucrose fermented.
Alkaline (red) butt, alkaline (red) slant: neither glucose, lactose, nor sucrose fermented.

Gas production

Presence of bubbles in the butt. With large amounts of gas, the agar may be cracked and displaced.

Hydrogen sulphide production

Hydrogen sulphide production from thiosulfate is indicated by a blackening of the butt as a result of the reaction of H_2S with the ferric ions to form black ferrous sulphide. Formation of H_2S requires an acidic environment; sometimes the butt will be entirely black; in such a case, it is assumed that butt portion of the tube is acid (yellow colour is masked by H_2S production).
All combinations of the reactions described above can be observed on Triple Sugar Iron Agar, therefore it is important to record the results of all the reactions (sugar fermentations, gas production, H_2S production). The following table, taken from MacFaddin[9] shows the reaction patterns of some *Enterobacteriaceae*.

Microorganism	Lac	Suc	Glu	Gas	H_2S
Edwarsiella	-	-	A	+	+
Escherichia coli	A [1]	V	A	V^+	-
Shigella	V^- [3]	V^- [1]	A	V^- [2]	-
Klebsiella	A	A	A	+	-
Enterobacter	V	V^+	A	V^- [6]	-
Hafnia	V^-	V^-	A	V^+	-
Serratia	V^-	A	A	V^-	-
Morganella	-	-	A	V^+	-
Proteus mirabilis	-	V^{-1}	A	+	+
Proteus vulgaris	-	A	A	V[7]	+
Salmonella	- [4]	-	A	V^+	+[5]
Salmonella arizonae	V^{+1}	V^-	A	+	+
Citrobacter amalonaticus	V	V^-	A	+	-
Citrobacter diversus	V	V^-	A	+	-
Citrobacter freundii	A[1]	V^-	A	+	+
Yersinia	-	V	A	V	-

Notes
Lac: lactose fermentation; Suc: sucrose fermentation; Glu: glucose fermentation; A: acid reaction; V: variable; V^+: variable, usually positive; V^-: variable, usually negative.
1: the reaction may by delayed; 2: *S. flexneri* ser.6 gas production positive (slight amount); 3: usually negative except *S. sonnei* (acid reaction may be delayed); 4: although rare, lactose positive variants of *S.* Typhi exist; 5: *S.* Typhi may have a ring of H_2S but its presence is not diagnostic. *S.* Paratyphi A if positive may be weak.; 6: *E. agglomerans* gas production variable; 7: if gas produced, a slight amount.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

E. coli ATCC 25922: growth, yellow slant, yellow butt, gas + H_2S -
S. flexneri ATCC 12022: growth, red slant, yellow butt, gas - H_2S -
S. Typhimurium ATCC 14028: growth, red slant, yellow butt, gas + H_2S +
Aerobic incubation at 35-37°C for 18-24 h.

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- It is necessary to inoculate the medium with a microbiological needle without breaking the agar (do not use loops).
- Perform the reading between 18 and 24 hours of incubation; early readings can induce false acidity results of the A/A type or there is not enough time for the sugar fermentation with consequent colour change of the indicator; delayed readings can give false K/K results due to the use of peptones and alkaline change of the medium.[9]
- H_2S production can mask the acid reaction in the butt, however the production of H_2S requires acidic conditions therefore the butt must be considered acid when there is blackening.
- Hydrogen sulphide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton[10] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader[11] found that not all H_2S-positive *Salmonella* are positive on TSI.
- An H_2S producing organism may exhibit blackening on SIM medium (positive) but none on TSI medium.[9]
- The medium does not contain inhibitors therefore a large variety of microorganisms can grow on it; for this reason, before inoculation, make sure that the organisms are catalase positive, Gram-negative bacilli.
- The addition of sucrose allows the earlier detection of coliform bacteria that ferment sucrose more rapidly than lactose. Adding sucrose also aids the identification of certain Gram-negative bacteria that could ferment sucrose but not lactose.[8]
- A pure culture is essential when inoculating the medium. If the culture is not pure, irregular results may be obtained.
- Some organisms such as the *Klebsiella-Enterobacter* group produce such an abundance of gas that the medium may be completely displaced by gas resulting in the medium being blown up into the cap. If this occurs, handle the culture with caution when sub-culturing to avoid contaminations.
- Make sure that the caps are loosened during incubation since for a correct medium performance a free exchange of air is necessary. If the caps are too closed, an acid reaction occurs only on the slant even in the presence of glucose fermentation.[9]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6-8 weeks.[9]

Ready-to-use tubes

Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Russell FF. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J Med Res 1911; 25:21
2. Kliger IJ. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am J Public Health 1917; 7:1042-1044
3. Krumwiede C, Kohn L. A triple sugar modification of the Russell Double Sugar medium. J Med Res1917; 37:225.
4. Sulkin SE, Willet JC. A triple sugar ferrous sulphate medium for use in identification of enteric organisms. J Lab Clin Med 1940; 25:649.
5. Hajna AA. Triple sugar iron agar medium for the identification of intestinal group of bacteria. J Bacteriol 1945; 49:516.
6. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition CRC Press,1997
7. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019
8. Lehman D. Triple sugar iron agar protocols. American Society for Microbiology 2015.
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
10. Bulmash JM, Fulton MD. Discrepant tests for hydrogen sulfide. J Bacteriol 1964; 88(2):1813
11. Padron AP, Dockstader WB. Selective medium for hydrogen sulfide production Appl Microbiol 1972; 23:1107

PACKAGING

Product	Type	REF	Pack
Triple Sugar Iron Agar	Dehydrated culture medium	4021412	500 g (8.2 L)
Triple Sugar Iron Agar	Ready-to-use tubes	552141	20 glass tubes with slanted medium, 17x125 mm

IFU rev 2, 2022/01

TRYPTIC GLUCOSE EXTRACT AGAR

Dehydrated culture medium

INTENDED USE

For microbial plate counts in milk, dairy products, water, and other samples of sanitary importance.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	5.0 g
Beef extract	3.0 g
Glucose	1.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In 1935, Bower and Hucker devised the composition of a medium (Tryptone Glucose Agar) for the bacteriological analysis of milk and reported higher plate counts and larger colony sizes.[1,2] Tryptic Glucose Extract Agar (TGEA) was originally suggested by the American Public Health Association in 1948[3] for the estimation of total viable counts in milk and dairy products and was later adopted for the analysis of water[4]. TGEA for many years remained the standard culture medium for microbial plate count. Currently it is recommended in the Compendium of Methods for the Microbiological Examination of Foods for performing the plate count of mesophilic aerobic endospore-forming bacilli[5] and for aerobic or heterotrophic plate count in bottled water[6].

Tryptone and beef extract provide nitrogen, carbon, minerals and amino acids for the microbial growth. Glucose is a source of carbon and energy; agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 24 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale beige, clear
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS

Milk, dairy products, water, and other samples of sanitary importance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Colony count by the pour plate technique.

1. Using a sterile pipette, dispense 1 mL of the liquid test sample, or 1 mL of an initial suspension in the case of other products, into an empty Petri dish and mix with the molten Tryptic Glucose Extract Agar pre-cooled to 44-46°C.
2. Prepare the other plates under the same conditions using decimal dilutions of the test sample or of the initial suspension.
3. Incubate the plates under aerobic conditions at 35 °C for 72 hours.

Colony count by the surface plating technique.

1. Dry the prepared plates before the use.
2. Using a sterile pipette, transfer 0.1 mL of the test sample, if the product is liquid, or of the initial suspension in the case of other products, to the centre of a Tryptic Glucose Extract Agar plate.
3. Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
4. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.
5. Incubate the plates under aerobic conditions at 35 °C for 72 hours.

Consult the references for information regarding the details of processing and inoculation of bottled water samples[4] and the plate count of mesophilic aerobic endospore-forming bacilli[5].

READING AND INTERPRETATION

After incubation, count all colonies obtained in the plates containing fewer than 300 colonies and calculate the number of microorganisms per gram or per millilitre of the test sample. Follow recommended procedures for the counting of colonies and the reporting of results.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 8739	37°C/72H/A	good growth
S. aureus ATCC 6538	37°C/72H/A	good growth
B. subtilis ATCC 6633	37°C/72H/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.[7]
- A potential source of error in plate count can result from the stack-pouring Petri dishes: in a stack of 3 plates, the middle and the top plates took too longer to cool, thereby resulting in lower counts. [7]
- Increasing the holding time of the dilutions in the diluent leads to higher count. [7]
- The Aerobic Plate Count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.[7]

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Bowers CS and Hucker GY. 1935. The composition of media for the bacteriological analysis of milk. Tech Bull. N.Y. State Agr. Expt. Sta. No. 228. 1935.
2. Bowers CS and Hucker GY. 1936. Further studies of the composition of media for the bacteriological analysis of milk. Am. J. of Public Health. 1936; 26:350–352
3. APHA Standard Methods for the Examination of Dairy Products. American Public Health Association, New York, N.Y. 9th ed. 1948.
4. APHA Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C. 15th ed. 1980
5. Stevenson KE, Lembke F. Mesophilic Aerobic Endospore-Forming Bacilli. In: Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
6. Posnick L, Feng P. Bottled Water. In: Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015
7. Petran R, Grieme LE, Foong-Cunningham S. Culture Methods for Enumeration of Microorganisms. In: Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

PACKAGING

Product	Type	REF	Pack
Tryptic Glucose Extract Agar	Dehydrated medium	4021442	500 g (20.8 L)

IFU rev 1, 2022/12

TRYPTIC GLUCOSE YEAST AGAR
(PLATE COUNT AGAR)
(STANDARD METHODS AGAR)
Dehydrated and ready-to-use culture medium

INTENDED USE
For microbial plate counts in foodstuffs, milk, dairy products, water, and other samples of sanitary importance.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM, READY-TO-USE PLATES, TUBES AND FLASKS

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Tryptic Glucose Yeast Agar also known as Plate Count Agar or Standards Methods Agar is recommended by regulatory authorities[1-7] for the enumeration of mesophilic or thermophilic aerobic organisms in foodstuffs, milk, dairy products, water, raw materials and other samples of sanitary importance, and for the evaluation of sanitary conditions of environmental samples in the area of food and feed production, and handling.
This test is based on the assumption that each viable cell, pair of cells, or small cluster of cells will form a visible colony, named a colony-forming unit (CFU), when mixed with the growth medium.[4]
Enumeration of microorganisms requires diluting samples to achieve a population that is countable by the chosen method.
Several techniques have been described and are available for aerobic plate counts: pour plate technique, surface spread plate method, membrane filtration, spiral plate method, calibrated loop method, drop plate method.[4] The choice of the most appropriate method must take into account the requirements of the regulatory authorities, the type of sample to be analysed, the expected microorganisms and level of contamination.
The International Standard ISO 4833-1 specifies a pour plate method for the enumeration of mesophilic organisms and is applicable to products that require a reliable count when a low limit of detection is specified or to products expected to contains spreading colonies.[1]
ISO 4833-2 specifies a surface plating technique applicable to products containing heat sensitive organisms or obligately aerobic bacteria.[2]
ISO 17410 describes a surface plating method for the enumeration of psychrotrophic microorganisms with incubation at 6.5°C.[3]
In USA, detailed procedures for determining the aerobic plate count have been developed by the APHA[4-6], the AOAC[7] and summarised in the FDA Bacteriological Analytical Manual for Foods.[8]
The formulation of Tryptic Glucose Yeast Agar complies with ISO Standards, FDA-BAM and other regulatory authorities. Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose is a source of carbon and energy.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)
Suspend 23.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS AND TUBES)
Liquefy the contents of the flask/tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle or the tube may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C, mix well and pour the medium into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale beige, clear
Final pH at 20-25 °C	7.0 ± 0.2

SPECIMENS
Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[1-7]

TEST PROCEDURE
Colony count by the pour plate technique.[1]
1. Using a sterile pipette, dispense 1 mL of the liquid test sample, or 1 mL of an initial suspension in the case of other products, into an empty Petri dish and mix with the molten Tryptic Glucose Yeast Extract Agar pre-cooled to 44-46°C.
2. Prepare the other plates under the same conditions using decimal dilutions of the test sample or of the initial suspension.
3. Incubate the plates under aerobic conditions at 30 °C for 72 h.

Colony count by the surface plating technique.[2,3]
1. Dry the prepared plates before the use.
2. Using a sterile pipette, transfer 0.1 mL of the test sample, if the product is liquid, or of the initial suspension in the case of other products, to the centre of a Tryptic Glucose Yeast Extract Agar plate.
3. Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
4. Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.
5. Incubate the plates under aerobic conditions at 30 °C for 72 h for the enumeration of mesophilic organisms or at 6.5°C for 10 days for the enumeration of psychrotrophic microorganisms.

Consult the appropriate International Standard for the details of the procedures.[1-7]

READING AND INTERPRETATION
After incubation, count all colonies obtained in the plates containing fewer than 300 colonies and calculate the number of microorganisms per gram or per millilitre of the test sample.
Follow recommended procedures for the counting of colonies and the reporting of results.[1-7]

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 8739	30°C/72H-A	good growth
S. aureus ATCC 6538	30°C/72H-A	good growth
B. subtilis ATCC 6633	30°C/72H-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the methods

- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.[4,9]
- A potential source of error in plate count can result from the stack-pouring Petri dishes: in a stack of 3 plates, the middle and the top plates took too longer to cool, thereby resulting in lower counts. [4,10]
- Increasing the holding time of the dilutions in the diluent leads to higher count. [4,11]
- The Aerobic Plate Count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.[4]

Storage conditions

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to ISO Standards, self-prepared flasks can be stored at +2 °C to +8 °C for up to 3 months and the self-prepared plates can be stored at +2 °C to +8 °C for up to 4 weeks.[1,3]

Ready to use plates, flasks and tubes

Store in their original pack at 2-8°C away from direct light.

References

1. ISO 4833-1:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 °C by the pour plate technique.
2. ISO 4833-2:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 °C by the surface plating technique.
3. ISO 17410:2019. Microbiology of the food chain — Horizontal method for the enumeration of psychrotrophic microorganisms
4. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
5. American Public Health Association. Standard Methods for the Examination of Water, 23rd ed. 2017. APHA, Washington, DC
6. American Public Health Association. Standard Methods for the Examination of Dairy Products. 17th ed. 2004. APHA, Washington, DC.
7. Association of Official Analytical Chemists. Official Methods of Analysis, 21st ed. 2019. AOAC, Arlington, VA.
8. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 3: Aerobic Plate Count. Rev 2001
9. Berry JM, McNeill DA, Witter LD. Effect of delay in pour plating on bacterial counts. J Dairy Sci 1969; 52:1456-1457
10. Koburger JA. Stack pouring of Petri plates: a potential source of error. J Food Prot. 1980; 43:561-562.
11. Huhtanen CN Brazis AR, Arledge WL et al. Effects of time of holding dilutions on counts of bacteria from raw milk. J Milk Food Technol. 1972; 35:126-130.

Packaging

Product	Type	REF	Pack
Tryptic Glucose Yeast Agar (Plate Count Agar), (Standard Methods Agar	Dehydrated medium	4021452 4021454	500 g (21.3L) 5 Kg (213 L)
Plate Count Agar	Ready to use medium in plates	542145	2 x 10 plates ø 90 mm
Plate Count Agar	Ready to use medium in plates	492145	3 x 10 plates ø 55 mm
Plate Count Agar	Ready to use medium in tubes	552145B	20 x 15 mL
Plate Count Agar	Ready to use medium in flasks	5121452 5121453	6 x 100 mL 6 x 200 mL

IFU rev 3, 2022/09

TRYPTIC SOY AGAR

Dehydrated and ready-to-use culture medium

Bacillus cereus on Tryptic Soy Agar

Intended use

In vitro diagnostics. General purpose medium for cultivation and maintenance of non-fastidious and moderately fastidious microorganisms. For microbial enumeration of non-sterile pharmaceutical products and cosmetics. Supplemented with defibrinated animal blood, Tryptic Soy Agar is intended for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for the determination of haemolytic properties.

Composition - typical formula * (after reconstitution with 1 L of water)

Dehydrated medium, ready-to-use plates, tubes and flasks

Pancreatic digest of casein	15 g
Soy peptone	5 g
Sodium chloride	5 g
Agar	15 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Tryptic Soy Agar (TSA) is one of the most widely used culture media in clinical and industrial microbiology. TSA has a multitude of uses in clinical and non-clinical laboratories including isolation, cultivation and purification of colonies of non-fastidious and moderately fastidious microorganisms

and maintenance of stock cultures.[1] As it doesn't contain the X and V factors, it is suitable for identification of *Haemophilus* sp. by adding on the agar surface discs or strips impregnated with X (Hemin) and V (NAD) factors.[2] It is recommended as a reference medium, when testing selective media, to measure the degree of inhibition.[3] TSA is the medium specified as "casein soya bean digest agar" in the harmonised EP, USP JP method[3] for microbial enumeration of non-sterile pharmaceutical products. It is recommended by ISO Standard 21149 for the enumeration and detection of aerobic mesophilic bacteria in cosmetics[5].
Tryptic Soy Agar may be supplemented with defibrinated animal blood (at concentrations between 5% and 7%) to provide a more nutritious medium for the growth of fastidious organisms; the addition of animal blood enables the determination of bacterial haemolytic properties, as a useful tool for the orientation of bacterial identification.
TSA may be supplemented with 0.7g/L lecithin and 5g/L Polysorbate 80, which neutralise the activity of quaternary ammonium compounds and other disinfectants, for determining the efficacy of sanitization of products, sanitary areas, containers.[6]
Tryptic Soy Agar with the addition of salt can be helpful in determining the halotolerance level of microorganisms.[6]
Tryptic Soy Agar is prepared with selected casein and soy peptones: the combination of casein and soy peptones renders the medium nutritious by supplying organic nitrogen in the form of amino acids and polypeptides. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent.

Directions for medium preparation (dehydrated medium)
Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C mix well and pour into sterile Petri dishes.
Notes. The medium may be also dispensed in tubes before sterilisation and cooled in slanted position. For the preparation of blood plates, to the medium autoclaved and cooled to 47-50°C, add 5-7% defibrinated animal blood, mix well and distribute into sterile Petri dishes.

Directions for medium preparation (ready-to-use flasks)
Liquefy the contents of the flask/tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle or the tube may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C, mix well and pour the medium into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Prepared plates, tubes and flasks	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

Specimens
Un-supplemented Tryptic Soy Agar should not be used for the direct inoculation of clinical specimens. Generally, TSA is used for the sub-culture of microorganisms grown on other culture media. Non-clinical samples analysed with Tryptic Soy Agar include non-sterile pharmaceutical products and cosmetics. Refer to the quoted literature for sample collection and preparation.[4,5]
If supplemented with animal blood, the poured plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens, related to specific infections.[7-9] Collect specimens before antimicrobial therapy where possible and apply good laboratory practices for collection, transport and storage of the clinical specimens; consult appropriate references for further information.[7]

Test procedure
Allow plates or tubes to come to room temperature and to dry the surface of the medium.
For the subculture of colonies, by means of a sterile needle or loop, inoculate an un-supplemented TSA plate with a colony cultivated on another isolation medium. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the inoculated organism and the local applicable protocols.
When using TSA supplemented with defibrinated animal blood, streak the clinical specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5 -10% CO_2, and record the results after 18-24, 48 and if necessary, 72 hours.
For the microbial enumeration in non-sterile pharmaceutical products and cosmetics consult the references.[4,5]

Reading and interpretation
After incubation, the presence of microorganisms is indicated by the appearance of colonies of various morphology and size on the un-supplemented medium surface. The characteristics of the growth are closely related to the type or types of cultivated microorganisms.
By cultivation on sheep blood agar plates prepared with Tryptic Soy Agar, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:
1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α-prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

Control strains			Incubation T°/ t / ATM	Expected results
S. aureus	ATCC	25923	35-37°C / 18-24H / A	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth

User quality control of TSA used for microbial enumeration in non-sterile pharmaceutical products and cosmetics should meet the requirements of EP[4] and ISO Standard[5]
A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- When using blood supplemented TSA, depending on the specimens analysed and the microorganisms being tested for, for the examination of clinical specimens, it is recommended to use also additional media such us selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic patterns other than expected.

- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood[10]; *Neisseria*, *Mycobacterium*, *Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the self-prepared plates can be stored at +2°C +8°C for up to 6-8 weeks.[11]

Ready to use plates, tubes and flasks

Store in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition CRC Press,1997
2. Ledeboer NA, Doern GV. Haemophilus. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.667.
3. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media
4. European Pharmacopoeia, current edition
5. ISO 21149:2017. Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
7. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
8. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
9. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
10. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999
11. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Tryptic Soy Agar	Dehydrated medium	4021502 4021504	500 g (12.5 L) 5 kg (125 L)
Tryptic Soy Agar	Ready-to-use plates	542150	2 x 10 plates ø 90 mm
Tryptic Soy Agar	Ready-to-use tubes	552150	20 glass tubes with slanted medium, 17x125 mm
Tryptic Soy Agar	Ready-to-use flasks	5121502 5121503	6 x 100 mL 6 x 200 mL

IFU rev 2, 2021/11

TRYPTIC SOY BLOOD AGAR BASE

Dehydrated culture medium

Tryptic Soy Blood Agar Base supplemented with sheep blood: *Streptococcus pyogenes*

INTENDED USE

In vitro diagnostic. Non selective, general-purpose medium to be used with defibrinated animal blood, for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical specimens and other materials and for determination of their haemolytic properties.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	14.5 g
Soy peptone	5.0 g
Sodium chloride	5.0 g
Agar	14.0 g
Growth factors	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The history of blood agar is uncertain. The inclusion of blood as a nutritive supplement in culture media may pre-date the use of agar[1]; in their 1903 Manual of Bacteriology, Muir and Ritchie[2] list its inclusion before they discuss “agar-agar” as a replacement for gelatine as a solidifying agent.[2] The term "blood agar", as we know it today, generally refers to an enriched base medium to which defibrinated mammalian blood has been added.

Tryptic Soy Blood Agar Base is a general-purpose medium, to be used with defibrinated sheep blood, to grow fastidious and non-fastidious organisms and to differentiate bacteria based on their haemolytic properties.

Tryptic Soy Blood Agar Base is prepared with selected casein and soy peptones for providing carbon, nitrogen and trace elements for bacteria, for improving the haemolytic reactions and it is supplemented with growth factors to achieve a bigger and faster growth of fastidious microorganisms. Sodium chloride maintains the osmotic balance. The addition of animal blood enables the determination of bacterial haemolytic properties, as a useful tool for the orientation of bacterial identification.

Tryptic Soy Blood Agar Base supplemented with sheep blood is useful for performing the CAMP (Christie-Atkins-Munch-Petersen) test for presumptive identification of *Streptococcus agalactiae* and for use with optochin and bacitracin discs for presumptive identification of group A streptococci.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C, and add 5-7% of sterile defibrinated sheep or horse blood. Mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Tryptic Soy Blood Agar Base supplemented with sheep blood and poured in plates can be directly inoculated with many clinical specimens collected from various normally sterile and non-sterile human sites. Refer to the quoted literature for specimens' types, related to specific infections.[3-5] Blood Agar plates are not suitable for direct inoculation of blood samples. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied; consult appropriate references for further information.[3]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate at 35-37°C in aerobic conditions with or without 5 -10% CO_2, and record the results after 18-24, 48 and if necessary, 72 hours.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.
CAMP test: a known haemolytic strain of *S. aureus* (ATCC 25923) is streaked in a straight line across the centre of the plate. Test inoculum is streaked in a straight line (2-3 cm in length) perpendicular to *S. aureus* streak but without touching it. A known Group B *Streptococcus* may also be streaked similarly as a positive control. Four-five test organisms may be tested per plate. The plate is incubated at 35-37°C for 18-24 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological, chromatic, haemolytic characteristics of the colonies.
By cultivation on sheep blood agar plates prepared with Tryptic Soy Blood Agar Base, bacteria can be differentiated based on their capacity to secrete haemolysins. The haemolysis will cause a clearing zone of the blood agar around the colonies. Bacteria can cause different types of haemolysis:

1. α-haemolysis: partial haemolysis of the red blood cells to produce a greenish-grey or brownish discoloration around the colonies.
2. β-haemolysis: complete haemolysis of red blood cells resulting in a clear zone around the colonies
3. γ or non-haemolysis: no haemolysis of red blood cells, no change of the medium under and surrounding the colonies.
4. α-prime haemolysis: a small zone of complete haemolysis that is surrounded by an area of partial lysis with green discoloration; this type of haemolysis is uncommon.

Here below are summarized the colonies characteristics of some microorganisms which can be isolated on blood agar sheep plates.[6]

- The colonies of Group A streptococci are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony.
- The colonies of group B streptococci are surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all.
- The appearance of surface or subsurface β-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of group A colonies to be of any value in identification.
- Group D streptococcal colonies are non-haemolytic.
- Pneumococcal colonies, when the culture has been incubated in CO_2 incubators, are surrounded by a fairly large zone of α-haemolysis.
- The viridans streptococcal colonies may be surrounded by a small zone of α-haemolysis or have no zone of haemolysis; rarely they show an α-prime haemolysis.
- Staphylococci colonies are yellow or white with or without the β-haemolysis zone.
- *Listeria* colonies are surrounded by a small β-haemolytic zone.

Once colonies have grown on blood agar plates, user must differentiate potential pathogens requiring identification and antimicrobial testing from contaminants that represent member of normal microbiota.
CAMP Test (with sheep blood agar plates): a positive test for CAMP factor appears as "arrowhead" haemolysis between the junction of growth of *S. aureus* and Group B *Streptococcus*. There is no enhanced or "arrowhead" haemolysis if the test isolate is not Group B Streptococcus.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[7]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. pyogenes	ATCC	19615	35-37°C / 18-24H / A or CO_2	good growth, beta haemolysis
S. pneumoniae	ATCC	6305	35-37°C / 18-24H / A or CO_2	good growth, alpha haemolysis
S. aureus	ATCC	25923	35-37°C / 18-24H / A or CO_2	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A or CO_2	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Depending on the specimens analysed and the microorganisms being tested for, it is recommended for the examination of clinical specimens to use also additional media such us selective media and Chocolate Agar.
- The growth and type of haemolysis depends on the metabolic requirements of organisms; it is possible that some strains do not grow and/or can demonstrate haemolytic models other than expected.
- *Haemophilus influenzae*, which requires both factor X and factor V, will not grow on this medium supplemented with sheep blood[8]; *Neisseria*, *Mycobacterium*, *Bordetella* and other microorganisms with highly specific nutritional requirements do not grow adequately; for the detection of these organisms, specific culture media should be used.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological, chromatic, haemolytic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the self-prepared plates with animal blood can be stored at +2°C /+8°C for up to 48 hours.[9]

REFERENCES

1. Buxton T. Blood agar plates and hemolysis protocols. ASM Science, 2005
2. Robert M, Ritchie J. 1903. Manual of Bacteriology. The MacMillan Company, London, 1903.
3. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
4. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve: World Health Organization.
5. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
6. Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) In Manual of Clinical Microbiology, 5th edition, Washington,DC: American Society for Microbiology; 1991.
7. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
8. Nye KJ, Fallon D, Gee B, Messer S, Warren RE, Andrews N. A comparison of blood Agar supplemented with NAD with plain blood agar and chocolated blood agar in the isolation of Streptococcus pneumoniae and Haemophilus Influenzae from sputum. Bacterial Methods Evaluation Group J Med Microbiol 48 (12), 1111-1114 Dec 1999
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Tryptic Soy Blood Agar Base	Dehydrated medium	4021512 4021514	500 g (12,5 L) 5 kg (125 L)

IFU rev 2, 2022/03

TRYPTIC SOY BROTH

Dehydrated and ready-to-use culture medium

INTENDED USE

In vitro diagnostics. General purpose medium for the sterility test and for the microbiological examination of pharmaceutical products according to the harmonized methods of EP, USP, JP. For suspension, enrichment and cultivation of microbial strains isolated from clinical specimens on other culture media.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE TUBES AND FLASKS

Pancreatic digest of casein	17.0 g
Soy peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Tryptic Soy Broth is a general-purpose medium that supports the growth of a wide variety of aerobic and facultative anaerobic bacteria and fungi.[1]
Tryptic Soy Broth is used for sterility testing and for the microbiological examination of pharmaceutical products with EP, USP, JP harmonized methods (casein soybean digest broth) and complies with the quality specifications reported therein.[2]
In clinical microbiology Tryptic Soy Broth is used for suspension, enrichment and cultivation of microbial strains isolated on other culture media and for the preparation of inocula in quality control test procedures.
Supplemented with 20% glycerol, Tryptic Soy Broth may be used for the long-term maintenance of microbial strains; supplemented with 0,1-0,15% of agar it may be used for enhancing growth of anaerobes.[1] Tryptic Soy Broth is used in food bacteriology as the basal medium to which a variety of selective compounds may be added for selective enrichment of pathogens. Tryptic Soy Broth may be used also for blood cultures.[1]
Pancreatic digest of casein and soy peptone are sources of carbon, nitrogen, vitamins and minerals for microbial growth; glucose is a source of energy; sodium chloride maintains osmotic balance, dipotassium hydrogen phosphate is included as a buffer system.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water. Warm until complete dissolution, distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Un-supplemented Tryptic Soy Broth should not be used for the direct inoculation of clinical specimens. In clinical microbiology the specimens consist of microbial colonies grown on other culture media. In pharmaceutical microbiology, samples consist of products on which to perform the sterility test or the detection for specific microorganisms. Refer to the European Pharmacopoeia for sample collection and transport procedures.[2]

TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube or bottle with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2° C for 18-24 is adequate for cultivation of common anaerobes and facultative anaerobes.
For sterility testing and for use of Tryptic Soy Broth as a pre-enrichment medium for the detection of specific microorganisms in pharmaceutical products, consult the European Pharmacopoeia.[2]

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.[3]

CONTROL STRAINS			INCUBATION T°/ t / ATM	EXPECTED RESULTS
S. aureus	ATCC	25923	35-37°C / 18-24H / A	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth

User quality control of TSB used for microbiological examination of pharmaceutical products should meet the requirements of EP[2]
A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Tryptic Soy Broth is not suitable for the cultivation of fastidious microorganisms (e.g. *Haemophilus* or *Neisseria* spp.) and for the cultivation of strict anaerobes.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place. According to MacFaddin, the medium prepared by the user in tubes or flasks can be stored at +2°C/+8°C for 6 months.[4]

Ready-to-use medium in tubes and flasks

Store in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. European Pharmacopoeia, current edition
3. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Tryptic Soy Broth	Dehydrated medium	4021552 4021554	500 g (16.7 L) 5 kg (167 L)
Tryptic Soy Broth	Ready-to-use tubes	552155	20 x 9 mL glass tubes
Tryptic Soy Broth	Ready-to-use flasks	5121552 5121553	6 x 100 mL 6 x 200 mL

IFU rev 2, 2021/11

Tryptic Soy Broth
from left: un-inoculated tube, growth of *B. subtilis*

TRYPTIC SOY BROTH γ- IRRADIATED

Dehydrated culture medium

INTENDED USE

Gamma-irradiated general-purpose medium, for the cultivation of microorganisms. For the microbiological validation of aseptic filling processes.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	17.0 g
Soy peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Tryptic Soy Broth is a general-purpose medium that supports the growth of a wide variety of aerobic and facultative anaerobic bacteria and fungi.[1] The medium is prepared according to the formulation recommended by the current European Pharmacopoeia and complies with the quality specifications reported therein.[2]

Tryptic Soy Broth γ-irradiated in 500 g and 5 kg packs is irradiated with gamma rays at a minimum dose of 25 kGy and maximum of 35 kGy, is cold filterable and triple-bagged allowing for safe introduction into controlled areas. Different doses can be applied based on specific agreements with the customer.

The broth is suitable for monitoring microbial contamination in sterile production lines during media fill tests.[3]

Peptones and glucose are sources of nitrogen, carbon, vitamins and trace elements needed for the growth of most non-fastidious and moderately fastidious microorganisms (bacteria, yeasts, fungi). Sodium chloride maintains osmotic balance, dipotassium hydrogen phosphate is included as a buffer system.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified sterile water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Use according to the purpose required.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Final pH at 20-25°C	7.3 ± 0.2
Gamma-irradiation	25 - 35 kGy

TEST PROCEDURE

Use the medium according to the purpose required. For “media fill” test[3] the culture medium is used in place of the product solution to test whether the aseptic procedures are adequate to prevent contamination during production process.

A media fill is one part of the validation of an aseptic manufacturing process.

After the final product container is filled and ready for release, it should be incubated in a temperature-controlled incubator. Any controlled temperature between 20 and 35° C would work for media fills. However, the “controlled temperature” should be specified in the procedures and be maintained within a range that does not exceed ± 2.5°C.

The incubation period of a media fill should be no less than 14 days and the containers should be examined every 2 or 3 days. If different incubation temperatures are chosen, it is recommended to incubate the containers filled with the medium for at least 7 days at the lowest temperature (e.g., 20- 25°C) and then 7 at the highest temperature (e.g., 30 - 35 ° C).

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[2]

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	20-25°C / 24-72h -A	good growth
A. brasiliensis ATCC 16404	20-25°C / 72-120h -A	good growth
B. subtilis ATCC 6633	30-35°C / 24 h -A	good growth
S. aureus ATCC 6538	30-35°C / 24 h -A	good growth
P. aeruginosa ATCC 9027	30-35°C / 24 h -A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Tryptic Soy Broth γ-Irradiated is not suitable for the cultivation of fastidious microorganisms (e.g., *Haemophilus* or *Neisseria* spp.) and for the cultivation of strict anaerobes.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- Due to the wide variety of production processes and devices to be examined with "media fill" test, it is the user's responsibility to validate this medium for the specific intended use.

STORAGE CONDITIONS

Upon receipt, store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. European Pharmacopoeia 11th Edition, 2022, Vol. 1.
3. FDA Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice

PACKAGING

Product	Type	REF	Pack
Tryptic Soy Broth γ-Irradiated	Dehydrated medium	402155G2 402155G4	500 g (16.7 L) Gamma-irradiated, triple bagged 5 kg (167 L) Gamma-irradiated, triple bagged

IFU rev 1, 2022/12

TRYPTIC SOY BROTH MODIFIED (mTSB)
NOVOBIOCIN ANTIMICROBIC SUPPLEMENT

Dehydrated culture medium and supplement, ready-to-use flasks

INTENDED USE

With the addition of novobiocin, Tryptic Soy Broth Modified (mTSB) is used for the selective enrichment of *Escherichia coli* O157 in foods.

COMPOSITION *

TRYPTIC SOY BROTH MODIFIED (MTSB), DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Pancreatic digest of casein	17.0 g
Soy peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	4.0 g
Glucose	2.5 g
Bile Salts N° 3	1.5 g

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT - VIAL CONTENTS

Novobiocin	10 mg

TSB MODIFIED, READY-TO-USE FLASKS
TYPICAL FORMULA

Tryptic Soy Broth Modified (mTSB)	33 g
Novobiocin	20 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

mTSB was devised by Doyle and Shoeni[1] by increasing the concentration of the phosphate buffer of Tryptic Soy Broth and adding bile salts n° 3 and novobiocin with the aim of developing a procedure that could specifically isolate *E. coli* O157:H7 from foods.
The medium is recommended by ISO 16654[2] for the preparation of the initial suspension of the sample and for the enrichment procedure for the detection of *E. coli* O157 in foodstuffs. It corresponds to medium n° 156 of FDA BAM.[3]
Pancreatic digest of casein and soy peptone provide nitrogen, carbon, and trace elements for microbial growth. Dipotassium hydrogen phosphate is used as buffering agent to control the pH in the medium. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium. Glucose is a source of carbon and energy. Selectivity is provided by the presence of bile salts n° 3 and novobiocin with a marked antibacterial activity against Gram-positive bacteria.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 33 g in 1000 mL of cold purified water. Mix well and, if necessary, heat slightly to completely dissolve the powder. Distribute 225 mL into flasks of suitable capacity and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature and to each 225 mL flask add, under aseptic conditions, 2.25 mL of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Final concentrations: 4.5 mg/225 mL or 20 mg/litre. The remaining novobiocin solution can be stored at 2-8 ° C for one month.[5]

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared flasks appearance	yellow, limpid
Freeze-dried selective supplement	short, dense, white pellet; colourless clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	7.4 ± 0.2

SPECIMENS

Foods, animal deeding stuffs, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[2]

TEST PROCEDURE

1. Prepare the initial suspension by adding the test sample portion to Tryptic Soy Broth Modified with novobiocin (mTSB-N), prewarmed to 41.5 ºC to obtain a ratio of 1/10 (e.g., 25 g of sample + 225 mL of mTSB-N)
2. Incubate at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h. The use of homogenisation bags with a filter strip is recommended.
3. *E. coli* O157 cells are separated and concentrated using immunomagnetic beads coated with antibodies to *E. coli* O157 after 6 h and again, if necessary, after a further 12 to 18 h incubation.
4. 50 µl of immunomagnetic concentrated broth are sub-cultured onto Mac Conkey Sorbitol Agar with CT Supplement (CT-SMAC) and onto a second selective isolation agar of laboratory choice (e.g. Mac Conkey Sorbitol MUG Agar REF 401669 or Chromogenic E. coli O157 Agar REF 405581). CT-SMAC is incubated at 37 ± 1°C for 18 to 24 h. The second agar of choice should be incubated following the IFU's recommended procedures.

READING AND INTERPRETATION
The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the liquid medium.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli O157 ATCC 43894	41.5°C / 18-24 H / A	growth
S. aureus ATCC 25923	41.5°C / 18-24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Collection of Type Cultures

LIMITATIONS OF THE METHOD
- No standard methods are available for all pathogenic *E. coli* and the existing methods are either adapted from general methods for isolating *E. coli* or are developed for a specific group of pathogenic *E. coli*. Regardless of methods, however, it is important that isolates are identified biochemically as *E. coli*, in addition to testing for virulence factors associated with the respective pathogenic group.[4]

STORAGE CONDITIONS
Dehydrated medium
Store at +10°C/+30°C away from direct light in a dry place.
Freeze-dried supplement
Store the product in the original package at +2°C/+8°C away from direct light. Novobiocin solution can be stored at +2°C/+8°C for one month.[5]
According to ISO 21567 the medium base and the novobiocin solution may be stored for up to one month at +2°C/+8°C.[5] At the time of use novobiocin should be added to the medium base.
Ready-to-use medium in flasks
Store flasks in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Doyle MP, Schoeni JL. Isolation of Escherichia coli 0157:H7 from retail fresh meats and poultry. App Environ Microbiol 1987; 53:2394-96.
2. ISO 16654:2001. Microbiology of food and animal feeding stuffs- Horizontal method for detection of E.coli O157.
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Media M156: Trypticase Soy Broth Modified (mTSB). Content current as of: 12/06/2017
4. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
5. ISO 21567:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.

PACKAGING
Product	Type	REF	Pack
Tryptic Soy Broth Modified (mTSB)	Dehydrated medium	402155M2	500 g (15.1 L)
Novobiocin Antimicrobic Supplement	Freeze-dried supplement	4240045	10 vials (10 mg/vial)
TSB Modified	Ready-to-use flasks	512155M3	6 x 225 mL

IFU rev 2, 2022/11

TRYPTIC SOY BROTH WITH NaCl 10% AND SODIUM PYRUVATE
Dehydrated culture medium

INTENDED USE
Selective broth the enumeration of *Staphylococcus aureus* in foods with MPN method.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Tryptone	17.0 g
Soy peptone	3.0 g
Sodium chloride	95.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Sodium pyruvate	10.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Staphylococcus aureus has been identified as the causative agent in many foods poisoning outbreaks. Processed foods may contain relatively small numbers of debilitated viable cells, whose presence must be demonstrated by appropriate methods.
Tryptic Soy Broth with NaCl 10% and sodium pyruvate is based on the ability of *S. aureus* to grow in presence of 10% NaCl.[1,2]
This liquid selective medium is recommended by FDA BAM[3,4] and AOAC[5] for the enumeration of *S. aureus* by MPN method in products in which small numbers of cells of the target organism are expected to be sub lethally injured or stressed by heating, freezing, or drying and in foods expected to contain large population of competing species. The formulation, with a concentration of 95 g/L of sodium chloride, meets the requirements of FDA-BAM.[4]
Essential growth factors are provided by tryptone and soy peptone. Sodium pyruvate aids in resuscitation of stressed cells. Glucose is a source of carbon and energy. Dipotassium hydrogen phosphate is used as buffering agent to control the pH in the medium. Sodium chloride at high concentration is the selective agent because it inhibits the growth of many competing bacteria without having an effect on staphylococcal growth.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 130 g in 1000 mL of cold purified water. Mix thoroughly and warm if necessary to completely dissolve the powder. Distribute 10 mL into test tubes and sterilise by autoclaving at 121ºC for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[3,5]

TEST PROCEDURE

1. Inoculate 3 tubes of Tryptic Soy Broth with NaCl 10% and Sodium Pyruvate with1 mL portions of decimal dilutions of each sample.
2. Incubate tubes 48 ± 2 hours at 35-37°C.
3. Using 3 mm loop, transfer 1 loopful from each tube showing growth (turbidity) to plate of Baird-Parker Agar medium with properly dried surface. Vortex-mix tubes before streaking if growth is visible only on bottom or sides of tubes.
4. Streak inoculum to obtain isolated colonies. Incubate plates 48 hours at 35-37°C.
5. From each plate showing growth, transfer at least 1 colony suspected to be *S. aureus* into a small tube containing 0.2 – 0.3 mL of BHI Broth and emulsify thoroughly.
6. After incubation at 35-37°C for 18-24 hours, perform coagulase test and ancillary identification tests (catalase test, anaerobic utilization of glucose and mannitol, lysostaphin sensitivity, thermostable nuclease production).[3]
7. Report as *S. aureus*/g as MPN/g according to appropriate MPN tables.

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the liquid medium.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538	37°C/24 H/A	growth
E. coli ATCC 25922	37°C/48 H/A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to FDA-BAM[4] the prepared medium may be stored up to 1 month at 4 ± 1°C.

REFERENCES

1. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.
2. Lancette GA, Bennet RW. Evaluation of an improved MPN medium for recovery of stressed and nonstressed Staphylococcus aureus. J Ass Off Chem 1986; 69:44-46
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. Chapter 12: Staphylococcus aureus. Content current as of: 12/16/2019
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. M154a: Trypticase (Tryptic) Soy Broth with10% NaCl and 1% Sodium Pyruvate. Content current as of:10/24/2017
5. AOAC International. Official Methods of Analysis, AOAC International Arlington, VA. 16th ed., 1995 sec. 987.09.

PACKAGING

Product	Type	REF	Pack
Tryptic Soy Broth with NaCl 10% and Sodium Pyruvate	Dehydrated medium	40215512	500 g (3.8 L)

IFU rev 1, 2022/11

TRYPTIC SOY YEAST EXTRACT AGAR (TSYEA)

Dehydrated and ready-to-use culture medium

INTENDED USE

General purpose medium for the cultivation of a wide variety of microorganisms, especially *Listeria* spp.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE PLATES

Enzymatic digest of casein	17.0 g
Enzymatic digest of soya meal	3.0 g
Yeast extract	6.0 g
Sodium chloride	5.0 g
Dipotassiurn hydrogen phosphate	2.5 g
Glucose	2.5 g
Agar	12.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Tryptic Soy Yeast Extract Agar (TSYEA) is based on the formulation of Tryptic Soy Broth to which yeast extract and agar are added. It is a general-purpose medium for the cultivation of a wide variety of microorganisms and it is recommended by ISO 11290[1,2] for the purification of the colonies cultivated on selective isolation media. Casein and soy peptones and yeast extract provide nitrogen, carbon, amino acids, vitamins and minerals required for the microbial growth. Glucose is a source of carbon and energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium while sodium chloride maintains the osmotic equilibrium.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 48 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	yellow, clear
Final pH at 20-25 °C	7.3 ± 0.2

SPECIMENS
Colonies cultivated on selective plating media

TEST PROCEDURE
Streak the selected colonies onto the surface of pre-dried plates of TSYEA, to allow the isolated colonies to develop.
Incubate at 37 °C for 18 h to 24 h or until growth is satisfactory.
The obtained colonies will then be used for biochemical identification tests.

READING AND INTERPRETATION
Typical colonies of *Listeria* spp. on Tryptic Soy Yeast Extract Agar are 1 mm to 2 mm in diameter, convex, colourless and opaque with an entire edge. When the plates are held to the light (artificial or natural) at about 45-degree angle, colonies exhibit a blue-grey colour and a granular surface.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
L. monocytogenes NCTC 7973	37°C/ 24 H/A	good growth
L. monocytogenes ATCC 13932	37°C/ 24 H/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Type Culture Collection

STORAGE CONDITIONS
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready to use plates
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
2. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.

PACKAGING
Product	Type	REF	Pack
Tryptic Soy Yeast Extract Agar (TSYEA)	Dehydrated medium	4021662	500 g (10.4 L)
Tryptic Soy Yeast Extract Agar (TSYEA)	Ready-to-use plates	542166	2 x 10 plates ø 90 mm

IFU rev 1, 2022/10

TRYPTIC SOY YEAST EXTRACT BROTH (TSYEB)
Dehydrated culture medium

INTENDED USE
General purpose medium for the cultivation of a wide variety of microorganisms, especially *Listeria* spp.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Enzymatic digest of casein	17.0 g
Enzymatic digest of soya meal	3.0 g
Yeast extract	6.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Tryptic Soy Yeast Extract Broth is based on the formulation of Tryptic Soy Broth to which yeast extract is added. It is a general-purpose medium for the cultivation of a wide variety of microorganisms and it is recommended by FDA-BAM[1] and APHA[2] for the cultivation of suspected *Listeria* spp. colonies isolated on selective media.
Casein and soy peptones and yeast extract provide nitrogen, carbon, amino acids, vitamins and minerals required for the microbial growth. Glucose is a source of carbon and energy. Dipotassium phosphate is used as buffering agent to control the pH in the medium while sodium chloride maintains the osmotic equilibrium.

Directions for medium preparation
Suspend 36 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute into tubes and sterilise by autoclaving at 121ºC for 15 minutes.

Physical characteristics
Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, clear
Final pH at 20-25 °C	7.3 ± 0.2

Specimens
Colonies cultivated on selective plating media.

Test procedure
Pick typical colony for inoculate a tube of TSYEB. Incubate at 35°C for 24 h. This culture may be kept at 4°C several days and used repeatedly as inoculum.[1]
Use this culture for carbohydrate fermentation test and motility test.[1,2]
Carbohydrate fermentation test: from TSYEB culture, inoculate the following carbohydrates in 0.5% solutions in Fermentation Broth Base (REF 1488) with Durham tubes: dextrose, esculin, maltose, rhamnose, mannitol, and xylose. Incubate 7 days at 35°C.[1]
Motility test: from TSYEB culture, inoculate a tube of SIM Bios Medium (REF 402036); observe for an umbrella-like growth pattern during 7 days of incubation at ambient temperature.[2]

Reading and interpretation
The presence of microorganisms in TSYEB is indicated by a varying degree of turbidity, specks and flocculation in the medium.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ T - ATM	Expected results
L. monocytogenes NCTC 7973	35 or 25°C/ 24 H/A	good growth
L. monocytogenes ATCC 13932	35 or 25°C/ 24 H/A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection; NCTC: National Type Culture Collection

Storage conditions
Store at +10°C /+30°C away from direct light in a dry place.

References

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods. Rev April 2022.
2. APHA Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington D.C. 5th Ed, 2015.

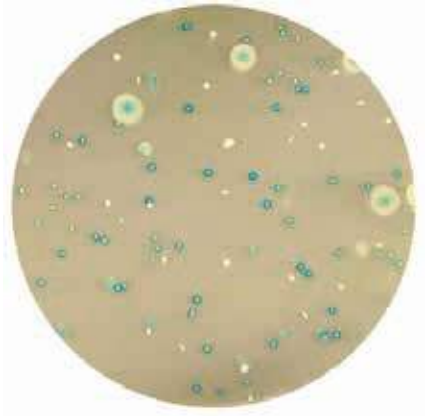
Packaging
Product	Type	REF	Pack
Tryptic Soy Yeast Extract Broth	Dehydrated medium	4021672	500 g (13.9 L)

IFU rev 1, 2022/10

ChromArt

TRYPTONE BILE X-GLUC (TBX) AGAR

Dehydrated and ready-to-use culture medium



TBX Agar: colonies of *E. coli* (blue-green) and *E. aerogenes* (white)

Intended use
For the enumeration of β-glucuronidase positive *Escherichia coli* in foods and animal feeding stuffs.

Composition - typical formula * (after reconstitution with 1 L of water)
Dehydrated medium, ready-to-use plates, tubes and flasks

Tryptone (enzymatic digest of casein)	20.0 g
Bile Salts No. 3	1.5 g
Agar	14.0 g
5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (X-GLUC) ^	75.0 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
^: cyclohexammonium salt

Principle of the method and explanation of the procedure
Tryptone Bile X-Glucuronide (TBX) Agar is based on the formulation of Tryptone Bile Agar originally devised for the enumeration of *E. coli* in food materials.[1,2] TBX Agar is a modification of Tryptone Bile Agar developed on the basis of many studies on first fluorogenic and then chromogenic substrates for the detection of the enzyme β-glucuronidase directly on the isolation media[3-7] and contains the chromogenic compound 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (X-GLUC).
TBX Agar is prepared in accordance with ISO 16649 and meets the requirements therein.[8-10]

The enumeration of β-glucuronidase-positive *E. coli* in foodstuffs may be performed by 1) poured plate technique in TBX Agar[8], 2) colony-count technique using membranes overlaid on Minerals Modified Glutamate Agar, and subsequently transferred on TBX Agar[9], 3) most probable number technique using Minerals Modified Glutamate Medium and the subculture onto TBX Agar[10]. The last two techniques involve a resuscitation step and are recommended for the examination of foodstuffs likely to contain sub-lethally injured cells.
Essential growth factors are provided by tryptone which is a source of nitrogen, carbon and minerals. Bile salts n° 3, inhibit the development of Gram-positive bacteria, especially bacilli and faecal streptococci, while at the same time promoting *E. coli* growth. The medium contains X-GLUC for the detection of β-glucuronidase enzyme: within the *Enterobacteriaceae*, *E. coli* and a few strains of *Salmonella* and *Shigella* are able to split the bond between the chromophore 5-bromo-4-chloro-3-indolyl and the ß-D-glucuronide; the released chromophore is coloured and builds up within the cells, causing *E. coli* colonies to be coloured blue-green.

Directions for medium preparation (Dehydrated medium)
Suspend 35.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and pour into sterile Petri dishes.

Directions for medium preparation (ready-to-use flasks and tubes)
Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes, under aseptic conditions.

Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	beige, clear
Final pH of complete medium (at 20-25°C)	7.2 ± 0.2

Specimens
Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

Test procedure
Enumeration of *E. coli* by poured plate technique (ISO 16649-2)[8]
1. Transfer 1 mL of the test sample in duplicate, into two sterile Petri dishes if liquid, or 1 mL of the initial suspension (10-1), in the case of other products. Repeat the procedure with further decimal dilutions if necessary.
2. Within 15 minutes, pour into each Petri dish about 15 mL of TBX Agar pre-cooled to 44-47°C.
3. Mix well the inoculum with the medium. Invert the inoculated dishes and incubate at 44°C for 18-24 hours. In cases where stressed colonies are suspected incubate for 4 hours at 37°C before incubation at 44°. Do not incubate over 45°C.

Enumeration of *E. coli* by membrane filtration technique (ISO 16649-1)[9]
1. Aseptically place a membrane onto the dried surface of an appropriate number of plates of Minerals Modified Glutamate Agar (MMGM REF 401737 supplemented with Agar) taking care to avoid trapping air bubbles beneath the membranes.
2. Transfer in the centre of the membrane 1 mL of the sample or 1 mL of the initial suspension and spread the inoculum on the surface of the membrane. Repeat the procedure with further decimal dilutions if necessary.
3. Using a sterile spreader, spread the inoculum evenly over the whole membrane surface, avoiding any spillage from the membrane.
4. Leave the plates at room temperature for 15 minutes in order the medium adsorbs the liquid sample.
5. Incubate the plates for 4 h ± 0.25 h at 37 °C, with the membrane/agar surface uppermost.
6. After this resuscitation step transfer the membranes onto TBX Agar plates and incubate at 44°C for 18-24 hours. Do not incubate over 45°C.

Enumeration of *E. coli* by MPN technique (ISO 16649-3)[10]
1. Inoculate 3 or 5 tubes containing 10 mL of double-strength Minerals Modified Glutamate Medium (MMGM REF 401737) with 10 mL aliquots of the test sample, if liquid, or with 10 mL aliquots of the initial suspension in the case of other products.
2. Inoculate 3 or 5 tubes containing 10 mL of single-strength MMGM with 1 mL aliquots of the test sample, if liquid or with 1 mL aliquots of the initial suspension in the case of other products.
3. Repeat the inoculation of the single strength liquid medium for each of the further decimal dilutions, using a fresh pipette for each dilution.
4. Incubate the tubes at 37°C for 24 ± 2 hours.
5. From each of the incubated tubes showing yellow colour subculture with a loop on a plate of TBX Agar by streaking to obtain isolated colonies and incubate at 44°C for 24 ± 2 hours.
6. Express the results as the Most Probable Number of *E. coli* on the basis of the presence of blue-green colonies on TBX plates.

Reading and interpretation
After incubation, examine the TBX Agar plates for the presence of typical, blue or blue-green colonies indicating the presence of β-glucuronidase-positive *E. coli*.
Calculate the number of β-glucuronidase-positive *E. coli* by counting the typical colonies in each plate containing less than 150 typical CFU and less than 300 total (typical and non-typical) CFU.
Multiply the numbers of colonies by the dilution factor and express the result as the number of *E. coli* per gram of sample.

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains			Incubation T°/ t - ATM	Expected results
E. coli	ATCC	25922	44°C / 18-24H / A	growth, blue-green colonies
C. freundii	ATCC	43864	44°C / 18-24H / A	growth, white to green-beige colonies
E. faecalis	ATCC	29212	44°C / 18-24H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive; only exceptionally this test is positive with *Providencia*, *Enterobacter* and *Yersinia* strains (1-5%).[11]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[12]
- Some strains of *E. coli* may grow poorly or not at all in media incubated at 44 °C. Consequently, some strains of *E. coli*, including pathogenic ones, will not be detected by the methods reported above taken from ISO Standards.[10]

STORAGE CONDITIONS

Dehydrated medium

Store at +2°C /+8°C away from direct light in a dry place.

According to ISO 16649-1 the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up for up to four weeks.[9] Immediately before use, dry the agar plates carefully.

Ready-to-use plates, tubes and flasks

Store in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:276-277.
2. Anderson JM, Baird-Parker AC A rapid and direct plate method for enumerating Escherichia coli biotype I in food. J Appl Bacteriol 1975; 39:111-7.
3. Kilian M, Bulow P. Rapid diagnosis of Enterobacteriaceae. Detection of bacterial glycosidases. Acta Pathol Microbiol. Scand. Sect. B. 1976; 84:245–251.
4. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of Escherichia coli. J Clin Microbiol 1984; 19 :172.
5. Ley AN, Bowers RJ, Wolfe S. Indoxyl-β-D-glucuronide, a novel chromogenic reagent for the specific detection and enumeration of Escherichia coli in environmental samples. Can J Microbiol 1988; 34: 690–693
6. Restaino L., Frampton EW, Lyon RH. Use of the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GLUC) for enumerating Escherichia coli in 24 h from ground beef. J Food Prot 1990; 53:508–510.
7. Ogden ID, Watt AJ. An evaluation of fluorogenic and chromogenic assays for the direct enumeration of Escherichia coli. Lett Appl Microbiol 1991; 13:212–215.
8. ISO 16649-2:2001 Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli -Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.
9. ISO 16649-1:2018. Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.
10. ISO 16649-3:2016. Microbiology of the food chain - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide.
11. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
12. Robison BJ. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl Environ Microbiol 1984; 48:285-288

PACKAGING

Product	Type	REF	Pack
Tryptone Bile X-GLUC (TBX) Agar	Dehydrated medium	4021562	500 g (14 L)
Tryptone Bile X-GLUC (TBX) Agar	Ready-to-use plates	542156	2 x 10 plates ø 90 mm
Tryptone Bile X-GLUC (TBX) Agar	Ready-to-use plates	492156	3 x 10 plates ø 55 mm
TBX Agar	Ready-to-use tubes	5521562S	20 x 15 mL
TBX Agar	Ready-to-use flasks	5121562	6 x 100 mL
TBX Agar	Ready-to-use flasks	5121563	6 x 200 mL

IFU rev 2, 2022/09

TRYPTONE SULFITE NEOMYCIN (TSN) AGAR

Dehydrated culture medium

INTENDED USE

For the isolation and enumeration of *Clostridium perfringens* in foodstuffs and other materials.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	15.00 g
Yeast extract	10.00 g
Sodium sulphite	1.00 g
Ferric citrate	0.50 g
Neomycin sulphate	0.05 g
Polymyxin B sulphate	0.02 g
Agar	13.50 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Food poisoning caused by *Clostridium perfringens* may occur when foods such as raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices are cooked and held without maintaining adequate heating or refrigeration before serving.[1]

In the 1950s and 1960s, several studies were carried out to develop a suitable culture medium that would allow the isolation and counting of *C. perfringens*.

Tryptone Sulfite Neomycin (TSN) Agar is prepared according to the formulation proposed by Marshall et al.[2] who modified Mossel's medium[3] for the enumeration of sulphite-reducing clostridia in foods. TSN Agar is used for the isolation and enumeration of *C. perfringens* in foodstuffs and other materials with the incubation at 46°C. The medium may be used by stabbing the tubes as well by streaking the plates.

Neomycin and polymyxin B inhibit the accompanying Gram-negative bacteria and are partially inhibitory for *Clostridium bifermentas*. The relatively high incubation temperature helps in the more specific detection of *C. perfringens*. Essential growth factors are provided by tryptone while the yeast extract is a source of vitamins, particularly of the B-group. Ferric citrate and sodium sulphite are indicators of sulphite reduction by *C. perfringens* which grows with black colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 40 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely the medium and sterilise by autoclaving at 115°C for 20 minutes. Cool to 47°C-50°C mix well and pour into sterile Petri dishes. If required, dispense before sterilisation 20 mL in 20x200 mm tubes and autoclave at 115°C for 20 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellowish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS
Foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE
Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.
Inoculate tubes or plates of the medium by stabbing deep tubes or streaking plates with the initial suspension of the specimen and its dilutions. Incubate for 18-24 hours at 46 ± 0.1°C in an anaerobic atmosphere.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
On TSN Agar, *C. perfringens* usually produce black or grey colonies as a result of the reduction of sulphite to sulphide.

USER QUALITY CONTROL
All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	46°C/ 18-24 H / AN	growth with black colonies
E. coli ATCC 25922	46°C/ 18-24 H / AN	inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATION OF THE METHOD
- Black colonies must be confirmed as *C. perfringens* by appropriate tests: motility (-), nitrate reduction (+), acid and gas from lactose (+), gelatin liquefaction (+).

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.
According to Marshall *et al.* TSN Agar must be used the same day of the preparation.[1]

REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. Marshall RS, Steenbergen JF, McClung LS, Mossel DAA. Rapid technique for the enumeration of Clostridium perfringens. Appl Microbiol 1965 Jul;13(4):559-63.
3. Mossel DAA. Enumeration of sulphite reducing clostridia occurring in foods. J Sci Food Agr 1959; 10:662-669.

PACKAGING
Product	Type	REF	Pack
Tryptone Sulfite Neomycin (TSN) Agar	Dehydrated medium	4021592	500 g (12.5 L)

IFU rev 1, 2022/08

TRYPTONE TRYPTOPHAN MEDIUM
Dehydrated and ready-to-use culture medium

INTENDED USE
Liquid medium to perform the indole test.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Tryptone	10 g
Sodium chloride	5 g
DL-tryptophan	1 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Bacteria that possess the enzyme tryptophanase are capable of hydrolysing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia.[1] The indole test is based on the formation of a red colour complex when indole reacts with aldehyde group of p-dimethylaminobenzaldehyde of Kovacs' Reagent, under acidic conditions.
The indole test is used as part of the IMViC procedures, a battery of tests designed to distinguish among members of the family *Enterobacteriaceae*.[1] Indole production is an important characteristic in the identification of many microorganisms, being particularly useful in separating *E. coli* (positive) from members of the *Klebsiella-Enterobacter-Hafnia-Serratia* group (mostly negative).
The chief requirement for culturing an organism prior to perform the indole test is that the medium contains a sufficient quantity of tryptophan.[2] Tryptone Tryptophan medium is prepared according to the formulation included in several ISO Standards.[3-5]
Essential growth factors are provided by tryptone which is carbohydrates free, rich in tryptophan and a source of nitrogen, carbon and minerals. The addition of 1 g/L tryptophan enhances the detection of tryptophanase by the indole reaction. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 16 g in 1000 mL of cold purified water. Dissolve the powder by mixing and by heating if necessary. Dispense 5 mL of medium in tubes and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tube appearance	pale yellow, limpid
Final pH at 20-25 °C	7.5 ± 0.2

SPECIMENS
The specimens consist of colonies grown on plated media.

TEST PROCEDURE
- Inoculate the Tryptone Tryptophan Medium using a light inoculum of an 18-24 h pure culture and incubate at:
- 44°C or 37 °C for 24 h for the confirmation test of *E. coli,*
- 37 °C for 24 h for the confirmation test of *E. coli* O157 according to ISO 16654,[3]
- 37 °C for 24 h, for the differentiation of *Salmonella* (generally indole negative) from *E. coli* (indole positive) according to ISO 6579,[4]
- 30 °C for 48 h, for the biotyping of *Y. enterocolitica* according to ISO 10273,[5]
- Add 1 mL of Kovács' Reagent and shake gently.
- Examine the upper layer of liquid after about 1 min for the appearance of a red-pink colour.

READING AND INTERPRETATION
Positive result: formation of a pink to red colour within 1 minute (occurring normally within a few seconds)
Negative result: no colour change, the reagent layer remains yellow or slightly cloudy

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.

Positive control: *Escherichia coli* ATCC 25922
Negative control: *Enterobacter aerogenes* ATCC 13048

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Indole test is an aid in the differentiation of microorganisms. For complete identification further suitable tests are recommended.
- Change in colour of the Kovacs Reagent from yellow to brown indicates improper storage, which may cause weaker reactions.

STORAGE CONDITIONS
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579, the self-prepared tubes can be stored at +2°C +8°C in the dark for up to 3 months.[4]
Ready-to-use medium in tubes
Store tubes in their original pack at 2-8°C away from direct light.

REFERENCES

1. Maria P. MacWilliams. Indole Test Protocol. ASM, 08 December 2009, American Society for Microbiology © 2016.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. ISO 16654:2001Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Escherichia coli O157.
4. ISO 6579-1:2017. Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella. Part 1: Detection of Salmonella spp.
5. ISO 10273:2017 -Microbiology of the food chain. Horizontal method for the detection of pathogenic Yersinia enterocolitica.

PACKAGING
Product	Type	REF	Pack
Tryptone Tryptophan Medium	Dehydrated culture medium	4021652	500 g (31.3 L)
Tryptone Tryptophan Medium	Ready-to-use tubes	552165	20 x 8 mL

IFU rev 1, 2022/09

TRYPTOSE (BIOTONE) BROTH
Dehydrated and ready-to-use culture medium

INTENDED USE
In vitro diagnostics. General purpose medium for the cultivation of nutritionally fastidious microorganisms.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM AND READY-TO-USE TUBES

Tryptose (Biotone)	20.000 g
Glucose	1.000 g
Sodium chloride	5.000 g
Thiamine HCl	0.005 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Tryptose (Biotone) Broth is a general purpose medium that supports the growth of a wide variety of aerobic and facultative anaerobic nutritionally fastidious microorganisms.[1,2] Tryptose (Biotone) Broth corresponds to Tryptose Vitamin B medium described in Diagnostic Procedures and Reagents APHA Manual.[3] Tryptose (formerly named Biotone by Biolife), is a mixture of enzymatic hydrolysates of proteins and is a source of carbon, nitrogen, vitamins and minerals for microbial growth; glucose is a source of energy; sodium chloride maintains osmotic balance. According to McCullough[4], thiamine HCl addition to Tryptose Broth enhances the recovery of *Brucella* species, especially *Brucella suis*. Tryptose Broth may be used for the preparation of enriched, selective, diagnostic media as described by WHO publication.[5]

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 26 g in 1000 mL of cold purified water, heat to dissolve, distribute and sterilise by autoclaving at 121 °C for 15 minutes. For specific uses, add the required enrichment.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25°C	7.2 ± 0.2

SPECIMENS

Tryptose (Biotone) Broth may be inoculated with a variety of clinical[1] and non-clinical[6] specimens for the cultivation/enrichment of microorganisms or with colonies cultivated on other isolation media. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.

TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube with the specimen or with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2°C for 18-24 hours is adequate for cultivation of common aerobes and facultative anaerobes.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS		INCUBATION T°/ t / ATM	EXPECTED RESULTS
S. aureus	ATCC 25923	35-37°C / 18-24H / A	good growth
E. coli	ATCC 25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- The preparation of enriched, selective diagnostic media with the addition of specific compounds[5] must be validated by the user.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the medium in tubes prepared by the user can be stored at +2°C/+8°C for up to 6 months.[1]
Ready-to-use medium in tubes
Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Atlas R, Parks LC. Handbook of Microbiological Media. 2nd edition.n. CRC Press,1997
3. Diagnostic Procedures and Reagents, 3rd Edition, APHA, New York; 1970.
4. McCullough WG, Mills RL, Herbst EJ, Roessler WJ and Brewer CR, J Bacteriol1947; 53:
5. OMS. La Brucellose: Techniques de Laboratoires. Serie de Monographies, 1968, N. 55
6. Standard Methods for the Microbiological Examination of Dairy Products, 9th Ed., APHA, New York. 1948

PACKAGING

Product	Type	REF	Pack
Tryptose (Biotone) Broth	Dehydrated medium	4011462 4011464	500 g (19.2) 5 kg (192)
Tryptose (Biotone) Broth	Ready-to-use tubes	551146	20 x 9 mL

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Tryptose (Biotone) Broth - from the left: un-inoculated tube, growth of *S. aureus*

TRYPTOSE (BIOTONE) AGAR

Dehydrated culture medium

INTENDED USE

General purpose medium for the cultivation of nutritionally fastidious microorganisms.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose (Biotone)	20.000 g
Glucose	1.000 g
Sodium chloride	5.000 g
Thiamine HCl	0.005 g
Agar	15.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Tryptose (Biotone) Agar is a general purpose medium that supports the growth of a wide variety of aerobic and facultative anaerobic nutritionally fastidious microorganisms.[1,2] Tryptose (Biotone) Agar corresponds to Tryptose Vitamin B medium described in Diagnostic Procedures and Reagents APHA Manual.[3] Tryptose (formerly named Biotone by Biolife), is a mixture of enzymatic hydrolysates of proteins and is a source of carbon, nitrogen, vitamins and minerals for microbial growth; glucose is a source of energy; sodium chloride maintains osmotic balance. According to McCullough[4], thiamine HCl addition to Tryptose medium enhances the recovery of *Brucella* species, especially *Brucella suis*. Tryptose Agar may be used for the preparation of enriched, selective, diagnostic media as described by WHO publication.[5]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41 g in 1000 mL of cold purified water, heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121 °C for 15 minutes. For specific uses, add the required enrichment.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25°C	7.2 ± 0.2

SPECIMENS

Tryptose (Biotone) Agar may be inoculated with a variety of specimens[1,6] for the cultivation/enrichment of microorganisms or with colonies cultivated on other isolation media. Good laboratory practices for collection, transport and storage of the specimens should be applied.

TEST PROCEDURE

Allow plates or tubes to come to room temperature and to dry the surface of the medium.
With a bacteriological needle or loop inoculate the medium with the specimen or with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2°C for 18-24 hours is adequate for cultivation of common aerobes and facultative anaerobes.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS			INCUBATION T°/ t / ATM	EXPECTED RESULTS
S. aureus	ATCC	25923	35-37°C / 18-24H / A	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- The preparation of selective diagnostic media with the addition of specific compounds[5] must be validated by the user.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the self-prepared plates can be stored at +2°C +8°C in the dark and protected against evaporation for up to 6-8 weeks.[1]

REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Atlas R, Parks LC. Handbook of Microbiological Media. 2nd edition.n. CRC Press,1997
3. Diagnostic Procedures and Reagents, 3rd Edition, APHA, New York; 1970.
4. McCullough WG, Mills RL, Herbst EJ, Roessler WJ and Brewer CR, J Bacteriol1947; 53:
5. OMS. La Brucellose: Techniques de Laboratoires. Serie de Monographies, 1968, N. 55
6. Standard Methods for the Microbiological Examination of Dairy Products, 9th Ed., APHA, New York. 1948

PACKAGING

Product	Type	REF	Pack
Tryptose (Biotone) Agar	Dehydrated medium	4011452	500 g (12.2 L)

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TRYPTOSE PHOSPHATE BROTH

Dehydrated culture medium

INTENDED USE

General purpose medium for the cultivation of nutritionally fastidious microorganisms.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptose	20.0 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	2.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Tryptose Phosphate Broth is an infusion-free buffered medium that supports the growth of a wide variety of aerobic and facultative anaerobic nutritionally fastidious microorganisms.[1,2] Tryptose Phosphate Broth corresponds to BAM media 168 for cell cultures.[3] It is useful in tissue culture procedures, where peptone content is considered a stimulating factor for cells.[4]
The addition of 0.1-0.2% agar to the medium aids in initiation of anaerobic growth from small inocula and retardation and dispersion of reducing substances and CO_2 formed in environment.[1]
Tryptose (formerly named Biotone by Biolife), is a mixture of enzymatic hydrolysates of proteins and is a source of carbon, nitrogen, vitamins and minerals for microbial growth; glucose is a source of energy; sodium chloride maintains osmotic balance. Disodium hydrogen phosphate is used as buffering agent to control the pH in the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 29.5 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	yellow, limpid
Final pH at 20-25°C	7.3 ± 0.2

SPECIMENS

Tryptose Phosphate Broth may be inoculated with a variety of specimens for the cultivation/enrichment of microorganisms or with colonies cultivated on other isolation media.

TEST PROCEDURE

With a bacteriological needle or loop inoculate the liquid medium in a test tube with the specimen or with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2°C for 18-24 hours is adequate for cultivation of common aerobes and facultative anaerobes.
The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the processed specimen, the requirements of organisms to be recovered and the local applicable protocols.

READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

CONTROL STRAINS		INCUBATION T°/ t / ATM	EXPECTED RESULTS
S. aureus	ATCC 25923	35-37°C / 18-24H / A	good growth
E. coli	ATCC 25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The nutritional requirements of microorganisms can be different, it is therefore possible that some microbial strains do not grow or grow scantily.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- The preparation of enriched, selective diagnostic media with the addition of specific compounds must be validated by the user.
- Biochemical, immunological, molecular, or mass spectrometry testing should be performed on isolates, from pure culture, for complete identification.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for 6 months.[1]

References

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. Atlas R, Parks LC. Handbook of Microbiological Media. 2nd edition. CRC Press,1997
3. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), online. BAM Media M168. January 2001.
4. Ginsberg HS, Gold E, Jordan Jr WS. Tryptose phosphate broth as supplementary factor for maintenance of HeLa cell tissue cultures. Proc Soc Exp Biol Med 1955; 89:66-71.

Packaging

Product	Type	REF	Pack
Tryptose Phosphate Broth	Dehydrated medium	4011502	500 g (16.9 L)

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TSC AGAR BASE
D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT
D-CYCLOSERINE 4-MUP SUPPLEMENT
TSC AGAR – TSC AGAR MUP

Dehydrated and ready to use culture medium and selective supplements

Intended use

For the isolation and enumeration of *Clostridium perfringens* in foods, waters and other materials.

Composition*

TSC Agar Base
Dehydrated and ready to use medium in flasks and tubes
Typical formula (after reconstitution with 1 L of water)

Enzymatic digest of casein	15 g
Soy Peptone	5 g
Yeast extract	5 g
Sodium metabisulphite anhydrous	1 g
Ferric ammonium citrate	1 g
Agar	15 g

TSC Agar - ready to use plates
Typical formula

TSC Agar Base	42 g
D-Cycloserine	400 mg
Purified water	1000 mL

D-Cycloserine Antimicrobic Supplement
(Vial content for 500 mL of medium)

D-Cycloserine	200 mg

D-Cycloserine 4-MUP Antimicrobic Supplement
(Vial content for 500 mL of medium)

D-Cycloserine	200 mg
4-methylumbelliferyl phosphate	50 mg

TSC MUP Agar - ready to use plates
Typical formula

TSC Agar Base	42 g
D-Cycloserine	400 mg
4-methylumbelliferyl phosphate	100 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Food poisoning caused by *Clostridium perfringens* may occur when foods such as raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices are cooked and held without maintaining adequate heating or refrigeration before serving.[1] The enumeration of *C. perfringens* in food samples plays a key role in the epidemiological investigation of food-borne disease outbreaks and for this purpose various culture media have been proposed since the 1950s.
Tryptose Sulfite Cycloserine (TSC) Agar was developed by Harmon[2] using the same basal medium as Shahidi-Ferguson-Perfringens (SFP) agar, but with 400 mg/L of D-cycloserine substituted for the kanamycin and polymyxin. The TSC agar method was improved by eliminating the egg yolk and using pour plates by Hauschild.[4] TSC Agar, with and without the addition of egg yolk, is probably the best medium of those currently available for the purpose of enumeration of *C. perfringens*.[5]
The complete medium TSC Agar with D-cycloserine and without egg yolk emulsion, meets the requirements given by ISO 7937[6] (will be replaced by ISO 15213-2 under development)[7] for the samples of the food chain and ISO 14189[8] for water. With and without egg yolk emulsion, TSC Agar meets the requirements of FDA-BAM[1].
TSC Agar Base supplemented with D-cycloserine and 4-methylumbelliferyl phosphate simplifies the enumeration of *C. perfringens* mainly when high number of small colonies are present.[9]
For the detection of sulphite reducing *Clostridium* spp. in food samples with Iron Sulfite Agar, consult ISO 15213-1.[10]
The enzymatic digest of casein and soy peptone provide nitrogen, carbon, minerals and amino acids for the microbial growth. The yeast extract is a source of vitamins particularly of the B-group. Ferric ammonium citrate and sodium metabisulfite are indicators of sulphite reduction by *C. perfringens* which produces black colonies. D-cycloserine is an antibiotic inhibiting cell-wall biosynthesis in bacteria which helps in the selective isolation of *C. perfringens* by inhibiting accompanying flora.
The detection of acid phosphatase has been shown to be a useful diagnostic tool for identifying *C. perfringens*.
C. perfringens can metabolize 4-methylumbelliferyl phosphate (MUP) using the enzyme acid phosphatase to produce 4-methylumbelliferone, which fluoresces when placed under long-wavelength (365-nm) ultraviolet light.[9]

Directions for medium preparation (dehydrated medium)

TSC Agar. Suspend 21 g of TSC Agar Base in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of D-Cycloserine Antimicrobic Supplement (REF 4240002), reconstituted with 5 mL of sterile purified water. If required, 25 mL of Egg Yolk Emulsion (REF 42111601) may be added to the precooled medium base. Mix well and pour into sterile Petri dishes.
TSC MUP Agar. Suspend 21 g of TSC Agar Base in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of D-Cycloserine 4-MUP Supplement (REF 4240049), reconstituted with 5 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (MEDIUM IN FLASKS AND IN TUBES)

Liquefy the contents of the flask or the tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottles and the tubes may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C. Reconstitute the contents of one vial of D-Cycloserine Antimicrobic Supplement (REF 4240002) or D-Cycloserine 4-MUP Supplement (REF 4240049) with 5 mL of sterile purified water and add to TSC Agar Base in the following quantities:
100 mL flasks: 1 mL/flask
200 mL flask: 2 mL/flask
15 mL tube: 0.15 mL/tube
Mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

TSC Agar Base	
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates/flasks/tubes appearance	yellow, limpid
Final pH at 20-25 °C	7.6 ± 0.2
D-Cycloserine Antimicrobic Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid
D-Cycloserine 4-MUP Supplement	
Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless limpid

SPECIMENS

Waters, foods and animal feeding stuffs. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[6-8]

TEST PROCEDURE

Enumeration of *C. perfringens* in foods with TSC Agar (ISO/DIS 15213-2)[7]

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.
2. If it is the intention to count only spores, heat the decimal dilution series to 80 °C in a water bath for 10 min ± 1 min.
3. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1mL of each decimal dilution, in duplicate, to the centres of empty Petri dishes.
4. Pour 12-15 mL of Egg Yolk free TSC Agar into each 90 mm dish and mix well with the inoculum. Pour 45 ml to 50 ml for 140 mm Petri dishes.
5. Carefully mix the inoculum with the medium by rotating the Petri dishes.
6. After complete solidification, pour about 5 ml of medium for 90 mm Petri dishes or 10 ml for 140 mm Petri dishes as overlay, to prevent the development of spreading colonies on the surface of the medium.
7. Allow to solidify and incubate in anaerobic jars or other suitable containers and incubate at 37 °C for 20 ± 2 hours. Longer incubation may result in excess blackening of the plates
8. Count the typical colonies in the plates containing less than 150 suspect colonies (90 mm Petri dishes) or less than 360 colonies (140 mm Petri dishes).
9. To confirm the presence of *C. perfringens,* choose one of the following two techniques:

Acid phosphatase test (REF192010) or SIM agar test (REF 402036)

Enumeration of *C. perfringens* in foods with TSC MUP Agar[11]

1. Transfer by means of sterile pipettes 0.1 mL of the test sample (if liquid) or 0.1 mL of the initial suspension and 0.1mL of each decimal dilution, in duplicate, to the surface of the TSC 4 MUP Agar plates.
2. Incubate in anaerobic jars or other suitable containers and incubate at 44°C for 22 ± 2 hours.
3. Count the fluorescent colonies observed under Wood's lamp (360 nm) on the plates containing between 15 and 150 characteristic colonies.
4. Confirm the suspected colonies with the catalase test (-) and with inverted CAMP Test (+).

Enumeration of *C. perfringens* in water with TSC Agar (ISO 14189)[8]

1. Filter a measured volume of sample, or a dilution of it, through a membrane with a pore size of 0.45 µm sufficient to retain spores of clostridia.
2. Using aseptic technique, roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
3. Incubate TSC Agar anaerobically at 44 ± 1 for 21 ± 3 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
On TSC Agar, *C. perfringens* usually produce black or grey to yellow brown colonies as a result of the reduction of sulphite to sulphide.
On TSC MUP Agar, *C. perfringens* usually produce black or grey to yellow brown colonies, fluorescent when observed under Wood's lamp.
For a complete explanation of the identification criteria and methods, refer to the quoted references.[1,6-8,10]

USER QUALITY CONTROL

All manufactured lots of the products are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control:

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. perfringens ATCC 13124	37°C/ 18-24 H / AN	growth, black colonies (TSC MUP Agar: fluorescent under Wood's Lamp)
B. subtilis ATCC 6633	37°C/ 18-24 H / AN	totally inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Black colonies must be confirmed as *C. perfringens* by appropriate tests. ISO Standards recommend the following tests: motility (-), nitrate reduction (+), acid and gas from lactose (+), gelatin liquefaction (+).

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplements
Store the products in the original package at +2°C /+8°C away from direct light.
According to ISO 14189[8] the TSC Agar plates with D-cycloserine prepared by the user may be stored at +2°C /+8°C for up to 7 days; the basal medium (without D-cycloserine) may be stored at +2°C /+8°C and used for up to 4 weeks.
Ready to use plates
Store plates in their original pack at +2°C /+8°C away from direct light.
Ready-to-use medium in flasks and tubes
Store flasks and tubes in their original pack at +2°C /+8°C away from direct light.

References

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM). Chapter 16: Clostridium perfringens.
2. Harmon SM, Kautter DA, Peeler JT. Improved medium for enumeration of Clostridium perfringens Appl Microbiol 1971 Oct;22(4):688-92.
3. Shahidi SA, Ferguson AR. New quantitative, qualitative, and confirmatory media for rapid analysis of food for Clostridium perfringens. Appl. Microbiol. 1971; 21:500-506.
4. Hauschild AH, Hilsheimer R. Evaluation and modifications of media for enumeration of Clostridium perfringens. Appl Microbiol 1974 Jan;27(1):78-82.
5. Mead GC. Selective and differential media for Clostridium perfringens. Int J Food Microbiol 1985; 2:89-98
6. ISO 7937:2004. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of Clostridium perfringens -- Colony-count technique
7. ISO/DIS 15213-2 6b Microbiology of the food chain — Horizontal method for the detection and enumeration of Clostridium spp. — Part 2: Enumeration of Clostridium perfringens by colony-count technique
8. ISO 14189:2013 Water quality — Enumeration of Clostridium perfringens — Method using membrane filtration
9. Adcock PW, Saint CP. Rapid Confirmation of Clostridium perfringens by Using Chromogenic and Fluorogenic Substrates. Appl Environ Microbiol. 2001 Sep; 67(9): 4382–4384.
10. ISO 15213-1:2023. Microbiology of the food chain - Horizontal method for the detection and enumeration of Clostridium spp. Part 1: Enumeration of sulfite-reducing Clostridium spp. by colony-count technique.
11. Manuel Suisse des Denrées Alimentaires (MSDA). Chapitre 56, Microbiologie. Juillet 2000.

Packaging

Product	Type	REF	Pack
TSC Agar Base	Dehydrated medium	4021582	500 g (11.9 L)
D-Cycloserine Antimicrobic Supplement	Freeze-dried supplement	4240002	10 vials, each for 500 mL of medium
D-Cycloserine 4-MUP Supplement	Freeze-dried supplement	4240049	10 vials, each for 500 mL of medium
TSC Agar	Ready-to-use plates	542158	2 x 10 plates ø 90 mm
TSC Agar	Ready-to-use plates	492158	3 x 10 plates ø 55 mm
TSC Agar MUP	Ready-to-use plates	492158X	3 x 10 plates ø 55 mm
TSC Agar Base	Ready-to-use tubes	552158B	20 x 15 mL
TSC Agar Base	Ready-to-use flasks	5121582	6 x 100 mL
TSC Agar Base	Ready-to-use flasks	5121584	6 x 200 mL

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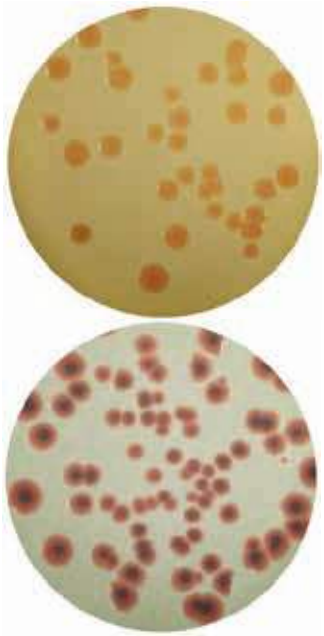
TTC TERGITOL 7 AGAR BASE
TTC TERGITOL 7 AGAR
TTC SOLUTIONS

Dehydrated culture medium, supplements and ready to use plates



TTC Tergitol 7 Agar- from top to bottom: colonies of E. *coli* and *Salmonella* sp. on membrane filters

Intended use

For the enumeration of coliforms and *Escherichia coli* in water samples by the membrane filtration technique.

Composition - typical formula *

Dehydrated TTC Tergitol 7 Agar Base (after reconstitution with 1 L of water)

Yeast extract	6.00 g
Peptone	10.00 g
Beef extract	5.00 g
Lactose	20.00 g
Bromothymol blue	0.05 g
Tergitol 7	0.10 g
Agar	13.00 g

TTC 0.05% Solution REF 421510 (bottle content)

Triphenyl tetrazolium chloride	0.0125 g
Purified water	25 mL

TTC 1% Solution REF 42111801 (bottle content)

Triphenyl tetrazolium chloride	0.3 g
Purified water	30 mL

TTC Tergitol 7 Agar (Ready-to-use plates)
Typical formula

TTC Tergitol 7 Agar Base	54 g
TTC 1% Solution	2.5 mL
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

Principle of the method and explanation of the procedure

Tergitol 7 agar was first introduced by Chapman[1] in 1947. The medium produced a consistent and characteristic colonial morphology with *E. coli, Enterobacter aerogenes*, and other Gram-negative bacteria. This medium was later modified by Chapman[2] to include 2,3,5-triphenyltetrazolium chloride and was recommended as the medium of choice for the quantitative detection of *E. coli* in drinking water.[3]
TTC Tergitol Agar was recommended by the ISO 9308-1:2000 Standard,[4] which was withdrawn and replaced by the 2014 version, for the detection and enumeration of *E. coli* and coliforms in drinking water using the membrane filtration method.
Peptone and beef extract provide nitrogen and minerals for microbial growth, yeast extract is a source of B-vitamins complex for growth stimulation, lactose is a fermentable carbohydrate and a source of carbon and energy. Tergitol-7 acts as surface-active agent which inhibits the growth of most Gram-positive bacteria as well as swarming of *Proteus*. Bromothymol blue is a pH indicator: the high concentration of acids produced during fermentation of lactose turns the bromothymol blue indicator from green to yellow. TTC is a sensitive indicator of dehydrogenase[5] and its reduction to insoluble formazan by lactose-negative bacteria produces dark red colonies. For typical *E. coli*, under the conditions of use, TTC is not a satisfactory hydrogen acceptor and it is reduced weakly, hence the colonies are yellow-orange.[5]

Directions for medium preparation

Suspend 27 g in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50 °C and add, under aseptic conditions, 1.25 mL of TTC 1% Solution (REF 42111801) or the content of one bottle (25 mL) of TTC 0.05% Solution (REF 421510). Mix well and pour into sterile Petri dishes.

Physical characteristics

Dehydrated medium appearance	pale blue, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	green, clear
TTC solutions appearance	colourless, clear
Final pH at 20-25 °C	7.2 ± 0.1

Specimens

Water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

Test procedure

1. Filter an appropriate volume of water depending on the expected microbial load onto two membrane filters.
2. Roll the membrane filters onto the surface of two TTC Tergitol 7 Agar plates, avoiding the formation of air bubbles between the filter and the agar surface.
3. Incubate one dish at 36 ± 2°C for 18-24 hours (total coliforms) and the other one at 44.0 ± 0.5 °C for 18-24 hours (*E. coli*).
4. Subculture the characteristic colonies in a non-selective agar such as Tryptic Soy Agar (REF 402150) and in Tryptone Tryptophan Medium (REF 402165).
5. Incubate the non-selective agar at 36 ± 2 °C for 18-24 h and perform the oxidase test.
6. Incubate the Tryptone Tryptophan Medium tube at 44.0 ± 0.5 °C for 18-24 h and examine indole production by adding few drops of Kovacs' reagent (REF 19171000).

Reading and interpretation

After incubation, examine the membranes and count as lactose-positive bacteria, the colonies developing a yellow colouration in the medium below the membrane.
Count all colonies that produce a negative oxidase reaction as coliforms.
Count all colonies producing a negative oxidase reaction and a positive indole reaction as *E. coli.*
Typical colonies on TTC Tergitol 7 Agar:
E. coli grows with yellow colonies with yellow zone sometimes with rust coloured centre.
Enterobacter/Klebsiella spp. exhibit greenish/yellow colonies.
Lactose negative organisms such as *Salmonella, Shigella, Proteus, Pseudomonas* grow with red colonies with bluish zones.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t - ATM	Expected results
E. coli ATCC 25922	37°/ 18-24 H-A	growth with yellow colonies and yellow zones
E. faecalis ATCC 19433	37°/ 18-24 H-A	partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- In special cases, identification of coliform bacteria may be necessary, e.g., to distinguish between faecal and aquatic/telluric strains.[5]

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Liquid supplements
Store the products in the original package at +2°C /+8°C away from direct light.
According to ISO 9308-1:2000 the self-prepared plates can be stored at +2°C /+8°C in the dark and protected against evaporation for up to 10 days.[4]
Ready to use plates
Sstore plates in their original pack at +2°C /+8°C away from direct light.

References

1. Chapman GH. A superior culture medium for the enumeration and differentiation of coliforms. J Bacteriol. 1947;53(4):504.
2. Chapman GH. A culture Mmedium for detecting and confirming Escherichia coli in ten hours. Am J Public Health Nations Health. 1951; 41(11 Pt 1): 1381.
3. Kulp W L, Mascoli C, Taushanjlian O. Use of Tergitol-7 Triphenyl Tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. Am J Public Health. 1953; 43:1111-1113.
4. ISO 9308-1 (2000) Withdraw. Water Quality: Detection and enumeration of Escherichia coli and coliform bacteria. Part 1: Membrane filtration method.
5. Kulp WL, Mascoli C, Tavshanjian O. Use of Tergitol-7 Triphenyl Tetrazolium Chloride Agar as the Coliform Confirmatory Medium in Routine Sanitary Water Analysis. Am J Public Health Nations Health. 1953 Sep; 43(9): 1111–1113.

PACKAGING

Product	Type	REF	Pack
TTC Tergitol Agar Base	Dehydrated medium	402160T2	500 g (9.3 L)
TTC 0.05% Solution	Liquid supplement	421510	10 x 25 mL
TTC 1% Solution	Liquid supplement	42111801	30 mL
TTC Tergitol Agar	Ready-to-use plates	492160T	3 x 10 piastre ø 55 mm

IFU rev 1, 2022/09

UREA AGAR BASE (CHRISTENSEN)

UREA 40% SOLUTION

Dehydrated culture medium, supplement and ready to use tubes

Urea Agar
From the left: *E. coli* urease -, *Proteus* sp. urease+

INTENDED USE

In vitro diagnostics. Base medium and urea supplement for the differentiation of microorganisms on the basis of urease activity.

COMPOSITION *

UREA AGAR BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	1.000 g
Glucose	1.000 g
Sodium chloride	5.000 g
Potassium dihydrogen phosphate	2.000 g
Phenol red	0.012 g
Agar	12.000 g

UREA 40% SOLUTION (VIAL CONTENT)

	REF 42211601	REF 4240096
Urea	20 g	2 g
Purified water	50 mL	5 mL

UREA AGAR, READY-TO-USE TUBES
TYPICAL FORMULA

Urea Agar Base	21 g
Urea 40% solution	50 mL
Purified water	950 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Urea Agar Base is prepared according to Christensen's[1] modification of the Rustigian and Stuart[2] formula and to the formulation recommended by ISO 6579[3] and FDA BAM[4]. The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease. Two units of ammonia are formed with resulting alkalinity in the presence of the enzyme, and the increased pH is detected by a colour change of the pH indicator from yellow (pH 6.8) to bright pink (pH 8.1).[5,6] The addition of peptone and glucose and the reduction of the phosphate buffer concentration of the Christensen formulation allow the differentiation between rapid (1-6 hours) urease-positive organisms (*Proteae*) and delayed (24 hours-6 days) urease-positive bacteria (some *Klebsiella, Enterobacter* and *Citrobacter* strains) and bacteria other than the *Enterobacteriaceae* (e.g. some *Bordetella* and *Brucella* species).[5] This test can be used for differentiation between the yeasts, *Candida albicans* and *Cryptococcus neoformans*: a presumptive identification of *C. neoformans* is based on rapid urease production, whilst *C. albicans* does not produce urease.[6]
The peptone provides the essential elements for microbial growth; glucose is a source of energy and allows rapid microbial growth and eliminates possible false negative reactions; potassium dihydrogen phosphate at a concentration of 0.2%, lower than in Stuart formulation, allows to detect small amounts of alkali; sodium chloride maintains the osmotic balance of the medium and phenol red is a pH indicator.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 21 g in 950 mL of cold purified water. Heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to approximately 47-50°C and add, under aseptic conditions, 50 mL of Urea 40% Solution (REF 42211601). Mix well and dispense the complete medium in quantities of 10 mL into sterile tubes. Cool in slanted position (long slant/short butt).

PHYSICAL CHARACTERISTICS

Urea Agar Base (Christensen)	
Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	pink-orange, limpid
Final pH at 20-25 °C	6.8 ± 0.2
Urea 40% Solution	
Solution appearance	colourless, limpid

SPECIMENS

Urea Agar shall not be used for the direct inoculation of clinical specimens. The samples consist of isolates from pure culture grown on solid medium.

TEST PROCEDURE

Inoculate the slope heavily (from an 18-24 hours pure culture) over the entire surface by streaking the surface of the agar. Do not stab the butt; it serves as a colour control.
Incubate inoculated tube with loosened cap at 35-37°C and observe the colour change of the medium to red-violet after 2, 4, 6, 18, 24 hours and daily for a total incubation time of 6 days.According to ISO 6579-1, incubate at 37°C for up to 24 h: the reaction is often apparent after 2 h to 4 h.

READING AND INTERPRETATION

After incubation, observe the colour change of the medium. The positive test (urea hydrolysis) is indicated by a bright pink (fuchsia) colour on the slant that may extend into the butt; any degree of pink is considered a positive reaction.[6]
The extent of colour indicates the rate of urea hydrolysis[5]:
- Strong positive: entire tube pink
- Positive: pink slant, no change in butt
- Weak positive: top of slant pink, remainder no change
- Negative results: no colour changes in agar slant

Regarding the development time of the pink alkaline reaction, some microbial categories may be observed:
- Rapid positive: 1-6 hours for all positive *Proteae* microorganisms *(Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains).
- Delayed positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days).[5-6]
- For bacteria other than the *Enterobacteriaceae* (e.g *Bordetella* and *Brucella* species, yeasts) the inoculated slope should be further incubated for up 4-6 days before it is considered negative. [5,6]

Once the test has been registered as positive, discard the tubes without prolonging the incubation.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Urease positive control: *P. vulgaris* ATCC 9484
Urease negative control: *E. coli* ATCC 25922
Incubation: 35-37°C for 18-24 hours

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The urea test is based on the alkalinisation of the culture medium and is therefore not specific for the urease enzyme. The utilisation of peptones, especially on the slope, for example by *P. aeruginosa*, may raise the pH to alkalinity, resulting in false positive reactions. To eliminate possible false positive, run a control test using the same strain and the test medium without urea.[5]
- Urease positive *Proteus* spp. cause a rapid alkalinisation of the medium. For the results to be valid for the detection of *Proteae*, the results must be read within the first 2-6 hours interval of incubation. *C. freundii* and *K. pneumoniae* convert Urea Agar within 24-48 hours. This medium detects rapid urease activity only of urease positive *Proteae*.[5]
- Do not inoculate Urea Agar slopes with cultures obtained from liquid media.
- Prolonged incubations could give rise to false positive results due to urea autolysis; when a long incubation is expected, incubate also an un-inoculated tube to verify the occurrence of urea autolysis.
- Even if the microbial colonies are differentiated on the basis of urea hydrolysis, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Urea supplement
Store the product in the original package at +2°C /+8°C away from direct light.
According to MacFaddin, the tubed medium prepared by the user can be stored at +2°C/+8°C for up to 6-8 weeks.[5]
Ready-to-use medium in tubes
Store tubes in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Christensen WB. J Bact 1946; 52:461-466
2. Stuart CA, Von Stratum E, Rustigian R. J Bact 1945; 48:437
3. ISO 6579-1:2017 Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.
4. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 07/2020
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Public Health England. UK Standards for Microbiology Investigations. Urease test. TP 36, Issue n° 4, 04/2019.

PACKAGING

Product	Type	REF	Pack
Urea Agar Base (Christensen)	Dehydrated medium	4021752	500 g (23.8 L)
Urea 40% Solution	Liquid supplement	42211601 4240096	50 mL 10 x 5 mL
Urea Agar	Ready-to-use tubes	552175	20 glass tubes with slanted medium, 17x125 mm

IFU rev 3, 2022/02

UREA BROTH BASE (STUART)
UREA 40% SOLUTION
Dehydrated culture medium and supplement

Urea Broth – from left:
uninoculated tube, *P. vulgaris*, *E. coli*

INTENDED USE
In vitro diagnostics. Basal medium and urea supplement for the determination of urease enzyme as an aid for the differentiation of members of the *Enterobacteriaceae* family.

COMPOSITION
UREA BROTH BASE (STUART)
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Yeast Extract	0.10 g
Potassium Dihydrogen Phosphate	9.10 g
Disodium Hydrogen Phosphate	9.50 g
Phenol Red	0.01 g

UREA 40% SOLUTION (VIAL CONTENT)

	REF 42211601	REF 4240096
Urea	20 g	2 g
Purified water	50 mL	5 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Urea Broth Base, prepared according to the formulation of Rustigian and Stuart[1], is a medium for the determination of the urease enzyme (urea amidohydrolase), as an aid for the differentiation of members of the *Enterobacteriaceae*. The medium provides positive results for *Proteus* spp., most *Morganella* species and some strains of *Providencia stuartii*.[2] Urea Broth is mainly used to differentiate *Proteus* spp. (urease positive) from *Salmonella* spp. and *Shigella* spp. (urease negative) and other enterobacteria that hydrolyse urea slowly and are negative on this medium.
Yeast extract at low concentrations (0.01%) provides the essential elements required for the growth of *Proteus* strains that are highly urease-producing and use ammonium ions, produced by urea hydrolysis, as their sole source of nitrogen; enterobacteria that hydrolyse urea slowly can only rely on the limited concentration of yeast extract for growth and thus test negative for urea on this medium. In addition, the strong buffer system tends to reduce pH changes in the presence of low levels of ammonium ion production.[3]
Urea, added to the base medium, is hydrolysed by the microorganisms with the formation of ammonium ions and subsequent alkaline reaction that induces the purple-red turn of phenol red when the pH of the medium exceeds 8.1.[3]

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 18.7 g in 950 mL of cold purified water. Heat to dissolve and sterilize by autoclaving at 121°C for 15 minutes. Cool to approximately 47-50°C and add, under aseptic conditions, 50 mL of Urea 40% Solution (REF 42211601). Mix well and dispense the complete medium in quantities of 3-5 mL into sterile tubes.

PHYSICAL CHARACTERISTICS
Urea Broth Base (Stuart)

Dehydrated medium appearance	pinkish, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	orange, limpid
Final pH at 20-25 °C	6.8 ± 0.1

Urea 40% Solution

Solution appearance	colourless, limpid

SPECIMENS
Urea Broth, supplemented with urea solution, shall not be used for the direct inoculation of clinical specimens. The samples consist of isolates from pure culture grown on solid medium.

TEST PROCEDURE
Inoculate the broth heavily with 3 loopfuls (2 mm loop) from an 18-24 h pure culture obtained on TSI or another appropriate medium. Shake the tube gently to suspend the colonies. Incubate the tubes with loosened caps at 35-37°C in an incubator or water bath for 8-48 hours. Examine broths for colour change at 2, 4, 6, 18, 24, and 48 hours of incubation.

READING AND INTERPRETATION
After incubation, observe the colour change of the medium.
The positive test (urea hydrolysis) is indicated by a bright pink (fuchsia) colour.
The negative test is indicated by the unchanged colour of the medium.
Proteus spp. induce rapid alkalinization of the medium. *P. vulgaris* and *P. mirabilis* are positive after about 8 hours of incubation, *P. rettgeri* after about 12 hours; *M. morganii* may require up to 36 hours of incubation and in case of interpretation doubts compare the colour with a non-inoculated tube or incubate for further 24 hours.
However, within 48 hours of incubation these strains will develop a positive reaction.[2]
Bacteria with low and delayed urease activity (e.g., *Enterobacter*) will not test positive for urease due to the high buffering capacity of the medium. Once the test has been recorded as positive, discard the tubes without prolonging the incubation.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.
Urease positive control: *P. vulgaris* ATCC 9484
Urease negative control: *E. coli* ATCC 25922
Incubation: 35-37°C for 18-24 hours

LIMITATIONS OF THE METHOD

- Urea Broth a highly buffered medium that requires large amounts of ammonia to raise the pH to 8.1 resulting in a colour change. Slowly and weakly urease-positive strains, due to the low concentration of yeast extract and a strong buffering system, appear as urease negative on Urea Broth (Stuart).[2,3]
- Purple-red turning occurs when the pH reaches 8.1; inoculation significantly affects the time required by the bacterial strain to develop these alkalinity values and thus a positive reaction.[3]
- The rate of urease reaction is also affected by the volume of liquid medium in the tube; Stuart et al.[1] report that with increasing volumes of 1.5 mL, 3 mL, 4.5 mL, 6 mL, for the same inoculum, the time of development of the positive reaction increases and that the minimum volume for the test is 1.5 ml.
- Urea Broth tubes are not suitable for quantitative evaluation of urea hydrolysis.
- Even if the microbial colonies are differentiated on the basis of urea hydrolysis, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Dehydrated medium
Store at 10-30°C away from direct light in a dry place.
Urea supplement
Store the product in the original package at +2°C /+8°C away from direct light.
According to MacFaddin, the tubed medium prepared by the user should be used within 48 hours.[3]

REFERENCES

1. Rustigian R, Stuart A. Decomposition of urea by *Proteus*. Proc Soc Exp Biol Med. 1941; *47*:108-112
2. Public Health England. UK Standards for Microbiology Investigations. Urease test. TP 36, Issue n° 4, 04/2019
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Urea Broth Base (Stuart)	Dehydrated medium	4021802	500 g (26.7 L)
Urea 40% Solution	Liquid supplement	42211601 4240096	50 mL 10 x 5 mL

IFU rev 1, 2022/02

UREAPLASMA DIFFERENTIAL AGAR A7

Ready-to-use plates

INTENDED USE

In vitro diagnostic device. Selective medium for the cultivation and isolation of *Ureaplasma urealyticum* and other members of the genus *Ureaplasma* from clinical specimens and for their differentiation from *Mycoplasma* spp.

COMPOSITION - TYPICAL FORMULA *

Pancreatic digest of casein	17.0 g
Soy peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Manganese sulphate	0.2g
Phenol red	0.002 g
Agar	14 g
Purified water	825 mL
Horse serum	200 mL
L-cysteine HCl 4% solution	2.5 mL
Biovitex ^	5 mL
Yeast extract 25% solution	10 mL
Urea 10% solution	10 mL
Antibiotic mix	17.5 mL

^ For Biovitex formulation see REF 42185011
* The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Following puberty, *Ureaplasma* spp., and *M. hominis* can be isolated from the lower genital tract in many healthy sexually active adults, but there is evidence that these organisms play etiological roles in some genital tract diseases.[1]
Ureaplasma Differential Agar A7, prepared according to a modification of the formulation described by Shepard and Lunceford[2,3], is used for the cultivation and isolation of *Ureaplasma urealyticum* and other species of the genus *Ureaplasma,* especially from urogenital samples and for their differentiation from *Mycoplasma hominis*.
Peptones, glucose, yeast extract, and horse serum supply the nutrients necessary for the growth of mollicutes. The addition of L-cysteine and Biovitex, a mixture of amino acids and vitamins, enhance the growth of *Ureaplasma* and Mycoplasma species. Urea, manganese sulphate and phenol red constitute the indicator and differential systems of the medium. Ammonia produced by *U. urealyticum*, by means of the urease enzyme, is detected by manganese indicator that produces a stable reaction product (manganese dioxide) which develops in and on the surface of individual colonies. Moreover, the alkaline pH induces the colour change of the phenol red pH indicator: as a consequence, the medium surrounding the *Ureaplasma* colonies changes to red. *Mycoplasma hominis* does not hydrolyse urea, fail to change the medium pH and grows with large colourless colonies. The antibiotic mixture inhibits most Gram-negative and Gram-positive bacteria, which may be present in the samples.

PHYSICAL CHARACTERISTICS

Medium appearance	Amber, limpid
Final pH at 20-25°C	6.1 ± 0.2

MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, jars and reagents for incubation in anaerobic / CO_2 conditions, ancillary culture media and reagents for the identification of the colonies.

SPECIMENS

Ureaplasma Differential Agar A7 is used for the bacteriological processing of clinical samples such as urines, vaginal and urethral specimens.[2,3,] Collect clinical specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[1]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Centrifuge the urine specimen and inoculate 0,03 mL drops of sediment. Several specimens per plate may be used.
The urethral and vaginal exudates must be inoculated directly by smearing on a small area of the agar surface.
Allow inocula to be absorbed into the medium before incubation.
Incubate inoculated plates in an atmosphere of air supplemented with 5-10% CO_2 or in anaerobic environment of 95% N_2 plus 5% CO_2; anaerobic jars with catalyst and disposable generator envelops are adequate if dedicated incubator is not available.[1]
Incubate at 35-37°C for 2-4 days. In certain cases, it may be necessary to prolong incubation.[1]

READING AND INTERPRETATION

After incubation, observe the plates macroscopically and under a microscope with direct light at low magnification (100 X), mainly at the edges of the inoculated area; record each specific morphological and chromatic characteristic of the colonies.
U. urealyticum is urease positive, grows with dark-golden or brown colonies with the typical "sea urchin" morphology. The area around the colonies turns to light red.
Mycoplasma spp. usually demonstrated a fried-egg appearance, are un-reactive and fail to produce the manganese dioxide, since they are urease negative.
Plates with characteristic growths should be subjected to confirmatory testing with appropriate techniques.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
U. parvum ATCC 27813	35-37°C / up to 6 days / AN	brown colonies
M. hominis ATCC 15488	35-37°C / up to 6 days / CO_2	fried-egg colourless colonies
E. faecalis ATCC 29212	35-37°C / up to 6 days / CO_2	inhibited

AN: anaerobic incubation; ATCC is a trademark of American Type Culture Collection;

LIMITATIONS OF THE METHOD

- Uniform circular "fried egg" appearance of *M. hominis* colonies is more characteristic of laboratory which is not always seen in primary clinical isolated strains. [3]
- Larger numbers of *U. urealyticum* are generally isolated in primary agar cultures from urethral exudates than from a urine specimen from the same urethritis patient.[3]
- A specific transport medium for *U. urealyticum* should be used for the transport of the specimens.[3]
- Unusual growth of *U. urealyticum* on A7 such as growth in association with colonies of other species of mycoplasmas, and growth associated with single or grouped urethral epithelial cells, urethral threads, and exudates are not artifacts.[3]
- If the medium is cut during inoculation of a clinical specimen, often *Ureaplasm*a organisms are entrapped and subsequent urease activity produces intense streaks of manganese reaction product in the agar.[2]
- If dark brown manganese accretion colonies are examined under low power by indirect transmitted or oblique light, colonies appear white.[2]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Waites KB, Bébéar C. Mycoplasma and Ureaplasma. *In* Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
2. Shepard MC, Lunceford, CD. Differential agar medium (A7) for identification of Ureaplasma urealyticum (human T mycoplasmas) in primary cultures of clinical material. J Clin Microbiol 1976; 3(6): 613-625
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Ureaplasma Differential Agar A7	Ready-to-use plates	542181	2 x 10 plates ø 90 mm

IFU rev 1, 2021/01

VANCOMYCIN SCREEN AGAR

Ready-to-use plates

Vancomycin Screen Agar:
Vancomycin resistant *Enterococcus faecalis*

INTENDED USE

In vitro diagnostic device. Screen agar for the detection of presumptive resistance of enterococci to vancomycin and presumptive reduced susceptibility of *Staphylococcus aureus* to vancomycin.

COMPOSITION -TYPICAL FORMULA *

Brain heart infusion and peptones	27.5 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	2.5 g
Agar	15.0 g
Vancomycin	6.0 mg
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Enterococci are common cause of urinary tract infections, intra-abdominal and intra-pelvic abscesses or post-surgery wound infections and blood-stream infections. The emergence and spread of resistant enterococci, particularly vancomycin resistant enterococci (VRE), represents a major public health problem. Glycopeptide resistance is mediated by different Vancomycin resistance (*Van*) gene operons: at least nine acquired resistance types and an additional type (*Van*C) that is intrinsic to *E. casseliflavus* and *E. gallinarum*.[1]
Staphylococcus aureus is a virulent microorganism responsible for many serious infections among the general population. The first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was identified by Hiramatsu *et al*. in 1997[2], and these strains have now been reported worldwide.
The Clinical and Laboratory Standards Institute (CLSI), recommends Brain Hearth Infusion Agar supplemented with 6 mg/L of vancomycin as a screening test for the presumptive detection of pure cultures of vancomycin resistant enterococci and of *S. aureus* strains with a reduced susceptibility to vancomycin.[3]
Vancomycin Screen Agar contains brain heart infusion and peptones as sources of nitrogen, carbon, vitamins and minerals for microbial growth; glucose provides an energy source, sodium chloride maintains osmotic balance, disodium hydrogen phosphate is included as a buffer system; vancomycin at the concentration of 6 mg/L is used to detect the resistance to vancomycin.

PHYSICAL CHARACTERISTICS

Prepared plates appearance	pale yellow, limpid
Final pH at 20-25°C	7.4 ± 0.2

SPECIMENS

Vancomycin Screen Agar is inoculated with pure cultures of clinical isolates, presumptively identified as enterococci or *S. aureus*. It is not intended as primary isolation medium for clinical specimens.

TEST PROCEDURE

Allow plates to come to room temperature before inoculation.
Select several well isolated colonies from a non-selective agar plate and prepare a suspension in Tryptic Soy Broth equivalent to 0.5 McFarland standard.
Preferably, using a micropipette, spot 1-10 µL drop (enterococci) or 10 µL drop (*S. aureus*) onto Vancomycin Screen Agar plate. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10-15 mm in diameter or streak a portion of the plate.
A blood agar plate should be also inoculated as a growth control, to check strain viability and purity.
Invert the plate and incubate at 35 ± 2°C, aerobically for a full 24 hours.

READING AND INTERPRETATION

Examine the incubated plates carefully with transmitted light.
Observe control plate for growth and purity that indicates viable test organisms in the inoculum.
Examine carefully the Vancomycin Screen Agar plate for growth.
Enterococci with a presumptive resistance to vancomycin: presence of > 1 colony.
S. aureus with a presumptive reduced susceptibility to vancomycin: presence of > 1 colony or light film of growth.
Additional testing and reporting (enterococci)
Perform vancomycin MIC on *Enterococcus* spp. that grow on Vancomycin Screen Agar and test for motility and pigment production to distinguish species with acquired resistance (e.g. *van*A and *van*B) from those with intrinsic intermediate-level resistance to vancomycin (e.g. *van*C), such as *E. gallinarum* and *E. casseliflavus* which often grow on the medium. In contrast to other enterococci, *E. gallinarum* and *E. casseliflavus* with vancomycin MICs of 8-16 µg/mL (intermediate) differ from vancomycin-resistant enterococci for infection prevention purposes.
Additional testing and reporting (*S. aureus*)
Perform a vancomycin MIC using a validated MIC method to determine vancomycin MICs on *S. aureus* that grows on Vancomycin Screen Agar.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[3]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. faecalis (VR) ATCC 51299	33-37°C / 24 h / A	growth
E. faecalis ATCC 29212	33-37°C / 24 h / A	no growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Testing on Vancomycin Screen Agar doesn't reliably detect all vancomycin-intermediate *S. aureus* strains: some strains for which the vancomycin MICs are 4µg/mL will fail to grow.[3]
- Perform vancomycin MICs with a reliable method on *Enterococcus* spp. and *S. aureus* that grow on Vancomycin Screen Agar for resistance confirmation and for determining the precise concentration of vancomycin to which the isolate is resistant.
- *E. gallinarum* and *E. casseliflavus,* with an intrinsic intermediate level resistance to vancomycin, often grow on the medium.[3]
- There are insufficient data to recommend the use of this medium for the detection of vancomycin resistant strains other than enterococci and *S. aureus*.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Orsi GB, Ciorba V. Vancomycin resistant enterococci healthcare associated infections. Ann Ig 2013; 25: 485-492
2. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J. Ant Chem 1997;40(1):135–6
3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 30th ed. CLSI supplement M100-S30. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

PACKAGING

Product	Type	REF	Pack
Vancomycin Screen Agar	Ready-to-use plates	549520	2 x 10 plates ø 90 mm

IFU rev 1, 2020/11

VEGETABLE PEPTONE BROTH

Dehydrated culture medium

INTENDED USE

General purpose medium with non-animal origin peptones, used as an alternative to Tryptic Soy Broth for the cultivation of non-fastidious and moderately fastidious microorganisms.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptones (non-animal origin)	20.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Vegetable Peptone Broth is a non-animal origin alternative to Soybean Casein Digest Medium also known as Tryptic Soy Broth. The use of raw materials of non-animal origin allows the elimination of the potential contamination risk with TSE/BSE.
In the pharmaceutical industry it may be used for sterility test and as an enrichment medium in the procedures for detection of target organisms.
In clinical microbiology Vegetable Peptone Broth is used for suspension, enrichment and cultivation of microbial strains isolated on other culture media and for the preparation of inocula in quality control test procedures.
This medium complies with the performance requirements of the harmonized chapters of the European, United States and Japanese Pharmacopoeias.[1] Vegetable Peptone Broth has the same formulation of Soybean Casein Digest Medium as described by harmonized method of European Pharmacopoeia[1] but contains peptones of non-animal origin instead of the recommended peptones.
Peptones and glucose are sources of nitrogen, carbon, vitamins and trace elements needed for the growth of most non-fastidious and moderately fastidious microorganisms (bacteria, yeasts, fungi). Sodium chloride maintains osmotic balance, dipotassium hydrogen phosphate is included as a buffer system.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified water. Mix carefully without heating until the medium is completely dissolved and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes or by filtration.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Final pH at 20-25°C	7.3 ± 0.2

SPECIMENS

Vegetable Peptone Broth should not be used for direct inoculation of clinical specimens. In clinical microbiology the specimens consist of microbial colonies grown on other culture media. In pharmaceutical microbiology, samples consist of products on which to perform the sterility test or the detection for specific microorganisms. Refer to the European Pharmacopoeia for sample collection and transport procedures.[1]

TEST PROCEDURE

Use the medium according to the purpose required.
With a bacteriological needle or loop inoculate the liquid medium in a test tube or bottle with a colony grown on another isolation medium. Incubate at the temperature and for the time required by laboratory procedures. Usually, an incubation temperature of 35 ± 2° C for 18-24 is adequate for cultivation of common aerobes and facultative anaerobes. For sterility testing and for use of Vegetable Peptone Broth as a pre-enrichment medium for the detection of specific microorganisms in pharmaceutical products, consult the European Pharmacopoeia.[1]

READING AND INTERPRETATION
The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[1]

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	20-25°C / 24-72h -A	good growth
A. brasiliensis ATCC 16404	20-25°C / 72-120h -A	good growth
B. subtilis ATCC 6633	30-35°C / 24 h -A	good growth
S. aureus ATCC 6538	30-35°C / 24 h -A	good growth
P. aeruginosa ATCC 9027	30-35°C / 24 h -A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Vegetable Peptone Broth is not suitable for the cultivation of fastidious microorganisms (e.g., *Haemophilus* or *Neisseria* spp.) and for the cultivation of strict anaerobes.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES
1. European Pharmacopoeia 11th Edition, 2022, Vol. 1.

PACKAGING
Product	Type	REF	Pack
Vegetable Peptone Broth	Dehydrated medium	4030012 4030014	500 g (16.7 L) 5 kg (167 L)

IFU rev 1, 2022/12

VEGETABLE PEPTONE BROTH γ IRRADIATED
Dehydrated culture medium

INTENDED USE
General purpose medium with non-animal origin peptones, for the cultivation of non-fastidious microorganisms. Cold filterable and gamma-irradiated. For the microbiological validation of aseptic filling processes.

COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Peptones (non-animal origin)	20.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Vegetable Peptone Broth is a non-animal origin alternative to Soybean Casein Digest Medium also known as Tryptic Soy Broth. The use of raw materials of non-animal origin allows the elimination of the potential contamination risk with TSE/BSE.
Vegetable Peptone Broth Irradiated is sterilized by gamma rays with a dose of at least 25 kGy, is cold filterable and triple bagged. In the pharmaceutical industry it is used for monitoring microbial contamination in sterile production lines during media fill tests.
This medium complies with the performance requirements of the harmonized chapters of the European, United States and Japanese Pharmacopoeias.[1]
Vegetable Peptone Broth has the same formulation of Soybean Casein Digest Medium as described by harmonized method of European Pharmacopoeia[1] but contains peptones of non-animal origin instead of the recommended peptones.
Peptones and glucose are sources of nitrogen, carbon, vitamins and trace elements needed for the growth of most non-fastidious and moderately fastidious microorganisms (bacteria, yeasts, fungi). Sodium chloride maintains osmotic balance, dipotassium hydrogen phosphate is included as a buffer system.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 30 g in 1000 mL of cold purified water. Mix carefully without heating until the medium is completely dissolved. Sterilize by membrane filtration or use as required.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Final pH at 20-25°C	7.3 ± 0.2
Gamma-irradiation	25 - 40 kGy

TEST PROCEDURE
Use the medium according to the purpose required. For “media fill” test[2] the culture medium is used in place of the product solution to test whether the aseptic procedures are adequate to prevent contamination during production process. A media fill is one part of the validation of an aseptic manufacturing process. After the final product container is filled and ready for release, it should be incubated in a temperature-controlled incubator. Any controlled temperature between 20 and 35° C would work for media fills. However, the “controlled temperature” should be specified in the procedures and be maintained within a range that does not exceed ± 2.5°C. The incubation period of a media fill should be no less than 14 days and the containers should be examined every 2 or 3 days. If different incubation temperatures are chosen, it is recommended to incubate the containers filled with the medium for at least 7 days at the lowest temperature (e.g., 20- 25°C) and then 7 at the highest temperature (e.g., 30 - 35 ° C).

READING AND INTERPRETATION
The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[1]

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	20-25°C / 24-72h -A	good growth
A. brasiliensis ATCC 16404	20-25°C / 72-120h -A	good growth
B. subtilis ATCC 6633	30-35°C / 24 h -A	good growth
S. aureus ATCC 6538	30-35°C / 24 h -A	good growth
P. aeruginosa ATCC 9027	30-35°C / 24 h -A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Vegetable Peptone Broth Irradiated is not suitable for the cultivation of fastidious microorganisms (e.g., *Haemophilus* or *Neisseria* spp.) and for the cultivation of strict anaerobes.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- Due to the wide variety of production processes and devices to be examined with "media fill" test, it is the user's responsibility to validate this medium for the specific intended use.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. European Pharmacopoeia 11th Edition, 2022, Vol. 1.
2. FDA Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice

PACKAGING
Product	Type	REF	Pack
Vegetable Peptone Broth γ Irradiated	Dehydrated medium	403001G2 403001G4	500 g (16.7 L) Gamma-irradiated, triple bagged 5 kg (167 L) Gamma-irradiated, triple bagged

IFU rev 1, 2022/11

VIOLET RED BILE AGAR (VRBL)
Dehydrated and ready-to-use culture media

INTENDED USE
For the detection and enumeration of coliform bacteria in food, animal feed and environmental samples

COMPOSITION*
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)
DEHYDRATED MEDIUM, READY-TO-USE PLATES, FLASKS AND TUBES

Peptone	7.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Bile salts No.3	1.5 g
Lactose	10.0 g
Neutral red	30.0 mg
Crystal violet	2.0 mg
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Coliforms are a group of closely related, mostly harmless bacteria that live in soil and water as well as the gut of animals. Coliforms count is a hygienic indicator and high level of coliform counts generally indicates unsanitary condition or poor hygiene practices during or after food production.
Violet Red Bile Agar, designed for the enumeration of bacteria of the coli-aerogenes group, is derived from MacConkey's[1] original formula. Violet Red Bile Agar is also known as Violet Red Bile Lactose (VRBL) Agar or Crystal Violet Neutral Red Bile Lactose Agar.
VRBL Agar is recommended by ISO 4832[2] for the detection and the enumeration of coliform bacteria, when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample and by FDA BAM for the enumeration of coliforms with a solid medium method.

In VRBL Agar, essential growth factors are provided by peptone and yeast extracts which are sources of nitrogen, carbon, vitamins and minerals; sodium chloride maintains the osmotic balance. The medium relies on the use of the selective inhibitory components crystal violet and bile salts which suppress the growth of Gram-positive bacteria and the indicator system lactose and neutral red.[3] Organisms which rapidly attack lactose produce purple colonies often surrounded by purple halos. Non-fermenters and late lactose fermenters exhibit pale or colourless colonies. Some Gram-negative bacteria other than *Enterobacteriaceae* may grow but may be limited by the overlay procedure.

Directions for medium preparation (dehydrated medium)

Suspend 41.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave and do not overheat. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

Directions for medium preparation (ready-to-use flasks and tubes)

Liquefy the contents of the flask in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes under aseptic conditions.

Physical characteristics

Dehydrated medium appearance	grey-violet, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	violet, clear
Final pH at 20-25 °C	7.4 ± 0.2

Specimens

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[2]

Test procedure

1. Prepare the test portion, initial suspension (primary dilution) and further dilutions in accordance with the specific International Standard appropriate to the product concerned.
2. Using a sterile pipette, transfer in the centre of two Petri dishes 1 mL of the test sample if the product is liquid, or 1 mL of the initial suspension in case of other products. Repeat the procedure described with the further dilutions.
3. Pour approximately 15 mL of the VRBL Agar into each Petri dish.
4. Carefully mix the inoculum with the medium and allow the medium to solidify, with the Petri dishes standing on a cool horizontal surface. If required, add a covering layer of approximately 5 mL to 10 mL of VRBL Agar to prevent spreading growth and to achieve semi-anaerobic conditions. Allow to solidify.
5. Invert the prepared dishes and incubate them at 30°C or 37°C for 24 h ± 2 h.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical coliforms colonies are pink to red or purple (with or without precipitation haloes).
Count the colonies in the Petri dishes with a number of colonies between 10 and 150.
Perform the confirmation tests in accordance with the specific International Standard appropriate to the product concerned.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 25922	30°C/24H-A	good growth, pink-red colonies with red halo
P. aeruginosa ATCC 27853	30°C/24H/A	colourless colonies
E. faecalis ATCC 19433	30°C/24H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the methods

- Occasionally enterococci grow on this medium; however, the colonies are pinpoint. If in doubt perform a Gram staining and a catalase test (Gram-positive cocci, catalase-negative).[4]
- Medium is not completely specific for enterics; other accompanying bacteria may give the same reactions. Further biochemical tests are necessary for positive identification.[4]
- Medium selectivity diminishes after 24 of incubation and organisms previously suppressed may exhibit growth.[4]
- Colonies of dubious colour can be expected on the medium in particular when dairy products containing sugars other than lactose are examined; in this case, the conversion of these sugars can give rise to colonies with an appearance similar to that of typical coliforms.[2]

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 4832, medium for the poured plate technique must be used within 4 hours after its preparation.[2]
Ready-to-use plates and flasks
Store plates and flasks in their original pack at +2°C /+8°C away from direct light.

References

1. MacConkey A. Lactose fermenting bacteria in faeces. J Hyg 1905; 5:333-379
2. ISO 4832-1:2006. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique.
3. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:282-284.
4. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Violet Red Bile Agar (VRBL)	Dehydrated medium	4021852 4021854	500 g (12 L) 5 Kg (120 L)
Violet Red Bile Agar	Ready-to-use medium in plates	542185	2 x 10 plates ø 90 mm
Violet Red Bile Agar	Ready-to-use medium in flasks	5121852 5121853	6 x 100 mL 6 x 200 mL

IFU rev 1, 2022/08

VIOLET RED BILE AGAR MUG

Dehydrated culture medium

INTENDED USE

For the detection and enumeration of coliform bacteria and *Escherichia coli* in food, animal feed and environmental samples.

COMPOSITION - TYPICAL FORMULA*
(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	7.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Bile salts No.3	1.5 g
Lactose	10.0 g
Neutral red	30.0 mg
Crystal violet	2.0 mg
4-methylumbelliferyl- β-D-glucuronide (MUG)	100.0 mg
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The presence of *E. coli* in food or water is accepted as indicative of recent faecal contamination and the possible presence of pathogens, while coliforms are used as an indicator of sanitary condition in the food-processing environment.
Violet Red Bile (VRBL) Agar, designed for the enumeration of bacteria of the coli-aerogenes group, is derived from MacConkey's[1] original formula. Trepeta and Edberg[2] modified the classical MacConkey Agar by incorporating the fluorogenic compound 4-methylumbelliferyl- β-D-glucuronide (MUG), for the rapid detection of *E. coli*, according to the preliminary studies of Dahlen and Linde[3] and of Kilian and Bulow[4].
VRBL Agar supplemented with MUG is recommended by FDA-BAM[5] for the enumeration of coliforms and *E. coli* in foodstuffs, with the poured plate technique.
In VRBL Agar MUG, essential growth factors are provided by peptone and yeast extracts which are sources of nitrogen, carbon, vitamins and minerals; sodium chloride maintains the osmotic balance; sodium chloride maintains the osmotic balance. The medium relies on the use of the selective inhibitory components crystal violet and bile salts which suppress the growth of Gram-positive bacteria and the indicator system lactose and neutral red.[6] Organisms which rapidly attack lactose produce purple colonies often surrounded by purple halos. Non-fermenters and late lactose fermenters exhibit pale or colourless colonies. The presence of MUG enables the enumeration of *E. coli* colonies by distinguishing them within the typical coliform colonies. MUG is cleaved by β-D-glucuronidase produced by *E. coli* to 4-methylumbelliferone and glucuronide; the fluorogenic 4-methylumbelliferone can be detected directly by using a long-wave ultraviolet light.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave and do not overheat. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green-violet, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	violet, clear
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[5]

TEST PROCEDURE

1. Prepare the test portion, initial suspension (primary dilution) and further dilutions in accordance with the specific International Standard appropriate to the product concerned.
2. Using a sterile pipette, transfer in the centre of two Petri dishes 1 mL of the test sample if the product is liquid, or 1 mL of the initial suspension in case of other products. Repeat the procedure described with the further dilutions.
3. Pour approximately 15 mL of the VRBL Agar MUG into each Petri dish.
4. Carefully mix the inoculum with the medium and allow the medium to solidify, with the Petri dishes standing on a cool horizontal surface. If required, add a covering layer of approximately 5 mL to 10 mL of VRBL Agar MUG to prevent spreading growth and to achieve semi-anaerobic conditions. Allow to solidify
5. Invert the prepared dishes and incubate them at 35°C for 24 h ± 2 h.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical coliforms colonies are pink to red or purple (with or without precipitation haloes).
Typical *E. coli* colonies are pink to red or purple, with precipitation haloes and exhibit a bluish fluorescence under a long-wave ultraviolet light (Wood's lamp). Perform the confirmation tests in accordance with the specific International Standard appropriate to the product concerned.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli ATCC 25922	35°C/24H-A	good growth, pink-red colonies with red halo, fluorescent under Wood's Lamp
E. aerogenes ATCC 13048	35°C/24H/A	good growth, pink-red colonies, not fluorescent under Wood's lamp
E. faecalis ATCC 19433	35°C/24H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- Occasionally enterococci grow on this medium; however, the colonies are pinpoint. If in doubt perform a Gram staining and a catalase test (Gram-positive cocci, catalase-negative).[7]
- Medium is not completely specific for enterics; other accompanying bacteria may give the same reactions. Further biochemical tests are necessary for positive identification.[7]
- Medium selectivity diminishes after 24 of incubation and organisms previously suppressed may exhibit growth.[7]
- Colonies of dubious colour can be expected on the medium in particular when dairy products containing sugars other than lactose are examined; in this case, the conversion of these sugars can give rise to colonies with an appearance similar to that of typical coliforms.[8]
- It has been reported that approximately 40% of *Shigella* species, various bio-serotypes of *Salmonella* (13% of *Salmonella* subgenus I) may be β-glucuronidase positive and fluorescent under Wood's Lamp; only exceptionally this test is positive with *Providencia, Enterobacter* and *Yersinia* strains (1-5%).[9]
- Approximately 3-4% of *E. coli* are β-glucuronidase negative, notably *E. coli* O157 strains.[9]
- Up to 10% of *E. coli* isolates have been reported to be slow or non-lactose fermenting.[10]

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 4832, the medium for the poured plate technique must be used within 4 hours after its preparation.[8]

REFERENCES

1. MacConkey A. Lactose fermenting bacteria in faeces. J Hyg 1905; 5:333-379
2. Trepeta RW, Edberg SC. Methylumbelliferyl- D-glucuronide-based medium for rapid isolation and identification of E. coli. J Clin Microbiol 1984; 19 :172.
3. Dahlén G, Linde A. Screening plate method for detection of bacterial beta-glucuronidase. Appl Microbiol 1973;26 (6): 863-6
4. Killian M, Bulow P. 1976. Rapid diagnosis of Enterobacteriaceae. Detection of bacterial glycosidases. Acta Pathol Microbiol Scand Sec B 1976; 84:245-251.
5. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 4: Enumeration of Escherichia coli and the Coliform Bacteria Rev 10/2020
6. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:282-284.
7. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
8. ISO 4832-1:2006. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique
9. Robison, B.J. 1984. Evaluation of a fluorogenic assay for detection of Escherichia coli in foods. Appl. Environ. Microbiol. 48:285-288
10. Gokul Yaratha, MD, Sarah Perloff, DO, Kinesh Changala, MBBS. Lactose vs non-lactose fermenting E. coli: Epidemiology, Clinical Outcomes, and Resistance. Open Forum Infect Dis 2017; V4 (Suppl 1).

PACKAGING

Product	Type	REF	Pack
Violet Red Bile Agar MUG	Dehydrated medium	4021862	500 g (12 L)

IFU rev1, 2022/08

VIOLET RED BILE GLUCOSE (VRBG) AGAR

Dehydrated and ready-to-use culture media

INTENDED USE

For the detection and enumeration of *Enterobacteriaceae* in food, animal feed and environmental samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES, TUBES, FLASKS

Peptone	7.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Bile salts No.3	1.5 g
Glucose	10.0 g
Neutral red	30.0 mg
Crystal violet	2.0 mg
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Enterobacteriaceae are usually considered by food manufacturers as hygiene indicators and thus used to monitor the effectiveness of preventive measures taken. This is also reflected in several national and international Standards or criteria in which *Enterobacteriaceae* are included as hygiene indicators.
Violet Red Bile Glucose (VRBG) Agar was designed by Mossel[1] for the enumeration of *Enterobacteriaceae,* by adding glucose to Violet Red Bile Lactose Agar. Later works of Mossel *et al.*[2,3] demonstrated that lactose could be omitted, resulting in the formulation known as VRBG Agar. Violet Red Bile Glucose Agar is recommended by ISO 21528-1[4] for the detection and the enumeration with a pre-enrichment step and with the MPN technique of *Enterobacteriaceae,* when the microorganisms sought are expected to need resuscitation, and when the number sought is expected to be below 100 per millilitre or per gram of test sample. It is recommended by ISO 21528-2[5] for the enumeration of *Enterobacteriaceae* with pour plate technique, when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample.

Peptone provides essential growth factors for bacterial growth; yeast extract is a source of B-vitamins complex for growth stimulation; sodium chloride maintains the osmotic balance. The medium relies on the use of the selective inhibitory components crystal violet and bile salts which suppress the growth of Gram-positive bacteria and the indicator system glucose and neutral red. The dissimilation of glucose causes acidification of the medium, with the consequent precipitation of bile salts and neutral red uptake. The *Enterobacteriaceae* grow with red-pink to red-violet colonies surrounded by a red precipitation zone. Non-glucose fermenters (e.g., *Pseudomonas, Acinetobacter, Alcaligenes* etc.) exhibit transparent, colourless colonies. Some Gram-negative bacteria other than *Enterobacteriaceae* may grow but may be limited by the overlay procedure.

Directions for medium preparation (dehydrated medium)

Suspend 41.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave and do not overheat. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

Directions for medium preparation (ready-to-use flasks/tubes)

Liquefy the contents of the flask/tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle or the tube may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes under aseptic conditions.

Physical characteristics

Dehydrated medium appearance	green-violet, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	violet, clear
Final pH at 20-25 °C	7.4 ± 0.2

Specimens

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling. Consult the appropriate references for sample collection, storage and preparation.[4,5]

Test procedure

Detection of *Enterobacteriaceae* after pre-enrichment.[4]

Inoculate VRBG Agar plates with a loop from each of the incubated cultures obtained after enrichment in Buffered Peptone Water.
Incubate the plates at 37 ± 1 °C for 24 h ± 2 h

Poured plates enumeration of *Enterobacteriaceae.*[5]

1. Using a sterile pipette, transfer to the Petri dish 1 mL of the test sample if the product is liquid, or 1 mL of the initial suspension in case of other products.
2. Repeat the procedure described with the further dilutions.
3. Add into each Petri dish approximately 15 mL of VRBG Agar.
4. Carefully mix the inoculum with the medium and allow the medium to solidify, with the Petri dishes standing on a cool horizontal surface.
5. After complete solidification of the mixture, add a covering layer of approximately 5 mL to 10 mL of VRBG Agar to prevent spreading growth and to achieve semi-anaerobic conditions. Allow to solidify.
6. Invert the prepared dishes and incubate them at 37 ± 1 °C for 24 h ± 2 h.
7. Count the colonies in the Petri dishes with less than 150 colonies.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Typical *Enterobacteriaceae* colonies are pink to red or purple (with or without precipitation haloes). Select well-isolated colonies from each of the incubated plates for the biochemical confirmation tests: oxidase reaction (-) and glucose fermentation (+).

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[4,5]

Control strains	Incubation T°/ t / ATM	Expected results
E. coli ATCC 25922	37°C/24H-A	good growth, pink-red colonies with red halo
E. faecalis ATCC 19433	37°C/24H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection.

Limitations of the methods

- Occasionally enterococci grow on this medium; however, the colonies are pinpoint. If in doubt perform a Gram staining and a catalase test (Gram-positive cocci, catalase-negative).[6]
- Medium is not completely specific for enterics; other accompanying bacteria may give the same reactions. Further biochemical tests are necessary for positive identification.[6]
- Medium selectivity diminishes after 24 of incubation and organisms previously suppressed may exhibit growth.[6]

Storage conditions

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 21528-1, self-prepared plates to be inoculated on the surface can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up 2 weeks.[4] According to ISO 21528-2 medium for the poured plate technique must be used within 4 hours after its preparation.[5]

Ready-to-use medium in plates, flasks and tubes

Store in their original pack at +2°C /+8°C away from direct light.

References

1. Mossel DAA, Mengerink WH, Scholts HH. Use of a modified MacConkey agar medium for the selective growth and enumeration of Enterobacteriaceae. J Bacteriol. 1962 Aug;84(2):381.
2. Mossel DAA, Eelderink I, Koopmans M, Van Rossem F. Lab Practice 1978; 27:1049.
3. Mossel DAA, Eelderink I, Koopmans M, Van Rossem F. Influence of Carbon Source, Bile Salts and Incubation Temperature on Recovery of Enterobacteriaceae from Foods Using MacConkey-type Agars. J Food Prot 1979 Jun;42(6):470-475.
4. ISO 21528-1:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae.
5. ISO 21528-2:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae —Part 2: Colony-count technique.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING

Product	Type	REF	Pack
Violet Red Bile Glucose (VRBG) Agar	Dehydrated medium	4021882 4021884	500 g (12 L) 5 Kg (120 L)
Violet Red Bile Glucose (VRBG) Agar	Ready-to-use medium in plates	542188	2 x 10 plates ø 90 mm
Violet Red Bile Glucose (VRBG) Agar	Ready-to-use medium in tubes	552188	20 x 15 mL
Violet Red Bile Glucose (VRBG) Agar	Ready-to-use medium in flasks	5121882	6 x 100 mL

IFU rev 3, 2022/08

VIOLET RED BILE GLUCOSE (VRBG) AGAR EP

Dehydrated and ready-to-use culture medium

INTENDED USE

For the detection and enumeration of bile-tolerant Gram-negative bacteria in pharmaceutical products.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE PLATES

Yeast extract	3.0 g
Pancreatic digest of gelatin	7.0 g
Bile salts No.3	1.5 g
Sodium chloride	5.0 g
Glucose monohydrate	10.0 g
Agar	15.0 g
Neutral red	30.0 mg
Crystal violet	2.0 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Violet Red Bile Glucose Agar was designed by Mossel[1] for the enumeration of *Enterobacteriaceae,* by adding glucose to Violet Red Bile Lactose Agar. Later works of Mossel *et al.*[2,3] demonstrated that lactose could be omitted, resulting in the formulation known as VRBG.
Violet Red Bile Glucose (VRBG) Agar EP meets the requirement of European Pharmacopoeia[4] and is recommended for presence/absence test and for the enumeration of bile-tolerant Gram-negative bacteria in non-sterile pharmaceutical products.
Pancreatic digest of casein provides carbon, nitrogen and trace elements for bacterial growth; sodium chloride maintains the osmotic balance. The selective agents of the medium are crystal violet and bile salts n° 3 which inhibits the growth of Gram-positive bacteria. The dissimilation of glucose causes acidification of the medium, with the consequent precipitation of bile salts and neutral red uptake. The *Enterobacteriaceae* grow with red-pink to red-violet colonies often surrounded by a red precipitation zone. Non-glucose fermenters (e.g. *Pseudomonas, Acinetobacter, Alcaligenes* etc.) exhibit transparent, colourless colonies.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 41.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave and do not overheat. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green-violet, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	violet, clear
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

Non-sterile pharmaceutical products. Consult the appropriate reference for sample collection, storage and preparation.[4]

TEST PROCEDURE

Prepare the sample suspension in Tryptic Soy Broth using at least 1 g or 1 mL of sample.
Incubate this suspension at 20°C / 25°C for 2-5 hours to ensure revitalisation but not multiplication of bacteria.

a- Test for absence

Inoculate a quantity of the initial suspension into Enterobacteriaceae Enrichment Broth Mossel EP (REF 401467) to ensure an inoculum of 1 g of sample and incubate at 30°C / 35°C for 24-48 hours
Subculture on plates of VRBG Agar EP and incubate 30°C / 35°C for 18-24 hours.

b- Quantitative test

Inoculate suitable quantities of Enterobacteriaceae Enrichment Broth Mossel EP (REF 401467) with the initial suspension and/or dilution of sample containing respectively 1 g, 0.1 g, 0.01 g and 0.001 g of the product to be examined.
Incubate at 30°C / 35°C for 24-48 hours.
Subculture each of the cultures on a plate of VRBG Agar EP (REF 402189) and incubate 30°C / 35°C for 18-24 hours

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Typical *Enterobacteriaceae* colonies are pink to red or purple (with or without precipitation haloes).
Typical Gram-negative non-glucose fermenters colonies are transparent and colourless.
Test for absence: according to European Pharmacopoeia the product complies with the test if there is no growth of colonies on VRBG Agar EP plates.
Quantitative test: refer to European Pharmacopoeia for the interpretation criteria of probable number of bacteria per g or mL of product.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[4,5]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
E. coli ATCC 8739	30-35°C/18-24H-A	good growth, pink-red colonies with red halo
P. aeruginosa ATCC 9027	30-35°C/18-24H-A	good growth, colourless colonies
E. faecalis ATCC 19433	30-35°C/18-24H-A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- Occasionally enterococci grow on this medium; however, the colonies are pinpoint. If in doubt perform a Gram staining and a catalase test (Gram-positive cocci, catalase-negative).[5]
- Medium is not completely specific for enterics; other accompanying bacteria may give the same reactions. Further biochemical tests are necessary for positive identification.[5]
- Medium selectivity diminishes after 24 of incubation and organisms previously suppressed may exhibit growth.[5]

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to ISO 21528-1, self-prepared plates to be inoculated on the surface can be stored at +2 °C to +8 °C in the dark and protected against evaporation for up 2 weeks.[6]

Ready-to-use plates

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Mossel DAA, Mengerink WH, Scholts HH. Use of a modified MacConkey agar medium for the selective growth and enumeration of Enterobacteriaceae. J Bacteriol. 1962 Aug;84(2):381.
2. Mossel DAA, Eelderink I, Koopmans M, Van Rossem F. Lab Practice 1978; 27:1049.
3. Mossel DAA, Eelderink I, Koopmans M, Van Rossem F. Influence of Carbon Source, Bile Salts and Incubation Temperature on Recovery of Enterobacteriaceae from Foods Using MacConkey-type Agars. J Food Prot 1979 Jun;42(6):470-475.
4. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. ISO 21528-1:2017 Microbiology of the food chain —Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae.

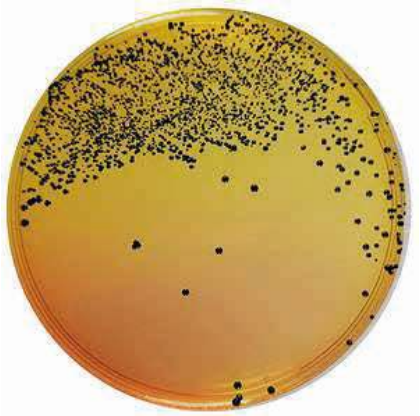
PACKAGING

Product	Type	REF	Pack
Violet Red Bile Glucose (VRBG) Agar EP	Dehydrated medium	4021892	500 g (12 L)
Violet Red Bile Glucose (VRBG) Agar EP	Ready-to-use plates	542189	2 x 10 plates ø 90 mm

IFU rev 1, 2022/11

VOGEL JOHNSON AGAR

Dehydrated culture medium



S. aureus on Vogel Johnson Agar

INTENDED USE

Selective medium for the isolation and differentiation of *Staphylococcus aureus.*

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.000 g
Yeast extract	5.000 g
Mannitol	10.000 g
Dipotassium hydrogen phosphate	5.000 g
Lithium chloride	5.000 g
Glycine	10.000 g
Phenol red	0.025 g
Agar	16.000 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Vogel Johnson Agar is prepared on the basis of the formula described by Vogel and Johnson[1] in 1960, as a modification of Tellurite Glycine Agar of Zebovitz, Evans and Niven[2] of 1955. Vogel and Johnson increased the mannitol content and added phenol red as a pH indicator.

Vogel Johnson Agar is a selective medium for the isolation and differentiation of *S. aureus*. Its use has been described for clinical specimens[3] for the examination of cosmetics[4,5], pharmaceutical products[6], swimming pool water[7], milk[8] and, supplemented with oxacillin, for the detection of methicillin resistant staphylococci, from clinical samples[9].

Tryptone and yeast extract provide nitrogen, carbon, minerals and vitamins for microbial growth. Potassium phosphate prevents pH changes. The selectivity of the medium is due to the presence of lithium chloride and glycine and to the addition of potassium tellurite, which allows a good growth of staphylococci and the inhibition of almost all normal upper respiratory tract flora, mainly within 24 hours of incubation.[3] Mannitol is included as a fermentable carbohydrate: *S. aureus* ferments mannitol producing the acidification around the colony, resulting in a yellow colour change of medium; potassium tellurite forms a black precipitate inside the colonies when reduced by staphylococci to metallic free tellurium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 61 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and aseptically add 20 mL of Potassium Tellurite 1% Solution (REF 42211501). A less selective medium may be prepared by adding 10 mL/L of 1% Potassium Tellurite Solution. Do not heat medium after addition of potassium tellurite.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-orange, slightly opalescent
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Vogel Johnson Agar is intended for the bacteriological processing of food, pharmaceutical, cosmetic and environmental samples. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied. For non-clinical samples, refer to the applicable international standards.

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Spread from 0.1 to 1.0 mL of diluted sample suspended in 0.1% Peptone Water over the surface of the well dried plate. Incubate at 35-37°C and examine after 24 and 48 hours.

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
S. aureus grows with large black convex shiny colonies, surrounded by a yellow halo.
Coagulase-negative staphylococci grow poorly with black colonies, without yellow zone; the medium surrounding colonies may be a deeper red colour due to utilisation of peptones with resultant alkalinity.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 25923	35-37°C / 18-24 H / A	growth, black colonies with a yellow halo
S. epidermidis ATCC 12228	35-37°C / 18-24 H / A	slight growth, black colonies
E. coli ATCC 25922	35-37°C / 18-24 H / A	growth inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- During the first 24 hours of incubation most organisms other than coagulase-positive staphylococci are inhibited; however, after this incubation period other organisms may exhibit slight growth, especially enterococci and *S. epidermidis.*
- *Proteus* spp. after 18 hours grow with black colonies and with a change in the colour of the medium to brown: however, after 48 hours of incubation there is an alkaline inversion of the pH with the development of a purple colour.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.
According to Baird RM et al., the self-prepared plates can be stored at +2°C +8°C for up to 7 days.[10]

REFERENCES

1. Vogel TA, Johnson M. A modification of the Tellurite-Glycine Medium for use in the identification of Staphylococcus aureus. Public Health Lab. 1960; 18:131.
2. Zebovitz E, Evans JB, Niven Jr CF. Tellurite-Glycine Agar; a selective plating medium for the quantitative detection of coagulase-positive staphylococci. J Bacteriol 1955; 70:686.
3. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
4. U.S. Food & Drug Administration.BAM: Bacteriological Analytical Manual, Chapter 23: Microbiological Methods for Cosmetics 10/31/2017
5. Curry, Graf and McEwen (ed.). 1993. CTFA microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
6. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
7. Alico RK, Dragonjact MF. Evaluation of culture media for recovery of Staphylococcus aureus from swimming pools. App Environ Microbiol 1986; 51:699-702.
8. Halpin-Dohnalek MI, Marth EH. Growth of Staphylococcus aureus in milks and creams with various amounts of milk fat. J Food Prot 1989; 52:540-543
9. Flournoy DJ, Wongpradit S, Silberg SL. Screening media for detection of Methicillin-Resistant Staphylococcus aureus from non-sterile body sites. Med Microbiol Immunol 1990; 179:25-30.
10. Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:195-6.

PACKAGING

Product	Type	REF	Pack
Vogel Johnson Agar	Dehydrated medium	4021922	500 g (8,2 L)

IFU rev 2, 2022/05

WL NUTRIENT MEDIUM

Dehydrated culture medium

INTENDED USE

For cultivation and enumeration of yeasts and bacteria in brewing and other fermentation industries.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	4.0 g
Tryptone	5.0 g
Glucose	50.0 g
Agar	20.0 g
Potassium dihydrogen phosphate	550.0 mg
Potassium chloride	425.0 mg
Calcium chloride	125.0 mg
Magnesium sulphate	125.0 mg
Bromocresol green	22.0 mg
Ferric chloride	2.5 mg
Manganese sulphate	2.5 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Fermentation is a well-known natural process used by humanity for thousands of years with the fundamental purpose of making alcoholic beverages, as well as bread and by-products. Fermentation processes to produce wines, beers and ciders are traditionally carried out with *Saccharomyces cerevisiae* strains, the most common and commercially available yeast.[1]

WL (Wallerstein Laboratory) Nutrient Medium is based on the formulation developed by Gray[2] and Green and Gray.[3-5] It is used for the cultivation and enumeration of yeasts and bacteria in microbiological control carried out in brewing and other fermentation industries.

Yeasts used in different fermentation processes have different growth requirements with regard to pH, atmosphere and incubation temperature. WL Nutrient Medium has a pH of 5.5, which is optimal for the enumeration of brewers' yeast. If baker's yeast or distillers' yeast is to be examined, the pH of the medium should be adjusted to 6.5. If the incubation is carried out under anaerobic conditions, brewer's cocci and lactobacilli develop; if it is incubated under aerobic conditions, aceto-acetic bacteria and thermobacteria grow. Incubation at 25°C is suitable for brewer's yeasts, incubation at 30°C for baker's yeasts.[6]

WL Nutrient Medium supports the growth of bacteria, but unless the number of yeast cells is reduced the bacteria cannot be detected. Because of this limitation, Green and Gray developed WL Differential Agar by adding 4 mg/L of cycloheximide which inhibits yeast growth.[4,5]

Yeast extract and tryptone provide nitrogen, carbon, minerals and vitamins for the microbial growth. Phosphate is used as buffering agent to control the pH in the medium. Potassium chloride, calcium chloride and ferric chloride are sources of electrolytes and maintains the osmotic equilibrium. Magnesium sulphate and manganese sulphate provide divalent cations for improving yeasts growth. Glucose at high concentration is the fermentable carbohydrate and a source of energy. Bromocresol green is a pH indicator, yellow at pH 4 and blue at pH 5.6.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 80 g in 1000 ml of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes.
WL Nutrient Medium at pH 6.5: before autoclaving add approximately 30 mL/L of sodium carbonate 1% aqueous solution.
WL Differential Medium: before autoclaving add 4 mg/L of cycloheximide.
Cool to 47-50°C, mix well and pour into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale blue, limpid
Final pH at 20-25 °C	5.5 ± 0.2

SPECIMENS

Samples from the fermentation process. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

Prepare 3 plates per specimen, one with WL Nutrient Medium and two with WL Differential Medium and inoculate with 0.1 mL of sample suspension and/or dilutions, streaked on the surface of the plates.[6]
Inoculation 1: WL Nutrient Medium, incubated aerobically for total count of mainly yeast colonies.
Inoculation 2: WL Differential Agar, incubated aerobically for growth of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacteria.
Inoculation 3: WL Differential Agar, incubated anaerobically for growth of lactic acid bacteria and *Pediococcus* spp.
pH and incubation temperature: brewing materials: pH 5.5, 25°C; baker's yeast and alcoholic mash: pH adjusted to 6.5, 30°C
Incubation time for all media: 1 week to 14 days depending of flora; make counts at various interval.
Note: the working scheme described is taken from the literature[6]; however, pH combinations and incubation conditions can be customised according to the analysis to be performed.

READING AND INTERPRETATION

Count the number of colonies per plate and calculate the microbial load.
Colony colouring varies greatly, from white or cream to various shades of green, with different shades of colour of the medium around the colonies depending on the yeast or bacterial strain isolated.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	25°/ 72 H-A	good growth
E. faecalis ATCC 19433	25°/ 72 H-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- Avoid over-heating and remelting of medium.
- The isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Maicas S. The Role of Yeasts in Fermentation Processes. Microorganisms 2020; 8:1142.
2. Gray PP. Some advances in microbiological control for beer quality. Wallerstein Lab Commun 1951; 14: 169.
3. Green SR, Gray PP. Paper read at American Society of Brewing Chemists Meeting. Wallerstein Lab Commun 1950; 12: 43.
4. Green SR, Gray PP. A differential procedure applicable to bacteriological investigation in brewing. Wallerstein Lab Commun 1950; 13: 357.
5. Green SR, Gray PP. A differential procedure for bacteriological studies useful in the fermentation industries. Wallerstein Lab Commun 1951; 14: 289.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

PACKAGING
Product	Type	REF	Pack
WL Nutrient Medium	Dehydrated medium	4021952	500 g (6.2 L)

IFU rev 1, 2022/08

WORT AGAR BASE
Dehydrated culture medium

INTENDED USE
For the cultivation of a wide variety of yeasts and filamentous fungi

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)
Malt extract	15.0 g
Peptone	0.78 g
Maltose	12.75 g
Dextrin	2.75 g
Dipotassium phosphate	1.0 g
Ammonium chloride	1.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Wort Agar Base is equivalent to the medium described by Parfitt[1] and is suitable for the cultivation and enumeration of fungi, especially yeasts, in butter, sugar and syrups, in lemonades and more generally in sweet or soft drinks.
Several modifications of the wort agar medium have been proposed for specific purposes.[2] According to Rapp[2], addition of certain indicator dyes allows differentiation between yeast and bacterial colonies. Scarr[3] employed a modified Wort Agar ('osmophilic agar') for the examination of sugar products for osmophilic yeasts. Wort or hopped-wort agar with monoiodacetic acid according to Šilhánková[4] is suitable for detection of non-*Saccharomyces* wild yeast; furthermore, monoiodacetic acid suppresses the growth of most bacteria. Wort Agar containing copper sulphate is also used for wild yeast assessment. Wort Agar supplemented with $CuSO_4$ (0.55 g/l) allowed for the growth of non-*Saccharomyces* wild yeasts.[6] Lowering the concentration to 0.2 g/L enables detection of some *Saccharomyces* wild yeasts.[7] According to Röcken et al.[8] incubation of Wort Agar at pH 4.5 at 37 °C is recommended for the detection of amylolytic yeast ("*S. diastaticus*") in bottom-fermenting yeast. The medium, which duplicates the composition of natural wort, contains peptone and malt extract which provide the growth factors for mycological growth. Maltose and dextrin are the fermentable carbohydrates and a source of carbon and energy. The medium acidity (pH 4.8) is favourable for yeast growth and inhibitory to most bacteria. For increasing the selective properties, the pH may be decreased to 3.5 by adding tartaric or lactic acid. Dipotassium phosphate buffers the medium while glycerol, added to the medium base, reduces the water activity.

DIRECTIONS FOR MEDIUM PREPARATION
Suspend 48.28 g in 1000 ml of cold purified water and add 2.35 mL of glycerol (REF 421015); heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes. Mix well and pour into sterile Petri dishes. Prolonged or excessive heating will diminish the gel strength of the agar.

PHYSICAL CHARACTERISTICS
Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	dark yellow, limpid
Final pH at 20-25 °C	4.8 ± 0.2

SPECIMENS
Butter, sugar and syrups, lemonades and more generally sweet or soft drinks. Refer to applicable International Standards and regulations for the collection of food samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE
Yeasts and moulds enumeration in butter.
1. Prepare the initial suspension of the sample and the decimal dilutions with quarter-strength Ringer solution.
2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1 mL of each decimal dilution in duplicate to the centre of each empty Petri dish.
3. Pour approximately 15 mL of Wort Agar, cooled to approximately 47°C into each dish.
4. Mix well the inoculum with the medium and allow the mixture to solidify.
5. Incubate for 5 days at 25°C

READING AND INTERPRETATION
Enumerate the number of colonies of yeasts and moulds per plate and calculate the microbial count.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	25°/ 72 H-A	good growth
S. aureus ATCC 25923	25°/ 72 H-A	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Avoid over-heating and remelting of medium.
- The isolated colonies on the plates should be identified with suitable tests.

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. Parfitt EH. J Dairy Sci. 1933; 16: 141-147.
2. Matoulková D, Kubizniaková P. Brewing Microbiology – Wild Yeasts and Methods of Their Detection. Kvasny Prum. 2013;59: 246–257
3. Rapp, M.: Indikatorzusätze zur Keimdifferenzierung auf Würze- und Malzextrakt-Agar. Milchwiss. 1974; 29:341-344.
4. Scarr MP J. Sci. Food Agric. 1959; 10(12):678-681.
5. Šilhánková L. A new method of quantitative determination of contamination of baker's yeast by wild yeasts or by dissociation forms of the production culture. Folia Microbiol. 1962; 7: 255–256.
6. Lin Y. Formulating and testing of cupric sulphate medium for wild yeast detection. J. Inst. Brew. 1981;87: 151–154.
7. Taylor GT, Marsh AS. MYGP + copper, a medium that detects both Saccharomyces and non-Saccharomyces wild yeast in the presence of culture yeast. J Inst Brew 1984; 90: 134–145.
8. Röcken W, Schulte S. Nachweis von Fremdhefen. Brauwelt 1986; 41: 1921–1927.

PACKAGING

Product	Type	REF	Pack
Wort Agar base	Dehydrated medium	4022032	500 g (10.3 L)

IFU rev 1, 2022/08

WORT BROTH BASE

Dehydrated culture medium

INTENDED USE

For the cultivation of a wide variety of yeasts and filamentous fungi

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Malt extract	15.0 g
Peptone	0.75 g
Maltose	12.75 g
Dextrin	2.75 g
Dipotassium phosphate	0.75 g
Ammonium chloride	1.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Wort Broth Base is equivalent to the medium described by Parfitt[1] and is suitable for the cultivation of fungi, especially yeasts, in butter, and syrups. Wort Broth is especially designed to propagate the multiplication of yeasts and often it is employed as an enrichment medium and for the production of yeasts suspensions.
The medium, which duplicates the composition of natural wort, contains peptone and malt extract which provide the growth factors for mycological growth. Maltose and dextrin are the fermentable carbohydrates and a source of carbon and energy. The medium acidity is favourable for yeast growth and inhibitory to most bacteria. For increasing the selective properties, the pH may be decreased to 3.5 by adding tartaric or lactic acid. Dipotassium phosphate buffers the medium, while glycerol, added to the medium base, reduces the water activity.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 33 g in 1000 mL of cold purified water and add 2.5 mL of glycerol (REF 421015); heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121ºC for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	dark yellow, limpid
Final pH at 20-25 °C	5.0 ± 0.2

SPECIMENS

Butter and syrups. Refer to applicable International Standards and regulations for the collection of food samples. Operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

TEST PROCEDURE

- Inoculate the medium with pure strains of yeasts or with a suspension of the sample and its decimal dilutions.
- Incubate for 5 days at 25°C

READING AND INTERPRETATION
After incubation the growth is evidenced by turbidity in the broth.

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	25°/ 72 H-A	good growth
S. aureus ATCC 25923	25°/ 72 H-A	growth partially inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS
Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES
1. Parfitt EH. J Dairy Sci. 1933; 16: 141-147.

PACKAGING
Product	Type	REF	Pack
Wort Broth Base	Dehydrated medium	4022022	500 g (15.1 L)

IFU rev 2, 2022/08

WURTZ AGAR
Ready-to-use plates

Wurtz Agar: lactose fermenting *E. coli* (yellow colonies)

INTENDED USE
In vitro diagnostic device. Culture medium for isolation and differentiation of enterobacteria from clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA *
Peptone	5.000 g
Beef extract	3.000 g
Sodium chloride	5.000 g
Lactose	10.000 g
Bromothymol blue	0.075 g
Agar	15.000 g
Purified water	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Wurtz agar is intended for the isolation, enumeration and differentiation of enterobacteria from samples of clinical and non-clinical origin.[1,2]
Animal peptones provide carbon, nitrogen, vitamins and trace elements for microbial growth. Sodium chloride maintains the osmotic balance while agar is the solidifying agent. Lactose and bromothymol blue differentiate lactose-fermenting from lactose non-fermenting bacteria: lactose-fermenting isolates acidify the medium with a colour change of bromothymol blue from green to yellow. Non-fermenting lactose bacteria grow with blue colonies because of alkalinisation of the medium due to the decomposition of peptones with production of ammonia.

PHYSICAL CHARACTERISTICS
Medium appearance	dark green, limpid
Final pH at 20-25°C	7.1 ± 0.2

SPECIMENS
Wurtz Agar is intended for the microbiological processing of clinical and non-clinical specimens when the differentiation of lactose fermenting and non-fermenting bacteria is required. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.

TEST PROCEDURE
Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a sterile loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Replace the lid and then incubate the streaked agar plate in aerobic atmosphere, at 35-37°C for 18-24 hours, in an inverted position to prevent condensation.

READING AND INTERPRETATION
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies.
The lactose fermenting bacteria grow on Wurtz Agar with yellow colonies of different sizes, with a more or less extensive yellow colour of the medium. Non-lactose fermenting bacteria grow with colourless to blue colonies.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS		INCUBATION T°/ T / ATM	EXPECTED RESULTS
E. coli	ATCC 25922	35-37°C / 18-24H / A	good growth, large yellow colonies
P. vulgaris	ATCC 6380	35-37°C / 18-24H / A	good growth, blue colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- Due to the inclusion of sodium chloride in the formulation, *Proteus* swarming is not limited on Wurtz Agar.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If required and relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

STORAGE CONDITIONS

Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Mazzone C, Rizzi M, Tauro L. Etiology and Incidence of positive Microbiological tests in the Department of Pneumology. Microbiologia Medica 2010; 25 (2):101
2. Corry JEL, Curtis GDW and Baird R M., (Eds.), Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, 1995, Elsevier, Amsterdam

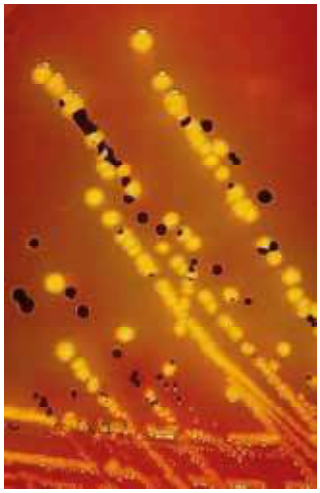
PACKAGING

Product	Type	REF	Pack
Wurtz Agar	Ready-to-use plates	542200	2 x 10 plates ø 90 mm

IFU rev 1, 2021/01

XLD AGAR

Dehydrated and ready-to-use culture medium



XLD Agar: *Salmonella* colonies with large black centre and *E. aerogenes* with yellow colonies

INTENDED USE

In vitro diagnostics. Selective and differential medium for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella,* from clinical and non-clinical specimens.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Xylose	3.50 g
L-lysine	5.00 g
Lactose	7.50 g
Sucrose	7.50 g
Sodium chloride	5.00 g
Yeast extract	3.00 g
Sodium deoxycholate	2.50 g
Sodium thiosulphate	6.80 g
Ferric ammonium citrate	0.80 g
Phenol red	0.08 g
Agar	13.50 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In the first half of the twentieth century, several culture media were developed and proposed for the isolation of enteric pathogens from faeces and other materials. Some of them were moderately selective and allowed the growth of faecal contaminants, others showed excessive toxicity for the growth of pathogens, especially of *Shigella.*[1] In 1965, Xylose Lysine Desoxycholate (XLD) agar was introduced by Welton I. Taylor for the enhanced recovery of *Shigella*.[2] Several clinical evaluations demonstrated the relatively high efficiency of XLD Agar in the primary isolation of *Shigella* and *Salmonella*.[3-5]

XLD Agar is a selective and differential medium intended for the isolation of Gram-negative enteric pathogens, especially *Salmonella* and *Shigella* from clinical specimens.[6-8] It is recommended for the detection of *Salmonella* in non-sterile pharmaceutical products according to harmonized EP, USP, JP method[9] and by FDA-BAM for detection of *Salmonella* in food[10]. The XLD formula recommended by ISO norms for the detection of *Salmonella* and *Shigella* in food and water contains a lower concentration of sodium deoxycholate and corresponds to Biolife medium XLD Agar ISO Formulation (REF 402208).

Yeast extract provides carbon, nitrogen, vitamins and trace elements for bacterial growth; sodium chloride maintains the osmotic balance in the medium; sodium deoxycholate is a selective agent for suppressing the growth of Gram-positive bacteria. XLD Agar contains three indicator systems: xylose, lactose, and sucrose combined with phenol red, lysine hydrochloride and again phenol red, sodium thiosulfate and ferric ammonium citrate. Target bacteria are tentatively grouped by reading the effect of carbohydrate fermentation, lysine decarboxylation and formation of hydrogen sulphide.

Sugars' fermentation lowers the pH and the phenol red indicator registers this by changing from red to yellow. Most enteric bacteria, including *Salmonella*, can ferment the xylose to produce acid; *Shigella* does not ferment the xylose, does not cause acidification of the medium, and therefore, grows with red colonies. After exhausting the xylose supply, *Salmonella* colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. To prevent similar pH reversion by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Moreover *Salmonella* spp. produce thiosulphate reductase that cause the release of a sulphide molecule from

the sodium thiosulfate present in the medium; this sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric ammonium citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 55 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, to dissolve completely. Do not autoclave. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red orange, limpid
Final pH at 20-25 °C	7.4 ± 0.2

SPECIMENS

XLD Agar is intended for the bacteriological processing of clinical specimens such as faeces, rectal swab, urine, bile,[6-8] non-sterile pharmaceutical products[9] and food[10]. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of clinical specimens should be applied.[11] Consult appropriate standard methods for details of collection and preparation of non-clinical specimens.[9,10]

TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Maximal recovery of *Salmonella* from faecal specimens is obtained by using the enrichment step in Selenite Broth followed by subculture to XLD Agar and to a second plating medium.[8]
For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised, followed by subculture on two different selective media: XLD Agar and a second less selective medium (Mac Conkey Agar).[8]
Incubate inoculated XLD Agar plates with the specimen or with a specimen enriched in liquid medium, in aerobic conditions at 35-37°C for 18-24 hours. Colonies on XLD agar may require 48 hours incubation for full colour and black precipitate development.
For the detection of *Salmonella* in non-sterile pharmaceuticals products the technique recommended by European Pharmacopoeia[9], and summarized below, should be followed:

- Prepare a sample using a 1:10 dilution of not less than 1 g of the product to be examined and use 10 mL or the quantity corresponding to 1 g or 1 mL to inoculate the suitable amount of Tryptic Soy Broth. Mix and incubate at 30-35°C for 18-24 h.
- Shake the container, transfer 0,1 mL of Tryptic Soy Broth to 10 mL of Rappaport Vassiliadis Enrichment Salmonella Broth EP (REF 401979) and incubate at 30-35°C for 18-24 h.
- Subculture on a plate of XLD Agar and incubate at 30-35 °C for 18-48 h.

Consult appropriate references for the detection of *Salmonella* in food.[10]

READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Do not examine areas of confluent growth as false negative fermentation reactions may occur.
Interpretation of colonies' colours[12]:
Red colonies: alkaline reaction, non-fermentation of xylose/sucrose/lactose, or fermentation of xylose followed by decarboxylation of lysine: possible *Shigella* or *Providencia* or *Pseudomonas* spp. or *Salmonella* sp. H_2S negative
Red colonies with black centre: xylose fermentation only, lysine positive, H_2S positive, rapid depletion of xylose and resultant alkalinity due to lysine decarboxylation, black centre due to H_2S production possible only in alkaline pH environment: suspect *Salmonella* H_2S positive.
Opaque yellow colonies: xylose fermentation, lysine negative and non-fermentation of lactose and sucrose, acid pH: possible *E. coli*, *Klebsiella/Enterobacter, Citrobacter*, *Serratia, Proteus* spp.
Yellow colonies: lactose or sucrose fermentation, lysine negative, acid pH: possible coliforms or sucrose-positive *P. vulgaris*.
For presumptive *Salmonella* spp. identification, it is advised to screen the colonies by testing the colonies with one drop of MUCAP reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of C_8 esterase enzyme, typical of *Salmonella* spp.[14]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[13]

CONTROL STRAINS			INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, red colonies with black centre
S. flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, red colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, yellow colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- A single medium is only rarely useful to recover all pathogens contained in a specimen. Therefore, additional media for the isolation of *Salmonella* and/or *Shigella*, with lower selectivity such as Mac Conkey Agar and with higher selectivity such as SS Agar, should be used; it is suggested to inoculate additional media for the isolation of other enteric pathogens with the specimen.[8]
- Non-enteric organisms such as *Pseudomonas* may grow; *Pseudomonas* and *Providencia rettgeri* may both exhibit red colonies. Some *Proteus* spp. may develop black centres. [12]
- S. Paratyphi A, S. Cholerae-suis, S. Pullorum and S. Gallinarum may form red colonies without black centre, thus resembling *Shigella* spp.
- Incubation exceeding 48 hours may lead to false positive results.[12]
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

STORAGE CONDITIONS

Dehydrated medium

Store at +10°C /+30°C away from direct light in a dry place.

According to MacFaddin, the self-prepared plates can be stored at +2°C +8°C for up to 6-8 weeks.[12]

Ready-to-use plates

Store plates in their original pack at +2°C/+8°C away from direct light.

REFERENCES

1. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010
2. Taylor WI. Isolation of shigellae I. Xylose lysine agars; new media for isolation of enteric pathogens. Am J Clin Pathol 1965; *44*:471-475
3. Taylor WI, Schelhart D. Isolation of shigellae VI. Performance of media with stool specimens. Appl Microbiol 1968; 16:1387-1393
4. Taylor WI, Schelhart D. Isolation of shigellae VIII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar and Eosin Methylene Blue Agar with stool specimens. Appl Microbiol 1971; 21:32-7
5. Zajc-Satler J, Gragas AZ. Xylose Lysine Deoxycholate Agar for the isolation of Salmonella and Shigella from clinical specimens. Zentralbl Bakteriol Orig 1977; A237:196-200
6. Vandepitte J Verhaegen J Engbaek K Rohner P Piot P Heuck CC. Basic laboratory procedures in clinical bacteriology. 2nd ed. 2003; Geneve:World Health Organization
7. Public Health England- UK Standards for microbiology investigations (UK SMI): searchable index. 9 January 2019
8. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. Escherichia, Shigella and Salmonella. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015. p.685.
9. European Pharmacopoeia, current edition.
10. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Rev 12/2019
11. Baron EJ, Specimen Collection, Transport and Processing: Bacteriology. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.270.
12. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
13. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004
14. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526.

PACKAGING

Product	Type	REF	Pack
XLD Agar	Dehydrated medium	4022062 4022064	500 g (9.1 L) 5 kg (91 L)
XLD Agar	Ready-to-use plates	542206	2 x 10 plates ø 90 mm

IFU rev 3, 2022/01

XLD AGAR ISO FORMULATION

Dehydrated and ready-to-use culture medium

INTENDED USE

Selective and differential medium for the detection of *Salmonella* and *Shigella* from foodstuffs and waters according to ISO Standards.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM AND READY-TO-USE PLATES

Xylose	3.75 g
L-lysine hydrochloride	5.00 g
Lactose	7.50 g
Sucrose	7.50 g
Sodium chloride	5.00 g
Yeast extract	3.00 g
Sodium deoxycholate	1.00 g
Sodium thiosulphate	6.80 g
Ferric ammonium citrate	0.80 g
Phenol red	0.08 g
Agar	14.50 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

In the first half of the twentieth century, several culture media were developed and proposed for the isolation of enteric pathogens from faeces and other materials. Some of them were moderately selective and allowed the growth of faecal contaminants, others showed excessive toxicity for the growth of pathogens, especially of *Shigella*.[1] In 1965, xylose lysine deoxycholate (XLD) agar was introduced by Welton I. Taylor for the enhanced recovery of *Shigella*.[2] Several evaluations demonstrated the relatively high efficiency of XLD Agar in the primary isolation of *Shigella* and *Salmonella*.[3-5]

XLD Agar ISO Formulation is a selective and differential medium intended for the detection of *Salmonella* in food chain samples according to ISO 6579,[6] in water samples according to ISO 19250[7] and for the detection of *Shigella* in foods according to ISO 21567[8].

XLD ISO Formulation contains a lower concentration of sodium deoxycholate and a slightly higher concentration of xylose than the standard XLD Agar formulation (REF 402206).

Yeast extract provides carbon, nitrogen, vitamins and trace elements for bacterial growth; sodium chloride maintains the osmotic balance in the medium; sodium deoxycholate is a selective agent for suppressing the growth of Gram-positive bacteria. XLD Agar contains three indicator systems: xylose, lactose, and sucrose combined with phenol red, lysine hydrochloride and again phenol red, sodium thiosulfate and ferric ammonium citrate. Target bacteria are tentatively grouped by reading the effect of carbohydrate fermentation, lysine decarboxylation and formation of hydrogen sulphide.

Sugars' fermentation lowers the pH and the phenol red indicator registers this by changing from red to yellow. Most enteric bacteria, including *Salmonella*, can ferment the xylose to produce acid; *Shigella* does not ferment the xylose, does not cause acidification of the medium, and therefore, grows with red colonies. After exhausting the xylose supply, *Salmonella* colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. To prevent similar pH reversion by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Moreover *Salmonella* spp. produce thiosulphate reductase that cause the release of a sulphide molecule from

the sodium thiosulfate; this sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric ammonium citrate, forming a precipitate, resulting in colonies that are black or have a black centre.

Directions for medium preparation
Suspend 54.9 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, to dissolve completely. Do not overheating, do not autoclave. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

Physical characteristics
Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red orange, limpid
Final pH at 20-25 °C	7.4 ± 0.2

Specimens
Food chain and water samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.[6-8]

Test procedure
Detection of *Salmonella* in foods[6]
The detection of *Salmonella* in foods and other samples of sanitary interest, necessitates four successive stages: pre-enrichment in non-selective liquid medium, enrichment in one or two selective liquid media, plating out and recognition, confirmation.
Allow plates to come to room temperature and to dry the surface of the medium.
From Rappaport Vassiliadis Soy (RVS) Broth (REF 4019819) or MSRV Medium (ref 401982) incubated at 41.5 °C ± 1 for 24 h ± 3 h and from MKTT Broth (REF 401745) incubated between 34 °C and 38 °C for 24 h ± 3 h, transfer a loopful of growth on a plate of XLD Agar ISO Formulation and on another selective medium for *Salmonella,* based on different diagnostic characteristics to those of XLD agar (e.g., Chromogenic Salmonella Agar REF 405350).
Incubate the XLD plates inverted between 34 °C and 38 °C and examined after 24 h. Incubate the second selective plating-out medium in accordance with the instructions for use
Detection of *Shigella* in foods[8]
From Shigella broth containing 0,5 µg/ml of novobiocin (Shigella Broth Base REF 4020402 supplemented with Novobiocin Antimicrobic Supplement REF 4240047), incubated anaerobically at 41.5 ±1°C for 16 h to 20 h, transfer a loopful of growth on plates of MacConkey Agar REF 401670 (low selectivity), XLD Agar ISO Formulation (moderate selectivity), and Hektoen Enteric Agar REF 401541 (greatest selectivity).
Incubate the plating-out media at 37 °C for 20 h to 24 h. If no typical colonies are seen and the growth of other microorganisms is weak (particularly on the more selective agar), re-incubate the plates for a further 24 h. Examine them again for typical *Shigella* colonies.

Reading and interpretation
After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Do not examine areas of confluent growth as false negative fermentation reactions may occur.
Typical colonies of *Salmonella* on XLD Agar ISO Formulation have a black centre and a lightly transparent zone of reddish colour due to the colour change of the indicator. *Salmonella* H_2S-negative variants grown with pink colonies with a darker pink centre. Lactose positive *Salmonella* grown with yellow colonies with or without blackening.
Typical colonies of *Shigella* on XLD Agar ISO Formulation are translucent with red/cerise centre, same colour as the agar.
The recognition of colonies of *Salmonella* is to a large extent a matter of experience, and their appearance can vary somewhat. Here below a summary of the interpretation criteria for colonies grown on XLD Agar[9]:
Red colonies: alkaline reaction, non-fermentation of xylose/sucrose/lactose, or fermentation of xylose followed by decarboxylation of lysine: possible *Shigella* or *Providencia* or *Pseudomonas* spp. or *Salmonella* sp. H_2S negative
Red colonies with black centre: xylose fermentation only, lysine positive, H_2S positive, rapid depletion of xylose and resultant alkalinity due to lysine decarboxylation, black centre due to H_2S production possible only in alkaline pH environment: suspect *Salmonella* H_2S positive.
Opaque yellow colonies: xylose fermentation, lysine negative and non-fermentation of lactose and sucrose, acid pH: possible *E. coli*, *Klebsiella/Enterobacter, Citrobacter*, *Serratia, Proteus* spp.
Yellow colonies: lactose or sucrose fermentation, lysine negative, acid pH: possible coliforms or sucrose-positive *P. vulgaris*.
For presumptive *Salmonella* spp. identification, it is advised to screen the colonies by testing with one drop of MUCAP Reagent (REF 191500) and observing after 3 to 5 min for the development of fluorescence under Wood's lamp, produced in the presence of C_8 esterase enzyme, typical of *Salmonella* spp.[10]

User quality control
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains			Incubation T°/ t / ATM	Expected results
S. Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, red colonies with black centre
S. flexneri	ATCC	12022	35-37°C / 18-24h / A	growth, red colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, yellow colonies

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method
- A single medium is only rarely useful to recover all pathogens contained in a specimen.
- Non-enteric organisms such as *Pseudomonas* may grow; *Pseudomonas* and *Providencia rettgeri* may both exhibit red colonies. Some *Proteus* spp. may develop black centres.[9]
- S. Paratyphi A, S. Cholerae-suis, S. Pullorum and S. Gallinarum may form red colonies without black centre, thus resembling *Shigella* spp.[9]
- Incubation exceeding 48 hours may lead to false positive results.[9]
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.

Storage conditions
Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
According to ISO 6579 the self-prepared plates can be stored at +2°C +8°C, protected from drying, for up to four weeks.[6]

Ready-to-use plates
Store plates in their original pack at 2-8°C away from direct light.

REFERENCES

1. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010
2. Taylor WI. Isolation of shigellae I. Xylose lysine agars; new media for isolation of enteric pathogens. Am J Clin Pathol 1965; *44:*471-475
3. Taylor WI, Schelhart D. Isolation of shigellae VI. Performance of media with stool specimens. Appl Microbiol 1968; *16:1387*-1393
4. Taylor WI, Schelhart D. Isolation of shigellae VIII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar and Eosin Methylene Blue Agar with stool specimens. Appl Microbiol 1971; 21:32-7
5. Zajc-Satler J, Gragas AZ. Xylose Lysine Deoxycholate Agar for the isolation of Salmonella and Shigella from clinical specimens. Zentralbl Bakteriol Orig 1977; A237:196-200
6. ISO 6579-1:2017. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella —Part 1: Detection of Salmonella spp.
7. ISO 19250:2010 Water quality — Determination of Salmonella species
8. ISO 21567:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.
9. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985
10. Ruiz J, Sempere MA, Varela C, Gomez J. Modification of the methodology of stool culture for Salmonella detection, J Clin Microbiol 1992; 30:525-526.

PACKAGING

Product	Type	REF	Pack
XLD Agar ISO Formulation	Dehydrated medium	4022082 4022084	500 g (9.1 L) 5 kg (91 L)
XLD Agar ISO Formulation	Ready-to-use plates	542208	2 x 10 plates ø 90 mm
XLD Agar ISO Formulation	Ready-to-use plates	502208	5 plates ø 150 mm

IFU rev 2, 2022/08

XLT4 AGAR BASE
XLT4 SUPPLEMENT
Dehydrated culture medium and selective supplement

INTENDED USE
XLT4 Agar Base is used with XLT4 Supplement for the preparation of XLT4 Agar, a highly selective medium for the detection of *Salmonella* spp. other than *Salmonella* Typhi.

COMPOSITION
XLT4 AGAR BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptone	1.60 g
Yeast extract	3.00 g
Xylose	3.75 g
Sucrose	7.50 g
Lactose	7.50 g
L-lysine	5.00 g
Sodium thiosulfate	6.80 g
Ferric ammonium citrate	0.80 g
Sodium chloride	5.00 g
Phenol red	0.08 g
Agar	17.00 g

XLT4 SUPPLEMENT (BOTTLE CONTENT)

Tergitol 4 (Niaproof 4/Sodium tetradecilsulfate)	100 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
In the first half of the twentieth century, several culture media were developed and proposed for the isolation of enteric pathogens from faeces and other materials. Some of them were moderately selective and allowed the growth of faecal contaminants, others showed excessive toxicity for the growth of pathogens.[1] In 1990, xylose-lysine-tergitol 4 (XLT4) agar was developed by Miller and Tate[2] as a modification of xylose lysine deoxycholate agar, for the enhanced recovery of non-Typhi *Salmonella* spp. Several evaluations demonstrated that XLT4 medium significantly improved *Salmonella* isolation from chicken farm environmental drag-swab samples over the other selective plating media.[3-5] XLT4 agar was found to strongly inhibit *Proteus, Pseudomonas, Providencia*, and many other non-salmonellae and to provide good differentiation between *Salmonella* and *Citrobacter.*[3]
XLT4 Agar is included in the FDA-BAM list of rapid methods and specialty substrate media for detection of foodborne bacteria.[6]
Yeast extract provides carbon, nitrogen, vitamins and trace elements for bacterial growth. XLT4 Agar contains three indicator systems: xylose, lactose, sucrose and lysine hydrochloride combined with phenol red, sodium thiosulfate and ferric ammonium citrate. Target bacteria are tentatively grouped by reading the effect of carbohydrate fermentation, lysine decarboxylation and formation of hydrogen sulphide.
Sugars' fermentation lowers the pH and the phenol red indicator registers this by changing from red to yellow. After exhausting the xylose supply, *Salmonell*a colonies will decarboxylate lysine, increasing the pH once again to alkaline. To prevent similar pH reversion by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Moreover *Salmonella* spp. produce thiosulphate reductase that cause the release of a sulphide molecule from the sodium thiosulfate; this sulphide molecule couples with a hydrogen ion to form H_2S gas that reacts with the ferric ammonium citrate, forming a precipitate, resulting in colonies that are black or have a black centre. The addition of low concentrations of peptone produces blacker *Salmonella* colonies in shorter incubation times (increased hydrogen sulphide production), while still maintaining strong inhibition of competing bacteria.[7] The selectivity relies on the use of the anionic surfactant Niaproof 4 (formerly Tergitol 4) that largely inhibits the unwanted accompanying flora. On XLT4 agar, *Salmonella* spp. produces black or black-centred colonies with a yellow or pink periphery. Other Gram-negative bacteria are markedly inhibited or produce yellow or pink colonies without black coloration.

Directions for medium preparation

Suspend 58 g in 1000 mL of cold purified water and add 4.6 mL of XLT4 Supplement (REF 4240097). Heat to boiling with frequent agitation, to dissolve completely. Do not overheating, do not autoclave. Cool to 47-50°C, mix well and distribute into sterile Petri dishes. Do not leave the medium more than 45 minutes into the water batch.

Physical characteristics

Dehydrated medium appearance	red-pink, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red, limpid
Final pH at 20-25 °C	7.4 ± 0.2

Specimens

Food samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

Test procedure

The detection of *Salmonella* in foods and other samples of sanitary interest, may require four successive stages: pre-enrichment in non-selective liquid medium, enrichment in one or two selective liquid media, plating out and recognition, confirmation.
Allow plates to come to room temperature and to dry the surface of the medium.
From a suitable *Salmonella* enrichment broth (e.g., Mueller Kauffman Tetrathionate Broth or Tetrathionate Broth or Selenite Cystine Broth) transfer a loopful of growth on a plate of XLT4 Agar.
Streak the inoculum over the four quadrants of the plate to obtain well isolated colonies.
Incubate between 34 °C and 38 °C and examined after 24 and 48 hours.

Reading and interpretation

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of isolated colonies. Do not examine areas of confluent growth as false negative fermentation reactions may occur.
Salmonella spp. ferment the xylose with acidification of the medium, and decarboxylate the lysine with consequent inversion of the pH of the medium to alkaline values. Typical colonies of *Salmonella* on XLT4 Agar after 18-24 hours incubation have a black centre and a lightly transparent zone of yellow colour. After 48 hours incubation *Salmonella* colonies became entirely black or pink to red with black centres. H_2S-negative variants grown with pinkish-yellow colonies. Lactose-positive *Salmonella* strains grown with yellow colonies with or without blackening.
Shigella is partially inhibited and grows with red colonies.
Coliforms bacteria such as *E. coli, Enterobacter, Citrobacter* are markedly inhibited and colonies appear yellow without blackening. Growth of other Gram-negative bacteria is markedly to completely inhibited. Gram positive bacteria are totally inhibited.

User quality control

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

Control strains			Incubation T°/ t / ATM	Expected results
S. Typhimurium	ATCC	14028	35-37°C / 18-24h / A	growth, colonies with black centre
E. coli	ATCC	25922	35-37°C / 18-24h / A	partially inhibited, yellow colonies
E. faecalis	ATCC	29212	35-37°C / 18-24h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

Limitations of the method

- A single medium is only rarely useful to recover all pathogens contained in a specimen.
- XLT4 agar is not intended for the detection of S. Typhi.
- Colonies of presumptive *Salmonella* must be sub cultured and their identity confirmed by means of appropriate biochemical and serological tests.
- Non-*Salmonella* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Salmonella.*

Storage conditions

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Supplement
Store the product in the original package at +10°C /+30°C away from direct light.

References

1. Jan Hudzicki. Hektoen Enteric Agar Protocol. American Society for Microbiology. 11 November 2010
2. Miller RG, Tate CR. XLT4: A highly selective plating medium for the isolation of Salmonella. The Maryland Poultryman, 1990 April: 2-7.
3. Tate CR, Mallinson ET, Scherrer JA. Xylose-lysine-tergitol 4: an improved selective agar medium for the isolation of Salmonella. Poult Sci. 1991; 70:2429.
4. Miller RG, Tate CR, Mallinson ET, Scherr JA. Xylose-lysine-tergitol 4: an improved selective agar medium for the isolation of Salmonella. Poult Sci. 1992; 71:398.
5. Miller RG, Tate CR, Mallinson ET. Evaluation of two Isolation and two non isolation methods for detecting naturally occurring Salmonellae from broiler flock environmental drag-swab samples. J Food Prot. 1992; 55:964-967.
6. U.S. Food and Drug Administration. Bacteriological Analytical Manual. Appendix 1, Rapid Methods for Detecting Foodborne Pathogens. Version: January 2001. Content current as of June 18, 2009.
7. Miller RG, Tate CR, Mallinson ET Improved XLT4 Agar: Small addition of peptone to promote stronger production of hydrogen-sulfide by Salmonellae. J Food Prot 1994; 57:854-858.

Packaging

Product	Type	REF	Pack
XLT4 Agar Base	Dehydrated medium	4022072	500 g (8.6 L)
XLT4 Supplement	Liquid supplement	4240097	100 mL

IFU rev 1, 2022712

YEAST EXTRACT AGAR

Dehydrated and ready-to-use culture medium

INTENDED USE

A highly nutritive medium for plate count of organisms in water samples.

COMPOSITION - TYPICAL FORMULA *
(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED MEDIUM, READY-TO-USE PLATES, TUBES AND FLASKS

Tryptone	6
Yeast extract	3
Agar	15

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Yeast Extract Agar is prepared according to the formula described by Windle Taylor[1] for the plate count of microorganisms in water. Yeast Extract Agar is recommended by ISO 6222[2] for the enumeration of aerobic bacteria, yeasts and moulds in water by pour plate method.
Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Agar is the solidifying agent.

DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 24 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

DIRECTIONS FOR MEDIUM PREPARATION (READY-TO-USE FLASKS/TUBES)

Liquefy the contents of the flask/tube in an autoclave set at 100 ± 2°C or in a temperature-controlled water bath (100°C). Alternatively, the bottle or the tube may be placed into a jar containing water, which is placed on a hot plate and brought to boiling. Slightly loosen the cap before heating to allow pressure exchange. Cool to 47-50°C and pour the medium into sterile Petri dishes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale yellow, clear
Final pH at 20-25 °C	7.2 ± 0.2

SPECIMENS

Water samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

TEST PROCEDURE

1. Using a sterile pipette, dispense a volume not exceeding 2 mL of test sample and/or its dilutions, into an empty Petri dish and mix with 15-20 mL of molten Yeast Extract Agar pre-cooled to 44-46°C.
2. Invert the plates and incubate one set under aerobic conditions at 36 ± 2 °C for 44 ± 4 hours; incubate the other set at 22 ± 2 °C for 68 ± 4 hours.

READING AND INTERPRETATION

For each incubation temperature, count all colonies obtained in the plates containing fewer than 300 colonies and calculate the number of microorganisms per millilitre of the test sample.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
B. subtilis ATCC 6633	37°C/44 H-A	good growth
A. brasiliensis ATCC16404	22°C/72 H-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHODS

- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.[3,4]
- A potential source of error in plate count can result from the stack-pouring Petri dishes: in a stack of 3 plates, the middle and the top plates took too longer to cool, thereby resulting in lower counts. [3,5]
- Increasing the holding time of the dilutions in the diluent leads to higher count. [3,6]
- The Aerobic Plate Count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.[3]

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Ready-to-use plates, flasks and tubes
Store in their original pack at 2-8°C away from direct light.

REFERENCES

1. Windle Taylor E. (1958) `The Examination of Waters and Water Supplies', 7th ed., Churchill Ltd., London, pp. 394-398 and 778.
2. ISO 6222: 1999 Water quality - Enumeration of culturable microorganisms-Colony count by inoculation in a nutrient agar culture medium
3. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
4. Berry JM, McNeill DA, Witter LD. Effect of delay in pour plating on bacterial counts. J Dairy Sci 1969; 52:1456-1457
5. Koburger JA. Stack pouring of Petri plates: a potential source of error. J Food Prot. 1980; 43:561-562.
6. Huhtanen CN Brazis AR, Arledge WL et al. Effects of time of holding dilutions on counts of bacteria from raw milk. J Milk Food Technol. 1972; 35:126-130

PACKAGING

Product	Type	REF	Pack
Yeast Extract Agar	Dehydrated medium	4022752	500 g (20.8 L)
Yeast Extract Agar	Ready-to-use medium in plates	492275	3 x 10 plates ø 55 mm
Yeast Extract Agar	Ready-to-use medium in tubes	552275B	20 x 15 mL
Yeast Extract Agar	Ready-to-use medium in flasks	5122752 5122753	6 x 100 mL 6 x 200 mL

IFU rev 1, 2022/11

YEAST NITROGEN BASE

Dehydrated culture medium

INTENDED USE

For the classification of yeasts based on the oxidative utilisation of the carbon containing compounds.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Nitrogen source	
Ammonium sulphate	5.00 g
Amino acids	
L-Histidine	10.00 mg
LD-Methionine	20.00 mg
LD-Tryptophan	20.00 mg
Vitamins	
Niacin -	0.40 mg
P-aminobenzoic acid	0.20 mg
Pyridoxine HCl	0.40 mg
Riboflavin	0.20 mg
Thiamine HCl	0.40 mg
Calcium pantothenate	0.40 mg
Inositol	2.00 mg
Biotin	20.00 µg
Folic acid	2.00 mg
Trace elements	
Boric acid	0.50 mg
Potassium iodide	0.10 mg
Ferric chloride	0.20 mg
Manganese sulphate	0.40 mg
Sodium molybdate	0.20 mg
Zinc sulphate	0.40 mg
Copper sulphate	40.00 µg
Salts	
Potassium dihydrogen phosphate	0.85 g
Dipotassium hydrogen phosphate	0.15 g
Magnesium sulphate	0.50 g
Calcium chloride	0.10 g
Sodium chloride	0.10 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom with great industrial importance and pathogenic implications. Primary identification of yeasts is achieved by an assortment of morphological, biochemical and physiological methods. The carbohydrate assimilation technique remains one of the most common and widely used for the definitive identification of yeasts.
Yeast Nitrogen Base is prepared according to the formulation devised by Wickerham[1-3] and modified by Van der Walt[4]. It containing all the growth factors for the yeasts, with the exception of the carbon source. It is suitable for the classification of the yeasts on the basis of the oxidative utilisation of the carbon containing compounds. The results are evident by growth in the liquid medium which is used for assimilation. Yeast Nitrogen Base supplemented with 13.0 g/L of agar, prepared according to Wickerham and Burton's formulation[3], may be used in an auxanographic technique for determining patterns of carbohydrate assimilation.

DIRECTIONS FOR MEDIUM PREPARATION

Dissolve 6.7 g in 100 mL of cold purified water and sterilise by filtration. The solution will be 10X strength; for use dilute 1:10 with a sterile solution of the chosen carbohydrate: dissolve 0.5 g of the carbohydrate in 90 mL of purified water, sterilise by filtration and aseptically add 4.5 mL of this solution to 0.5 mL of Yeast Nitrogen Base.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	white, fine, homogeneous, free-flowing powder
Solution and prepared tubes appearance	colourless, clear
Final pH at 20-25 °C	5.6 ± 0.2

SPECIMENS

Pure cultures of yeasts.

TEST PROCEDURE

Inoculate the tubed medium very lightly with the test organism.
Incubate at 25°C for 6-7 days or, if necessary, for 20-24 days. After incubation shake the tubes and read for growth.

READING AND INTERPRETATION

After incubation, the presence of bacterial growth is evidenced by the presence of turbidity compared to an un-inoculated control.
Observe the growth of the yeasts by placing the tubes against a white card where black lines (thickness: 3-4mm) have been drawn. If the lines are poorly visible through the culture the test is positive. The yeast growth is often yellow because of the presence of riboflavin.
Refer to the literature for yeast identification schemes based on the results of the carbohydrate assimilation test.[5]

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below is listed a test strain useful for the quality control of Yeast Nitrogen Base supplemented with glucose.

CONTROL STRAIN	INCUBATION T°/ T / ATM	EXPECTED RESULTS
C. albicans ATCC 18804	25°C /72h /A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

STORAGE CONDITIONS

Store at +2°C /+8°C away from direct light in a dry place.

REFERENCES

1. Wickerham LJ. A simple technique for the detection of melibiose-fermenting yeasts. J Bacteriol 1943; 46:501-505.
2. Wickerham LJ. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J Bacteriol 1946; 52:293-301.
3. Wickerham LJ, Burton KA. Carbon assimilation tests for the classification of yeasts. J Bacteriol 1948; 56:363-371.
4. Van der Walt J P. Criteria and methods used in classification. In: «The Yeasts» ed. Lodder, J. ch.2, pp.84-113. Amsterdam: North Holland. 1971.
5. Borman AM, Johnson EM. Candida, Cryptococcus and other yeasts of medical importance. In Carroll KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Vol 2, 2019. Washington, DC: American Society for Microbiology.

PACKAGING

Product	Type	REF	Pack
Yeast Nitrogen Base	Dehydrated medium	4022552	500 g (74.5 L)

IFU rev 2, 2022/12

YERSINIA ITC BROTH BASE
POTASSIUM CHLORATE SUPPLEMENT
TICARCILLIN IRGASAN ANTIMICROBIC SUPPLEMENT
YERSINIA ITC BROTH

Dehydrated culture medium, selective supplements and ready-to-use flasks

INTENDED USE

Liquid enrichment medium for the detection of *Yersinia enterocolitica* in samples of the food chain.

COMPOSITION*

YERSINIA ITC BROTH BASE, DEHYDRATED MEDIUM
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	10.00 g
Yeast extract	1.00 g
Magnesium chloride anhydrous	28.10 g^
Sodium chloride	5.00 g
Malachite green	0.01 g

TICARCILLIN IRGASAN ANTIMICROBIC SUPPLEMENT
(VIAL CONTENTS FOR 250 ML OF MEDIUM)

Ticarcillin	0.25 mg
Irgasan	0.25 mg

POTASSIUM CHLORATE SUPPLEMENT
(VIAL CONTENTS FOR 250 ML OF MEDIUM)

Potassium chlorate 5% solution	5 mL

YERSINIA ITC BROTH, READY-TO-USE FLASKS
TYPICAL FORMULA

Tryptone	10.00 g
Yeast extract	1.00 g
Magnesium chloride anhydrous	28.10 g^
Sodium chloride	5.00 g
Malachite green	0.01 g
Potassium chlorate	1.00 g
Ticarcillin	1.00 mg
Irgasan	1.00 mg
Purified water	1000 mL

*The formulas may be adjusted and/or supplemented to meet the required performances criteria.
^Equivalent to magnesium chloride hexahydrate 60 g/L.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The genus *Yersinia* comprises Gram-negative coccobacilli, of which three species, *Yersinia pestis*, *Yersinia pseudotuberculosis*, and certain strains of *Yersinia enterocolitica* are of pathogenic importance for humans. *Y. enterocolitica* is ubiquitous, being isolated frequently from soil, water, animals, and a variety of foods.[1] The most common form of disease due to *Y. enterocolitica* is gastroenteritis associated with consumption of contaminated food or water.[2]
The detection of *Y. enterocolitica* can involve up to four successive steps: first enrichment, second enrichment, plating out, confirmation/identification.
Irgasan Ticarcillin Chlorate (ITC) broth is a selective enrichment liquid medium derived from the modified Rappaport base, developed by Wauters *et al*.[3] It was found to be the most efficient method for the recovery of *Y. enterocolitica* biotype 4, serotype 0:3, which is the most common clinical serotype in Europe.[3,4]
ITC broth is included in ISO 10273[5] procedure for the detection of *Y. enterocolitica* in samples of the food chain.

The detection method of pathogenic *Y. enterocolitica* recommended by ISO 10273[5] involves the homogenisation of the sample into PSB broth followed by: 1) direct inoculation onto CIN agar plates, 2) incubation of PSB broth, 3) second enrichment step in ITC broth, 3) alkaline treatment, 4) plating out of the treated enrichment broths onto CIN Agar.
Yersinia ITC Broth Base includes tryptone, providing nitrogen, amino acids and trace elements for microbial growth, yeast extract, a source of vitamins, particularly of the B-group, sodium chloride which contributes to maintaining the osmotic balance of the medium, and the basic selective compounds malachite green and magnesium chloride. Selectivity is improved by the antimicrobials included into selective supplements: irgasan with inhibitory properties against Gram negative bacteria, ticarcillin with bactericide activity mainly on Gram-negative but also on Gram-positive bacteria and potassium chlorate, inhibitory for *Enterobacteriaceae* possessing type A nitrase.[4]

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 11 g in 250 mL of cold purified water. Heat to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 47-50 °C and aseptically add the contents of one vial of Potassium Chlorate Supplement (REF 4240065) and one vial of Ticarcillin Irgasan Antimicrobic Supplement (REF 4240070) reconstituted with 2 mL of sterile purified water. Mix well and dispense the medium aseptically in 90 mL amounts into flasks of suitable capacity, so as to obtain the minimum area/volume ratio (relative anaerobiosis).

PHYSICAL CHARACTERISTICS

Yersinia ITC Broth Base	
Dehydrated medium appearance	beige-green, fine, homogeneous, free-flowing powder
Solution appearance	blue-green, limpid
Ticarcillin Irgasan Antimicrobic Supplement	
Freeze-dried supplement appearance	short, fragile, white pellet
Reconstituted supplement appearance	colourless, limpid
Potassium Chlorate Supplement	
Solution appearance	colourless, limpid
Final pH of complete medium at 20-25 °C	6.9 ± 0.2

SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling. Refer to applicable International Standards and regulations[5] for the collection, transport, storage, preparation of samples and operate in accordance with good laboratory practice.

TEST PROCEDURE

Detection of *Y. enterocolitica* according to ISO 10273[3]

1. Initial suspension: homogenize 25 g of sample into 225 mL of PSB Broth (REF 402270).
2. Transfer 10 mL of PSB suspension into 90 mL of ITC broth and mix.
3. Using the initial PSB suspension, divide a total volume of 1 mL onto two to four CIN agar plates^ and spread it over the plates.
4. Invert the CIN plates and incubate at 30 °C for 24 h ± 2 h.
5. Incubate the initial suspension in PSB broth and ITC broth at 25 ° for 44 h ± 4 h.
6. Perform the alkaline treatment by transferring 0.5 mL of the incubated PSB and ITC broths into 4.5 mL of KOH 0.5% in saline solution and by mixing.
7. After 20 ± 5 seconds of the addition of the PSB/ITC enrichments to the KOH solution, streak by means of a loop, the surface of a CIN agar plate and the surface of a chromogenic agar plate§ to obtain well-separated colonies.
8. Incubate CIN agar plates at 30 °C for 24 h ± 2 h. Incubate the chromogenic plates according to the instructions for use.
9. Perform the confirmation and bio typing tests according to the methods described by the ISO Standard.

Notes
^ CIN Agar Base (REF 401302) + Yersinia Selective Supplement (REF 4240011)
§ Chromogenic Yersinia Agar Base (REF 408050) + Chromogenic Yersinia Supplement (REF 4240095).

READING AND INTERPRETATION

Bacterial growth in ITC Broth is evidenced by the development of turbidity.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Y. enterocolitica ATCC 23715 + *P. aeruginosa* ATCC 27853+ *P. mirabilis* ATCC 29906	25°C/ 44h/ A	> 10 typical colonies after subculture onto CIN Agar

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The recovery and identification of pathogenic *Yersinia* may be influenced by the type of samples, the enrichment and plating media, level and type of background microflora, the level of pathogenic and non-pathogenic *Yersinia*, serotype of pathogenic *Yersinia* present in foods, and loss of virulence genes during incubation.[6]
- ITC broth performs well for the pathogenic *Y. enterocolitica* serotype O:3 but is less appropriate for other serotypes.[4]
- In foodborne outbreaks investigation, the cold enrichment procedure could be necessary to supplement the general procedure.[5]

STORAGE CONDITIONS

Dehydrated medium
Store at +10°C /+30°C away from direct light in a dry place.
Freeze-dried supplement and liquid supplement
Store the products in the original package at +2°C /+8°C away from direct light.
According to Curtis *et al.* the self-prepared complete medium in flasks may be stored at least 1 month at 2-8°C in screw-capped containers.[4]
Ready-to-use medium in flasks
Store flasks in their original pack at +2°C /+8°C away from direct light.

REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 8: Yersinia enterocolitica. Rev 10/2017
2. Petersen MJ, Gladney LM, Schriefer ME. Yersinia. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
3. Wauters G, Goossens V, Janssens M, Vandepitte J. New enrichment method for isolation of pathogenic Yersinia enterocolitica serogroup O:3 from pork. Appl Environ Microbiol. 1988; 54: 851–854.
4. Curtis GDW, Baird RM. Pharmacopoeia of Culture Media for Food Microbiology: Additional Monographs (II). Proceedings of the 6th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Heidelberg 30 March-3 April, 1992. Int J Food Microbiol 1993; 17:260-1.
5. ISO 10273:2017 Microbiology of the food chain-Horizontal method for the detection of pathogenic Yersinia enterocolitica.
6. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.

PACKAGING

Product	Type	REF	Pack
Yersinia ITC Broth Base	Dehydrated medium	4022652	500 g (11.3 L)
Ticarcillin Irgasan Antimicrobic Supplement	Freeze-dried supplement	4240060	10 vials, each for 250 mL of medium
Potassium Chlorate Supplement	Liquid supplement	4240065	10 vials, each for 250 mL of medium
Yersinia ITC Broth	Ready-to-use flasks	5122652	6 x 90 mL

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YERSINIA PSB BROTH

Dehydrated culture medium

INTENDED USE

Liquid enrichment medium for the detection of *Yersinia enterocolitica* in food, water, environmental samples.

COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptone	5.00 g
Sorbitol	10.00 g
Sodium chloride	5.00 g
Disodium hydrogen phosphate	8.23 g
Sodium dihydrogen phosphate	1.20 g
Bile salts n° 3	1.50 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The genus *Yersinia* comprises Gram-negative coccobacilli, of which three species, *Yersinia pestis*, *Yersinia pseudotuberculosis*, and certain strains of *Yersinia enterocolitica* are of pathogenic importance for humans. *Y. enterocolitica* is ubiquitous, being isolated frequently from soil, water, animals, and a variety of foods.[1] The most common form of disease due to *Y. enterocolitica* is gastroenteritis associated with consumption of contaminated food or water.[2]

The detection of *Y. enterocolitica* can involve up to four successive stages: first enrichment, second enrichment, plating out, identification.

Peptone Sorbitol Bile (PSB) Broth is a selective enrichment liquid medium included in ISO 10273[3], FDA-BAM[1] and APHA[4] procedures for the detection of *Y. enterocolitica* in food, water, and environmental samples.

The detection method of pathogenic *Y. enterocolitica* recommended by ISO 10273[3] involves the homogenisation of the sample into PSB broth followed by: 1) direct inoculation onto CIN agar plates 2) incubation of PSB broth 3) second enrichment step in ITC Broth 3) alkaline treatment, 4) plating out of the treated enrichment broths onto CIN Agar. The detection method recommended by FDA-BAM[1] and APHA[4] involves the enrichment in PSB broth followed by plating onto selective media (MacConkey Agar and CIN Agar plates).

Yersinia PSB Broth includes a peptone providing nitrogen, amino acids and trace elements for microbial growth. Sorbitol is a fermentable carbohydrate, source of carbon and energy. Bile salts n° 3 limits the growth of Gram-positive bacteria. Sodium chloride contributes to maintaining the osmotic balance of the medium. Phosphates are used as buffering agents to control the pH in the medium.

DIRECTIONS FOR MEDIUM PREPARATION

Suspend 31 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Dispense into tubes or flasks of suitable capacity to obtain portions appropriate for the test samples. Sterilise by autoclaving at 121°C for 15 minutes.

PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Prepared tubes appearance	pale yellow, limpid
Final pH at 20-25 °C	7.6 ± 0.2

SPECIMENS

Food, water, environmental samples. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.[1,3,4]

TEST PROCEDURE

Detection of *Y. enterocolitica* according to ISO 10273[3]

1. Initial suspension: homogenize 25 g of sample into 225 mL of Yersinia PSB Broth.
2. Transfer 10 mL of PSB suspension into 90 mL of ITC broth* and mix.
3. Using the initial PSB suspension, divide a total volume of 1 mL onto two to four CIN agar plates^ and spread it over the plates.
4. Invert the CIN plates and incubate at 30 °C for 24 h ± 2 h.
5. Incubate the initial suspension in PSB broth and the ITC broth flasks at 25 ° for 44 h ± 4 h.
6. Perform the alkaline treatment by transferring 0.5 mL of the incubated PSB and ITC broths into 4.5 mL of KOH 0.5% in saline solution and by mixing.
7. After 20 ± 5 seconds of the addition of the PSB/ITC enrichments to the KOH solution, streak by means of a loop, the surface of a CIN agar plate and the surface of a chromogenic agar plate§ to obtain well-separated colonies.
8. Incubate CIN agar plates at 30 °C for 24 h ± 2 h. Incubate the chromogenic plates according to the instructions for use.
9. Perform the confirmation and biotyping tests according to the methods described by the ISO Standard.

Notes
* Yersinia ITC Broth Base (REF 402265) + Potassium Chlorate Supplement (REF 4240065) + Ticarcillin Irgasan Antimicrobic Supplement (REF 4240060).
^ CIN Agar Base (REF 401302) + Yersinia Selective Supplement (REF 4240011)
§ Chromogenic Yersinia Agar Base (REF 408050) + Chromogenic Yersinia Supplement (REF 4240095).

READING AND INTERPRETATION

Bacterial growth in Yersinia PSB Broth is evidenced by the development of turbidity.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Y. enterocolitica ATCC 23715 + *P. aeruginosa* ATCC 27853	25°C/ 44h/ A	> 10 typical colonies after subculture onto CIN Agar

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The recovery and identification of pathogenic *Yersinia* may be influenced by the type of samples, the enrichment and plating media, level and type of background microflora, the level of pathogenic and non-pathogenic *Yersinia*, serotype of pathogenic *Yersinia* present in foods, and loss of virulence genes during incubation.[4]
- In foodborne outbreaks investigation, the cold enrichment procedure could be necessary to supplement the general procedure.[3]

STORAGE CONDITIONS

Store at +10°C /+30°C away from direct light in a dry place.

REFERENCES

1. U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM) Chapter 8: Yersinia enterocolitica. Rev 10/2017
2. Petersen MJ, Gladney LM, Schriefer ME. Yersinia. *In* Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015.
3. ISO 10273:2017 Microbiology of the food chain-Horizontal method for the detection of pathogenic Yersinia enterocolitica.
4. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.

PACKAGING

Product	Type	REF	Pack
Yersinia PSB Broth	Dehydrated medium	4022702	500 g (16 L)

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Section 3

Selective Supplements and Enrichments
Agar, Peptones and other Biological Materials
Miscellaneous Products

SELECTIVE SUPPLEMENTS AND ENRICHMENTS

AEROMONAS SELECTIVE SUPPLEMENT (AMPICILLIN)

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Ampicillin	5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of m-Aeromonas Selective Agar Base (Havelaar) REF 401019, autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.
USEPA methods require the addition of vancomycin hydrochloride: reconstitute one vial of Vancomycin Selective Supplement CSB (REF 4240057C) with 5 mL of sterile purified water; to 500 mL of medium prepared as described above with ampicillin, add 1 mL of Vancomycin Selective Supplement CBS (final concentration of vancomycin: 2 mg/L).

Refer to the monograph of m-Aeromonas Selective Agar Base (Havelaar) REF 401019 for the intended use, test procedure, and other information.

PACKAGING

Product	Type	REF	Pack
Aeromonas Selective Supplement-Ampicillin	Freeze-dried selective supplement	4240012	10 vials, each for 500 mL of medium

ALOA ENRICHMENT - SELECTIVE SUPPLEMENTS

COMPOSITION

ALOA® SELECTIVE SUPPLEMENT	**(VIAL CONTENTS FOR 500 mL OF MEDIUM)**
Nalidixic acid. sodium slat	0.010 g
Ceftazidime	0.010 g
Cycloheximide	0.025 g
Polymyxin B sulphate	38,350 UI

ALOA® ENRICHMENT SUPPLEMENT	**(VIAL CONTENTS FOR 500 mL OF MEDIUM)**
L-α-phosphatidylinositol	1.0 g

DIRECTIONS

Dissolve the contents of one vial of ALOA Selective Supplement (REF 423501) with 5 mL of sterile purified water, under aseptic conditions. Add to 500 mL of Agar Listeria acc. to Ottaviani and Agosti (REF 401605), autoclaved at 121°C for 15 minutes and cooled to 47-50°C; also add the contents of one vial of ALOA Enrichment Supplement (REF 423501), pre-warmed to 48-50°C. Mix well and distribute in sterile Petri dishes.

Refer to the monograph of ALOA-Agar Listeria acc. to Ottaviani and Agosti (REF 401605) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
ALOA® Enrichment Selective Supplements	Freeze-dried and liquid supplements	423501	4+4 vials, each for 500 mL of medium

BACILLUS CEREUS ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Polymyxin B sulphate	50,000 IU

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions.
Use for the supplementation of the following basal media cooled to 44-47°C:
450 mL of Bacillus Cereus Agar Base (MYP) REF 401111 + 50 mL of Egg Yolk Emulsion REF 42111601,
470 mL of Bacillus Cereus Agar Base (PEMBA) REF 401112 + 25 mL of Egg Yolk Emulsion REF 42111601.

Refer to the monographs of the relevant basal media for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Bacillus Cereus Antimicrobic Supplement	Freeze-dried supplement	4240001	10 vials, each for 500 mL of medium

BCSA SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	1.25 mg
Gentamicin	5 mg
Polymyxin B	300,000 UI

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of BCSA Burkholderia Cepacia Selective Agar Base (REF 401153), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of BCSA Burkholderia Cepacia Selective Agar Base (REF 401153) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
BCSA Selective Supplement	Freeze-dried supplement	4240073	10 vials, each for 500 mL of medium

BIOVITEX -RESTORING FLUID

COMPOSITION

BIOVITEX - VIAL CONTENTS	**FOR 500 mL**	**FOR 5 L**
Diphosphopyridine nucleotide	1.250 mg	12.50 mg
Cocarboxylase	0.500 mg	5.00 mg
p-Aminobenzoic acid	0.065 mg	0.65 mg
Thiamine HCl	0.015 mg	0.15 mg
Vitamin B_{12}	0.050 mg	0.50 mg
L-Glutamine	50.000 mg	500.00 mg
L-Cystine	5.500 mg	55.00 mg
L-Cysteine HCl	129.500 mg	1295.00 mg
Adenine	5.000 mg	50.00 mg
Guanine HCl	0.150 mg	1.50 mg
Ferric nitrate	0.100 mg	1.00 mg
RESTORING FLUID - VIAL CONTENTS	**FOR 500 mL**	**FOR 5 L**
Glucose	0.5 g	5 g
Purified water	5.0 mL	50 mL

DIRECTIONS

Aseptically reconstitute the freeze-dried Biovitex with the contents of one vial of Restoring fluid.
Prepare 500 mL (or 5000 mL) of GG Medium Base or another suitable base medium, supplemented with haemoglobin or chocolatized blood cooled to 47-50°C. Add the Biovitex and any other selective supplements (VCN, VCNT etc) and mix. Normally the working concentration for Biovitex is 1 %; however, a 2 % concentration can be achieved by adding the vial contents to only 250 mL (or 2500 mL) of base medium. This higher concentration gives increased stimulation of *Neisseria* growth.

Refer to the monograph of the following media for the intended use, test procedure and other information.
GC Medium Base dehydrated medium, REF 4015202
Chocolate Agar Bacitracin, ready-to-use plates, REF 541519
Chocolate Agar Enriched, ready-to-use plates, REF 541521
Modified Thayer Martin (MTM) Medium, ready-to-use plates, REF 541522
Ureaplasma Differential Agar A7, ready-to-use plates, REF 542181

PACKAGING

Product	Type	REF	Pack
Biovitex-Restoring Fluid	Enrichment supplement	4240009	5 + 5 vials, each for 500 mL of medium
Biovitex-Restoring Fluid	Enrichment supplement	42185011	1 vial of Biovitex+1 vial of Restoring Fluid for 5000 mL of medium

BOLTON BROTH SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cefoperazone	10 mg
Vancomycin	10 mg
Trimethoprim lactate	10 mg
Amphotericin B	5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of 50% ethanol/sterile purified water, using aseptic conditions. Add to 500 mL of Campylobacter Bolton Enrichment Broth Base (REF 401286B), autoclaved at 121°C for 15 minutes and cooled below 47°C. Also add 25 mL of lysed horse blood (REF 90HLX100). Mix well and distribute into sterile tubes or flasks.

Refer to the monograph of Campylobacter Bolton Enrichment Broth Base (REF 401286B) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Bolton Broth Selective Supplement	Freeze-dried supplement	4240025	10 vials, each for 500 mL of medium

BOLTON CCDA ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cefoperazone	16 mg
Amphotericin B	5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of Campylobacter Blood Free Medium Base Bolton (m-CCDA) (REF 401282), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Campylobacter Blood Free Medium Base Bolton (m-CCDA) (REF 401282) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Bolton CCDA Antimicrobic Supplement	Freeze-dried supplement	4240020	10 vials, each for 500 mL of medium

BRILLIANT GREEN 0.1% SOLUTION

COMPOSITION - FLASK CONTENTS

Brilliant green	50 mg
Purified water	50 mL

DIRECTIONS

The solution is ready to use. Brilliant Green 0.1% Solution may be used for the supplementation of the following media:
Muller Kauffmann Tetrathionate Broth Base, dehydrated culture medium, REF 401743, 9.5 mL/litre
Tetrathionate Broth Base, dehydrated culture medium, REF 402125, 10 mL/litre

Refer to the monographs of relevant basal media for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Brilliant Green 0.1% Solution	Liquid supplement	421505	50 mL

CAMPYLOBACTER GROWTH SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Sodium pyruvate	125 mg
Sodium metabisulphite	125 mg
Ferrous sulphate	125 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions.
Use for the supplementation of the following basal media cooled to 44-47°C:
Columbia Agar Base, dehydrated medium, REF 401136 (for the preparation of Skirrow medium)
Nutrient Broth n° 2, dehydrated medium, REF 401286B (for the preparation of Preston Broth)

Refer to the monographs of relevant basal media for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Campylobacter Growth Supplement	Freeze-dried supplement	4240021	10 vials, each for 500 mL of medium

CEFIXIME TELLURITE O157 SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cefixime	0.025 mg
Potassium tellurite	1.250 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions.
Use for the supplementation of the following basal media cooled to 44-47°C:
Mac Conkey Sorbitol Agar, dehydrated medium, REF 401669S2; Mac Conkey Sorbitol MUG Agar, dehydrated medium, REF 4016692
Chromogenic E. coli O157 Agar, dehydrated medium, REF 4055812

Refer to the monographs of relevant basal media for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Cefixime Tellurite O157 Supplement	Freeze-dried supplement	42ISEC	10 vials, each for 500 mL of medium

CFC PSEUDOMONAS SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cetrimide	5 mg
Fusidic acid	5 mg
Cephalosporin	25 mg

DIRECTIONS

Dissolve the contents of one vial with 2 mL of sterile purified water/ethanol (1:1), using aseptic conditions. Add to 500 mL of Pseudomonas Agar Base (REF 401960), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Pseudomonas Agar Base, REF 401960, for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
CFC Pseudomonas Supplement	Freeze-dried supplement	4240075	10 vials, each for 500 mL of medium

CHLORAMPHENICOL ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS

Chloramphenicol	50 mg

DIRECTIONS

Dissolve the contents of one vial with 3 mL of a mixture of sterile purified water-ethanol (1:1), using aseptic conditions.
Use for the supplementation of the following basal media before or after autoclaving:
DG 18 Agar Base, dehydrated medium, REF 401394
DRBC Agar Base, dehydrated medium, REF 4013932
Rose Bengal Agar Base, dehydrated medium, REF 4019912
Sabouraud Dextrose Agar, dehydrated medium, REF 2005
In yeast and mould isolation media, chloramphenicol is used at a concentration of 50 mg/L although its concentration can be increased to 100-500 mg/L.

Refer to the monographs of relevant basal media for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Chloramphenicol Antimicrobic Supplement	Freeze-dried supplement	4240003	10 vials, each for 500 mL of medium

CHROMART CRE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Antimicrobials mix	0.21 g

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of CRE-ESBL Agar Base (REF 408025), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of CRE-ESBL Agar Base (REF 408025), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
ChromArt CRE Supplement	Freeze-dried supplement	4240082	10 vials, each for 500 mL of medium base

CHROMART ESBL SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Antimicrobials mix	0.21 g

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of CRE-ESBL Agar Base (REF 408025), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Consult the monograph of CRE-ESBL Agar Base (REF 408025), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
ChromArt ESBL Supplement	Freeze-dried supplement	4240082	10 vials, each for 500 mL of medium base

CHROMOGENIC BACILLUS CEREUS SUPPLEMENTS

COMPOSITION

CHROMOGENIC B. CEREUS SELECTIVE SUPPLEMENT (4240090S)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Antimicrobial mix	75 mg

CHROMOGENIC B. CEREUS ENRICHMENT SUPPLEMENT (4240090E)
VIAL CONTENTS FOR 500 mL OF MEDIUM

Phospholipids	10 mL

DIRECTIONS

Dissolve the contents of one vial of Chromogenic B. Cereus Selective Supplement (REF 4240090S) with 5 mL of sterile purified water, under aseptic conditions. Add to 500 mL of Chromogenic B. Cereus Agar Base (REF 408020), autoclaved at 121°C for 15 minutes and cooled to 42-45 °C; also add the contents of one vial of Chromogenic B. Cereus Enrichment Supplement (REF 4240090E). Mix well and distribute in sterile Petri dishes

Refer to the monograph of Chromogenic B. Cereus Agar Base (REF 408020) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Chromogenic B. Cereus Supplements	Freeze-dried and liquid supplements	4240090	4 + 4 vials (each vial is for 500 mL of medium base)

CHROMOGENIC YERSINIA SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Antibiotic mix	0.0875 g

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of Chromogenic Yersinia Agar Base (REF 408050), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Chromogenic Yersinia Agar Base, (REF 408050), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Chromogenic Yersinia Supplement	Freeze-dried supplement	4240095	10 vials, each for 500 mL of medium

CLOSTRIDIUM BOTULINUM ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

D-cycloserine	125 mg
Trimethoprim	2 mg
Sulphamethoxazole	38 mg

DIRECTIONS

Dissolve the contents of one vial with 2.5 mL of sterile purified water followed by 2.5 mL of acetone, using aseptic conditions. Add to 450 mL of Clostridium Botulinum Agar Base (REF 401306), autoclaved at 121°C for 15 minutes and cooled to 47°C-50°C; also add 50 mL of Egg Yolk Emulsion (cat. N° 4244160). Mix well and pour into sterile Petri dishes.

Refer to the monograph of Clostridium Botulinum Agar Base, dehydrated medium (REF 401306), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Clostridium Botulinum Antimicrobic Supplement	Freeze-dried supplement	4240066	10 vials, each for 500 mL of medium

CN PSEUDOMONAS SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cetrimide	100.0 mg
Nalidixic acid	7.5 mg

DIRECTIONS

Dissolve the contents of one vial with 2 mL of sterile purified water/ethanol (1:1), using aseptic conditions. Add to 500 mL of Pseudomonas Agar Base (REF 401960) supplemented with 5 g of glycerol (REF 421025), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Pseudomonas Agar Base, REF 4019602, for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
CN Pseudomonas Supplement	Freeze-dried supplement	4240046	10 vials, each for 500 mL of medium

CNA ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Colistin	5.0 mg
Nalidixic Acid	7.5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of Columbia Agar Base (REF 401136) or other suitable medium, autoclaved at 121°C for 15 minutes and cooled to 47°C-50°C; also add 25 mL of defibrinated animal blood. Mix well and pour into sterile Petri dishes.

Refer to the monographs of Columbia Agar Base, (REF 401136) and Columbia CNA Agar Base (REF 4011361), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
CNA Antimicrobic Supplement	Freeze-dried supplement	4240018	10 vials, each for 500 mL of medium

D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

D-cycloserine	200 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions.
Use for the supplementation of the following basal media autoclaved and cooled to 47°C-50°C:
500 mL TSC Agar Base. If required, 25 mL of Egg Yolk Emulsion (REF 42111601) may be added to the precooled medium base.
475 mL of Clostridium Perfringens Agar Base (REF 401307) + 25 mL Egg Yolk Emulsion (REF 42111601)

Refer to the monographs of TSC Agar Base (REF 402158) and Clostridium Perfringens Agar Base (REF 401307) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
D-Cycloserine Antimicrobic Supplement	Freeze-dried supplement	4240002	10 vials, each for 500 mL of medium

D-CYCLOSERINE 4-MUP ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

D-cycloserine	200 mg
4-methylumbelliferyl phosphate	50 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of TSC Agar Base (REF 402158), autoclaved at 121°C for 15 minutes and cooled to 47°C-50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of TSC Agar Base (REF 402158), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
D-Cycloserine 4-MUP Supplement	Freeze-dried supplement	4240049	10 vials, each for 500 mL of medium

DERMATOPHYTE ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Chlortetracycline HCl	50 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of Dermatophyte Selective Medium -DTM- (Taplin) (REF 4013691), autoclaved at 115°C for 10 minutes and cooled to 47°C-50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Dermatophyte Selective Medium -DTM- (Taplin) (REF 4013691), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Dermatophyte Antimicrobic Supplement	Freeze-dried supplement	4240024	10 vials, each for 500 mL of medium

DMLIA NOVOBIOCIN SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Novobiocin	7.5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 500 mL of Double Modified Lysine Iron Agar (DMLIA) (REF 401325), cooled to 47°C-50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of dehydrated medium Double Modified Lysine Iron Agar (DMLIA) (REF 4013691), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
DMLIA Novobiocin Supplement	Freeze-dried supplement	4240029	10 vials, each for 500 mL of medium base

EGG YOLK EMULSION 50%

COMPOSITION - BOTTLE CONTENTS

EGG YOLK EMULSION 500%, 50 mL, REF 42111601

Egg yolk	25 mL
Saline solution	25 mL

EGG YOLK EMULSION 50%, 200 mL, REF 42111600

Egg yolk	100 mL
Saline solution	100 mL

EGG YOLK EMULSION 50%, 100 mL, REF 42111605

Egg yolk	50 mL
Saline solution	50 mL

DIRECTIONS

The emulsion is ready to use. Egg Yolk Emulsion may be used for the supplementation of the following media:
Bacillus Cereus Agar Base - MYP, REF 401111 (50 mL/450 mL)
Bacillus Cereus Agar Base - PEMBA, REF 401112 (25 mL/470 mL)
Clostridium Botulinum Agar Base, REF 401306 (50 mL/450 mL)
Clostridium Perfringens Agar Base REF 401307 (50 mL/450 mL

Consult the monographs of relevant basal media for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Egg Yolk 50% Solution	Liquid supplement	42111601 42111605 42111600	50 mL 100 mL 200 mL

EGG YOLK TELLURITE EMULSION 20%

COMPOSITION - BOTTLE CONTENTS

EGG YOLK TELLURITE EMULSION 20% 50 mL, REF 42111601

Egg yolk	10 mL
Potassium tellurite	105 mg
Saline solution	40 mL

EGG YOLK TELLURITE EMULSION 20%, 200 mL, REF 42111600

Egg yolk	40.0 mL
Potassium tellurite	420.0 mg
Saline solution	160 mL

EGG YOLK TELLURITE EMULSION 20%, 100 mL, REF 42111605

Egg yolk	20 mL
Potassium tellurite	210 mg
Saline solution	80 mL

DIRECTIONS

The emulsion is ready to use. Egg Yolk Tellurite Emulsion 20% may be used for the supplementation of Baird Parker Base Agar REF 401116.
Prepare 1000 mL of Baird Parker Base Agar, autoclaved and cooled to 47-50°C. Using aseptic conditions, add 50 mL of Egg Yolk Tellurite Emulsion 20%, mix well and pour into sterile Petri dishes
Consult the monographs of Baird Parker Base Agar REF 401116, for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Egg Yolk Tellurite Emulsion 20%	Liquid supplement	423700 423701 423702	50 mL 100 mL 200 mL

EGG YOLK TELLURITE EMULSION 50%

COMPOSITION - BOTTLE CONTENTS

EGG YOLK TELLURITE EMULSION 50% 50 mL, REF 42111602

Egg yolk	25 mL
Potassium tellurite	105 mg
Saline solution	25 mL

EGG YOLK TELLURITE EMULSION 50%, 100 mL, REF 42111603

Egg yolk	50 mL
Potassium tellurite	210 mg
Saline solution	50 mL

EGG YOLK TELLURITE EMULSION 50%, 200 mL, REF 42111604

Egg yolk	100.0 mL
Potassium tellurite	420.0 mg
Saline solution	100 mL

DIRECTIONS

The emulsion is ready to use. Egg Yolk Tellurite Emulsion 20% may be used for the supplementation of Baird Parker Base Agar REF 401116. Consult the monographs of Baird Parker Base Agar REF 401116, for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Egg Yolk Tellurite Emulsion 50%	Liquid supplement	42111602 42111603 42111604	50 mL 100 mL 200 mL

EUGON LT SUPPLEMENT

COMPOSITION - BOTTLE CONTENTS FOR 1 LITRE OF MEDIUM

Sodium laureth sulphate	1.56 g
Egg lecithin	1.00 g
Polysorbate 80	15.00 g
Purified water	85.00 mL

DIRECTIONS

Suspend 30.4 g of Eugon Broth REF 401643 in 900 mL of cold purified water. Heat to boiling stirring constantly and add the content of one flask of Eugon LT Supplement (100 mL). Distribute in tubes or bottles and sterilize by autoclaving at 121°C for 15 minutes. After autoclaving, gently shake the medium to mix the phases that may have formed.

Consult the monograph of Eugon Broth REF 401643, for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Eugon LT Supplement	Liquid supplement	421540	6 x 100 mL

FRASER HALF SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 225 mL OF MEDIUM

Ferric ammonium citrate	112.50 mg
Nalidixic acid	2.25 mg
Acriflavine HCl	2.81 mg

DIRECTIONS

Dissolve the contents of one vial with 3 mL of ethanol/ sterile purified water (1:1), using aseptic conditions. Add to 225 mL of Fraser Broth Base (REF 401495), autoclaved at 121°C for 15 minutes and cooled to room temperature.

Refer to the monograph of Fraser Broth Base (REF 401495), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Fraser Half Selective Supplement	Freeze-dried supplement	4240044	10 vials, each for 225 mL of medium

FRASER SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Ferric ammonium citrate	250.0 mg
Nalidixic acid	10.0 mg
Acriflavine HCl	12.5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of ethanol/ sterile purified water (1:1), using aseptic conditions. Add to 500 mL of Fraser Broth Base (REF 401495), autoclaved a at 121°C for 15 minutes nd cooled to room temperature. Mix well and pour into sterile tubes or flasks under aseptic conditions.

Refer to the monograph of Fraser Broth Base (REF 401495), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Fraser Selective Supplement	Freeze-dried supplement	4240043	10 vials, each for 500 mL of medium

GARDNERELLA ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Gentamicin	2 mg
Nalidixic Acid	15 mg
Amphotericin B	1 mg

DIRECTIONS

Dissolve the contents of one vial with 2 mL of a 1:1 (v/v) mixture of ethanol and purified water, using aseptic conditions. Add to 500 mL of Columbia Agar Base (REF 401136), autoclaved at 121°C for 15 minutes and cooled to 47°C-50°C; also add 25 mL of human, sheep or horse blood. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Columbia Agar Base, (REF 401136), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Gardnerella Antimicrobic Supplement	Freeze-dried supplement	4240019	10 vials, each for 500 mL of medium

GENTAMICIN ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Gentamicin	25 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, under aseptic conditions. Add to 500 mL of OGYE Agar Base, REF4013932, autoclaved at 115ºC for 15 minutes and cooled to 47°C- 50°C; add also the contents of one vial of Oxytetracycline Antimicrobic Supplement (REF 424000). Mix well and pour into sterile Petri dishes.

Refer to the monograph of OGYE Agar Base (REF4013932), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Gentamicin Antimicrobic Supplement	Freeze dried supplement	4240004	10 vials, each for 500 mL of medium

GLYCEROL

COMPOSITION - FLASK CONTENTS

Glycerol	100 mL

DIRECTIONS

The solution is ready to use. Glycerol may be used for the supplementation of the following media:
DG 18 Agar Base, REF 401394, 220 g/litre
DG 18 Chloramphenicol Agar Base, REF 401394C, 220 g/L
Pseudomonas Agar Base, REF 401960, 10 mL/L
STAA Agar Base (REF 402079), 15 g/L

Refer to the monographs of the relevant basal media for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Glycerol	Liquid supplement	421015	100 mL

IODINE SOLUTION

COMPOSITION - FLASK CONTENTS

Iodine	10.0 g
Potassium iodide	12.5 g
Purified water	50 mL

DIRECTIONS

The solution is ready to use. Iodine Solution may be used for the supplementation of the following media:
Muller Kauffmann Tetrathionate Broth Base, dehydrated culture medium, REF 401743, 19 mL mL/litre
Muller Kauffmann Tetrathionate Broth Base ISO Formulation, Dehydrated culture medium, REF401745, 20 mL/litre
Tetrathionate Broth Base, dehydrated culture medium, REF 402125, 20 mL/litre

Refer to the monographs of relevant basal media for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Iodine Solution	Liquid supplement	421501	50 mL

KANAMYCIN POLYMYXIN B ANTIMICROBIC SUPPLEMENT (SFP)

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Kanamycin sulphate	6 mg
Polymyxin B	15,000 U.I.

DIRECTIONS

Preparation of SFP Agar

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Add to 450 mL of Clostridium Perfringens Agar Base (REF 401307) autoclaved at 121°C for 15 and cooled to 47°C-50°C; also add 50 mL of Egg Yolk Emulsion (REF 42111601). Mix well and pour into sterile Petri dishes.

Refer to the monograph of Clostridium Perfringens Agar Base, dehydrated medium (REF 401307) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Kanamycin Polymyxin B Antimicrobic Supplement	Freeze-dried supplement	4240005	10 vials, each for 500 mL of medium

KANAMYCIN SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Kanamycin sulphate	10 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, using aseptic conditions. Use for the supplementation of the following basal media autoclaved and cooled to 44-47°C:
500 mL Kanamycin Aesculin Azide Agar Base, REF 401552;
500 mL E.C.O.A.gar, REF 401430

Refer to the monographs of relevant basal media for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Kanamycin Selective Supplement	Freeze-dried supplement	4240055	10 vials, each for 500 mL of medium

KARMALI ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cefoperazone	16 mg
Vancomycin	10 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water. Add to 500 mL of Campylobacter Blood Free Medium Base Karmali (REF 401283), autoclaved at 118°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Campylobacter Blood Free Medium Base Karmali (REF 401283), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Karmali Antimicrobic Supplement	Freeze-dried supplement	4240035	10 vials, each for 500 mL of medium

LEGIONELLA SUPPLEMENTS

LEGIONELLA GVPC SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Glycine	1.5 g
Vancomycin HCl	0.5 mg
Polymyxin B	40.000 IU
Cycloheximide	40.0 mg

LEGIONELLA AB SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefazolin	4.5 mg
Polymyxin B	40,000 UI
Pimaricin (natamycin)	35 mg

LEGIONELLA MWY SELECTIVE SUPPLEMENT (ISO)
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Glycine	1.5 g
Vancomycin HCl	0.5 mg
Polymyxin B	25.000 UI
Anisomycin	40 mg
Bromothymol blue	5.0 mg
Bromocresol purple	5.0 mg

LEGIONELLA BCYE α-GROWTH SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

ACES Buffer/Potassium hydroxide	6.4 g
α–ketoglutarate	0.5 g
Ferric pyrophosphate	125.0 mg
L-Cysteine HCl	200.0 mg

LEGIONELLA BCYE α-GROWTH SUPPLEMENT W/O CYSTEINE
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

ACES Buffer/Potassium hydroxide	6.4 g
α–ketoglutarate	0.5 g
Ferric pyrophosphate	125.0 mg

DIRECTIONS FOR MEDIA PREPARATION

Suspend12.5 g of Legionella BCYE Agar Base (REF 401582) in 450 mL of cold purified water. Heat to boiling with agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C. Add the suitable growth and selective supplements. After supplements addition, keeping the medium under stirring, distribute into sterile Petri dishes.

SELECTIVE MEDIUM BCYE-GVPC

To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella GVPC Selective Supplement (REF 423215) reconstituted with 10 mL of sterile purified water.

SELECTIVE MEDIUM BCYE-AB

To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella AB Selective Supplement (REF 423225), reconstituted with 5 mL of sterile purified water.

SELECTIVE MEDIUM BCYE-MWY (WITH ANISOMYCIN)

To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella MWY Selective Supplement (ISO) (REF 423220), reconstituted with 10 mL of sterile purified water.

NON-SELECTIVE MEDIUM WITH CYSTEINE: BCYE W/ L-CYSTEINE

To the medium base cooled to 47-50°C add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210), reconstituted with 50 mL of sterile purified water.

NON-SELECTIVE MEDIUM WITHOUT CYSTEINE: BCYE W/O L-CYSTEINE

To the medium base cooled to 47-50°C add the contents of one vial of Legionella BCYE α-Growth Supplement w/o Cysteine (REF 423212), reconstituted with 50 mL of sterile purified water.

Refer to the monograph of Legionella Agar Base (REF 401582), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Legionella BCYE α-Growth Supplement	Lyophilized supplement	423210	4 vials, each for 500 mL of medium
Legionella BCYE α-Growth Supplement w/o Cysteine	Lyophilized supplement	423212	4 vials, each for 500 mL of medium
Legionella GVPC Selective Supplement	Lyophilized supplement	423215	4 vials, each for 500 mL of medium
Legionella AB Selective Supplement	Lyophilized supplement	423225	10 vials, each for 500 mL of medium
Legionella MWY Selective Supplement (ISO)	Lyophilized supplement	423220	4 vials, each for 500 mL of medium

LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)

COMPOSITION

	VIAL CONTENTS FOR 500 mL OF MEDIUM REF 4240056	VIAL CONTENTS FOR 5 L OF MEDIUM REF 42185056 and 42185056A
Ferric Ammonium Citrate	0.25 g	2.5 g

DIRECTIONS

Vial for 500 mL of medium

Dissolve the contents of one vial with 5 mL of purified water, using aseptic conditions. Add to 500 mL of Listeria Fraser Broth Base (REF 401596), autoclaved at 121°C for 15 minutes and cooled to room temperature. Mix well and pour into sterile tubes or flasks under aseptic conditions.

Vial for 5000 mL of medium

Dissolve the contents of one vial with 20 mL of purified water, using aseptic conditions. Add to 5000 mL of Listeria Fraser Broth Base (REF 401596), autoclaved and cooled to room temperature. Mix well and pour into sterile tubes or flasks under aseptic conditions.

Refer to the monograph of Fraser Broth Base (REF 401495), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	4240056	10 vials, each for 500 mL of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056	1 vial for 5 L of medium
Listeria Fraser Supplement (Fe Ammonium Citrate)	Freeze-dried supplement	42185056A	9 vials, each for 5 L of medium

LISTERIA MOX-COL ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Moxalactam	10.0 mg
Colistin sulphate	5.0 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, under aseptic conditions. Add to 500 mL of Listeria Oxford Agar Base (REF 401600), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Consult the monograph of Listeria Oxford Agar Base (REF 401600), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Listeria MOX-COL Antimicrobic Supplement	Freeze-dried supplement	4240039	10 vials, each for 500 mL of medium

LISTERIA OXFORD ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cycloheximide	200.0 mg
Colistin sulphate	10.0 mg
Cefotetan	1.0 mg
Fosfomycin	5.0 mg
Acriflavine	2.5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of a solution of 1:1 ethanol/sterile purified water, under aseptic conditions. Add to 500 mL of Listeria Oxford Agar Base (REF 401600), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Consult the monograph of Listeria Oxford Agar Base (REF 401600), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Listeria Oxford Antimicrobic Supplement	Freeze-dried supplement	4240038	10 vials, each for 500 mL of medium

LISTERIA PALCAM ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Polymyxin B sulphate	5 mg
Ceftazidime	10 mg
Acriflavine HCl	2.5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of a solution of 1:1 ethanol/sterile purified water, under aseptic conditions. Add to 500 mL of Listeria PALCAM Agar Base (REF 401604), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Listeria PALCAM Agar Base (REF 401604), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Listeria PALCAM Antimicrobic Supplement	Freeze-dried supplement	4240042	10 vials, each for 500 mL of medium

LYSED HORSE BLOOD

COMPOSITION - FLASK CONTENTS

Lysed (laked) horse blood	100 mL

DIRECTIONS

The product is ready to use. Lysed horse blood should be used for the supplementation of a variety of culture media, including *Campylobacter* detection media: Campylobacter Blood Agar Base REF 401285, Campylobacter Enrichment Broth Base, REF 401286, Campylobacter Bolton Enrichment Broth Base REF 401286B, Preston broth (Nutrient Broth n° 2 REF 401812S2), Skirrow medium (Columbia Blood Agar REF 401136)

Refer to the monographs of relevant basal media for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Lysed Horse Blood	Liquid supplement	90HLX100	100 mL

m-CP SUPPLEMENTS

COMPOSITION

M-CP ANTIMICROBIC SUPPLEMENT
(VIAL CONTENT FOR 500 mL OF MEDIUM BASE)

Polymyxin B sulphate	12.5 mg (105,000 IU)
D-cycloserine	200 mg

M-CP SUPPLEMENT A
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Phenolphthalein diphosphate	750 mg

M-CP SUPPLEMENT B
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Ferric chloride	700 mg

M-CP SUPPLEMENT C
(VIAL CONTENTS FOR 500 G OF MEDIUM BASE)

Indoxyl β-D glucoside	450 mg

DIRECTIONS

Suspend 34.7 g of m-CP Agar Base (REF 401320) in 500 mL of cold purified water, heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add and the contents of one vial of m-CP Antimicrobic Supplement (REF 4240070), reconstituted with 5 mL of sterile purified water. Aseptically add the following fresh filter sterilised solutions, prepared with the powdered compounds supplied with the vials of m-CP Supplements A, B and C dissolved in purified water:

Phenolphthalein diphosphate (REF 4240070A)	0.5% solution	10 mL in 500 mL of medium base
Ferric chloride hexahydrate (REF 4240070B)	4.5% solution	1 mL in 500 mL of medium base
Indoxyl β -D-glucoside (REF 4240070C)	0.75% solution	4 mL in 500 mL of medium base

Mix well and pour into sterile Petri dishes.

Refer to the monograph of m-CP Agar Base (REF 401320), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
m-CP Agar Base	Dehydrated medium	4013202	500 g (7.2 L)
m-CP Antimicrobic Supplement	Freeze-dried supplement	4240070	10 vials, each for 500 mL of medium
m-CP Supplement A (Phenolphthalein diphosphate	Powdered supplement	4240070A	1 vial (750 mg for 500 g of medium)
m-CP Supplement B (Ferric chloride)	Powdered supplement	4240070B	1 vial (700 mg for 500 g of medium)
m-CP Supplement C (Indoxyl β -D-glucoside)	Powdered supplement	4240070C	1 vial (450 mg for 500 g of medium)
m-CP Agar	Ready-to-use plates	491320	3 x 10 plates, ø 55 mm

NOVOBIOCIN ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS

Novobiocin 10 mg

DIRECTIONS

MSRV MEDIUM

Suspend 31.6 g of Rappaport Vassiliadis Semisolid Medium Modified (MSRV) REF 40198, in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely. Do not autoclave. Cool to approximately 47-50°C.

MSRV medium ISO 6579-1 formulation

Add the contents of one vial of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 10 mg/L

MSRV medium original De Smedt formulation

Add the contents of two vials of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Mix well and pour 15-20 mL into sterile Petri dishes and leave to dry for one hour. Novobiocin concentration in final medium: 20 mg/L

SHIGELLA BROTH

Suspend 31.5 g of Shigella Broth Base REF 402040 in 1000 mL of cold, purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 225 mL in bottles and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature.

Dissolve the content of one vial of Novobiocin Antimicrobic Supplement (REF 4240045) with 4 mL of sterile purified water (novobiocin concentration: 2.5 mg/mL). Add a volume of novobiocin solution to the basic medium to obtain the required antibiotic concentration:

Shigella Broth according to ISO 21567 and FDA-BAM for *S. sonnei*: add 50 µL of solution to 225 mL of Shigella Broth Base (final concentration of 0,5 µg/mL broth after 25 g or 25 mL of sample is added).

Shigella Broth according to FDA-BAM for other *Shigella* species: add 300 µL of solution to 225 mL of Shigella Broth Base (final concentration 3 µg/mL broth after 25 g or 25 mL of sample is added).

M-TSB

Suspend 33 g of Tryptic Soy Broth Modified (mTSB), REF 402155M2, in 1000 mL of cold purified water. Mix well and, if necessary, heat slightly to completely dissolve the powder. Distribute 225 mL into flasks of suitable capacity and sterilise by autoclaving at 121°C for 15 minutes. Cool to room temperature and to each 225 mL flask add, under aseptic conditions, 2.25 mL of Novobiocin Antimicrobic Supplement (REF 4240045), reconstituted with 5 mL of sterile purified water. Final concentrations: 4.5 mg/225 mL or 20 mg/litre.

After reconstitution of the supplement, the remaining novobiocin solution can be stored at 2°C-8°C for one month

Refer to the monographs of relevant basal media for the intended use, test procedures and other information.

PACKAGING

Product	Type	REF	Pack
Novobiocin Antimicrobic Supplement	Freeze-dried supplement	4240045	10 vials, 10 mg/vial

NOVOBIOCIN MKTT SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS

Novobiocin 20 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, under aseptic conditions. Add to 500 mL of Muller Kauffmann Tetrathionate Broth Base ISO Formulation (REF 401745), cooled to below 47°C. Also add 10 mL of Iodine Solution (ref. n° 421501. Mix well and aseptically distribute 10 mL into sterile tubes.

Consult the monograph of Muller Kauffmann Tetrathionate Broth Base ISO Formulation (REF 401745), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Novobiocin MKTT Selective Supplement	Freeze-dried supplement	4240047	10 vials, each for 500 mL of medium

OXYTETRACYCLINE ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Oxytetracycline HCl 50 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water, under aseptic conditions. Add to 500 mL of OGYE Agar Base, REF4013932, autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of OGYE Agar Base (REF4013932), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Oxytetracycline Antimicrobic Supplement	Freeze dried supplement	4240000	10 vials, each for 500 mL of medium

PENICILLIN 500 IU SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 100 mL OF MEDIUM

Penicillin G sodium salt 500 IU

DIRECTIONS

Dissolve the contents of one vial with 2 mL of sterile purified water, under aseptic conditions. Add to 100 mL of Sugar Free Agar Base, REF402098, autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Sugar Free Agar Base (REF402098), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Penicillin G 500 IU Selective Supplement	Freeze-dried supplement	4240050	10 vials, each for 100 mL of medium

POTASSIUM CHLORATE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 250 mL OF MEDIUM

Potassium chlorate 5% solution 5 mL

DIRECTIONS

The solution is ready to use.
Prepare 250 mL of Yersinia ITC Broth Base, REF 402265, autoclaved at 121°C for 15 minutes and cooled to approximately 47-50 °C; aseptically add the contents of one vial of Potassium Chlorate Supplement (REF 4240065) and one vial of Ticarcillin Irgasan Antimicrobic Supplement (REF 4240070) reconstituted with 2 mL of sterile purified water. Mix well and dispense the medium aseptically in 90 mL amounts into flasks of suitable capacity, so as to obtain the minimum area/volume ratio (relative anaerobiosis).

Refer to the monograph of Yersinia ITC Broth Base (REF402265), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Potassium Chlorate Supplement	Liquid supplement	4240065	10 vials, each for 250 mL of medium

POTASSIUM TELLURITE SOLUTIONS

COMPOSITION – BOTTLE CONTENTS

Potassium Tellurite 1% Solution
Potassium Tellurite 0.3 g
Purified water 30 mL

Potassium Tellurite 3.5% Solution
Potassium Tellurite 1.05 g
Purified water 30 mL

DIRECTIONS

The solutions are ready to use. They can be used for the supplementation of the following media:
Baird Parker Agar Base, Giolitti Cantoni Staphylococci Broth, Vogel Johnson Agar.

Refer to the monographs of relevant basal media for the intended use, test procedures and other information.

PACKAGING

Product	Type	REF	Pack
Potassium Tellurite 1% Solution	Liquid supplement	42211501	30 mL
Potassium Tellurite 3.5% Solution	Liquid supplement	42211502	30 mL

PP PSEUDOMONAS SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cetrimide 100.0 mg
Nalidixic acid 7.5 mg

DIRECTIONS

Dissolve the contents of one vial with 2 mL of sterile purified water. Add to 500 mL of Pseudomonas Agar Base (REF 401960), autoclaved at 121°C for 15 minutes and cooled to 47°C- 50°C. Mix well and pour into sterile Petri dishes.

Consult the monograph of Pseudomonas Agar Base, REF 4019602, for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
PP Pseudomonas Supplement	Freeze-dried supplement	4240048	10 vials, each for 500 mL of medium

PRESTON ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Polymyxin B	2500 IU
Cycloheximide	50 mg
Rifampicin	5 mg
Trimethoprim lactate	5 mg

DIRECTIONS

Dissolve the contents of one vial with 2 mL of 50% acetone/sterile purified water, using aseptic conditions. Add to 500 mL of Campylobacter Enrichment Broth Base (REF 401286), autoclaved at 121°C for 15 minutes and cooled below 47°C. Also add 25 mL of lysed horse blood (REF 90HLX100). Mix well and distribute into sterile tubes or flasks under aseptic conditions.

Refer to the monograph of Campylobacter Enrichment Broth Base (REF 401286) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Preston Antimicrobic Supplement	Freeze-dried supplement	4240017	10 vials, each for 500 mL of medium

PRESTON ANTIMICROBIC SUPPLEMENT II

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Polymyxin B	2500 IU
Amphotericin B	5 mg
Rifampicin	5 mg
Trimethoprim	5 mg

DIRECTIONS

Dissolve the contents of one vial with 2 mL of 50% acetone/sterile purified water, using aseptic conditions. Add to 500 mL of Nutrient Broth n° 2 (REF 401812), autoclaved at 121°C for 15 minutes and cooled below 47°C. Also add 25 mL of lysed horse blood (REF 90HLX100). If required add the contents of 2 vials of Campylobacter Growth Supplement (REF 4240021), reconstituted with 5 mL of sterile purified water. Mix well and distribute into sterile tubes or flasks under aseptic conditions.

Refer to the monograph of Nutrient Broth n° 2 (REF 401286) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Preston Antimicrobic Supplement II	Freeze-dried supplement	4240022	10 vials, each for 500 mL of medium

ROSOLIC ACID

COMPOSITION - VIAL CONTENTS

Rosolic Acid	1 g

DIRECTIONS

Transfer the contents of one vial to 100 mL volumetric flask. Add 100 mL of 0.2 N NaOH solution and stir to dissolve completely. Use this solution for the preparation of m-FC Agar and m-FC Broth, by adding 10 mL of rosolic acid solution to 1000 mL of the basal medium and boiling until complete solution is obtained.

Refer to the monographs of m-Faecal Coliform Agar (m-FC Agar), (REF401487) and m-Faecal Coliform Broth (m-FC Broth), (REF401486) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Rosolic Acid	Powdered supplement	4211901	10 x 1 g

RPF SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 200 mL OF MEDIUM

Fibrinogen	380.0 mg
Trypsin Inhibitor	1.5 mg
Coagulase Plasma	3.0 ml
Potassium Tellurite	15.0 mg

DIRECTIONS

Dissolve the contents of one vial with 50 ml of sterile purified. Mix thoroughly to completely dissolve the freeze-dried materials. Add to 200 mL Baird Parker Agar Base, REF 401116, autoclaved at 121°C for 15 minutes and cooled to approximately 47-50 °C. Mix well and pour into sterile Petri dishes.
Note: RPF Supplement is prepared according to the formulation given by Manuel Suisse des Denrèes Alimentaires (1985).

PACKAGING

Product	Type	REF	Pack
RPF Supplement	Freeze-dried supplement	423101	1 vial, for 200 mL of medium

RPF SUPPLEMENT II

COMPOSITION - VIAL CONTENTS

	FOR 100 mL OF MEDIUM	FOR 200 mL OF MEDIUM
Fibrinogen	380.0 mg	760 mg
Trypsin Inhibitor	2.5 mg	5 mg
Coagulase Plasma	2.5 ml	5 mg
Potassium Tellurite	2.5 mg	5 mg

DIRECTIONS

RPF Supplement II for 100 mL of medium (REF 433102)
Dissolve the contents of one vial with 10 ml of sterile purified. Mix thoroughly to completely dissolve the freeze-dried materials. Add to 90 mL Baird Parker Agar Base, REF 401116, autoclaved at 121°C for 15 minutes and cooled to approximately 47-50 °C. Mix well and pour into sterile Petri dishes.
RPF Supplement II for 100 mL of medium (REF 433102D)
Dissolve the contents of one vial with 20 ml of sterile purified. Mix thoroughly to completely dissolve the freeze-dried materials. Add to 180 mL Baird Parker Agar Base, REF 401116, autoclaved at 121°C for 15 minutes and cooled to approximately 47-50 °C. Mix well and pour into sterile Petri dishes.
Note: RPF Supplement II is prepared according to the formulation given by ISO 6888-2.

PACKAGING

Product	Type	REF	Pack
RPF Supplement II	Freeze-dried supplement	423102 423102D	4 vials of 10 mL, each for 100 mL of medium 4 vials of 20 mL, each for 200 mL of medium

SALMONELLA SELECTIVE SUPPLEMENTS

COMPOSITION

SALMONELLA SELECTIVE SUPPLEMENT VIAL A (LIQUID SUPPLEMENT READY-TO-USE)
VIAL CONTENTS FOR 500 ML OF MEDIUM

Emulsifying agents	5.7 mL

SALMONELLA SELECTIVE SUPPLEMENT VIAL B (FREEZE-DRIED SUPPLEMENT)
VIAL CONTENTS FOR 500 ML OF MEDIUM

Cefsulodin	2.5 mg

DIRECTIONS

Suspend 19 g of Chromogenic Salmonella Agar Base REF 405350 in 500 mL of cold purified water; add the contents of one vial of Salmonella Selective Supplement Vial A, heat to boiling stirring constantly and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the contents of one vial of Salmonella Selective Supplement Vial B, reconstituted with 2 mL of sterile purified water. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Chromogenic Salmonella Agar Base REF 405350 for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Salmonella Selective Supplements	Selective supplements	4240013	5 vials A + 5 vials B, each for 500 mL of medium

SENECA EE-EC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Antimicrobial compounds	4.50 mg
Chromogenic substrate	6.25 mg

DIRECTIONS

Dissolve the contents of one vial with 1 mL of ethanol, mix and then add 1 mL of sterile purified water under aseptic conditions. Add to 500 mL of SENECA Base (REF 405582S), autoclaved at 121°C for 15 minutes and cooled to 47-50°. Mix well and pour into sterile Petri dishes.

Refer to the monograph of SENECA Base (REF 405582S) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
SENECA EE-EC Supplement	Freeze-dried supplement	4240023	10 vials, each for 500 mL of medium

SKIRROW ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Polymyxin B	1250 IU
Trimethoprim	2.5 mg
Vancomycin	5.0 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water under aseptic conditions. Add to 500 mL of Columbia Agar Base (REF 401136), autoclaved at 121°C for 15 minutes and cooled to 47-50°. Also add 50 mL of defibrinated sheep blood or 25 mL of lysed horse blood and the contents of one vial of Campylobacter Growth supplement (REF 4240021). Mix well and pour into sterile Petri dishes.

Refer to the monograph of Columbia Agar Base (REF 401136) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Skirrow Antimicrobic Supplement	Freeze-dried supplement	4240016	10 vials, each for 500 mL of medium

SODIUM BISELENITE

COMPOSITION – BOTTLE CONTENTS

Sodium biselenite (sodium acid selenite)	100 g

DIRECTIONS

Dissolve 4 g of Sodium Biselenite in 1 litre of cold purified water and then add 19 g of Selenite Broth Base (REF 402025B) or 19 g of Selenite Cystine Broth Base (REF 402026B). Warm until complete dissolution and distribute into sterile tubes. Do not overheat or autoclave.

Refer to the monographs of Selenite Broth Base (REF 402025B) or Selenite Cystine Broth Base (REF 402026B) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Sodium Biselenite	Raw material	4123651	100 g (25 L)

SODIUM GLUTAMATE

COMPOSITION – BOTTLE CONTENTS

Sodium glutamate	300 g

DIRECTIONS

Single strength medium

Dissolve 2.5 g of ammonium chloride in 1000 mL of cold purified water. Add 11.4 g of Minerals Modified Glutamate Medium Base (REF 401737), and 6.35 g of Sodium Glutamate.

Double strength broth

Dissolve 5 g of ammonium chloride in 1000 mL of cold purified water. Add 22.7 g of Minerals Modified Glutamate Medium Base (REF 401737), and 12.7 g of Sodium Glutamate (REF 4123642).

Mix and heat if necessary to dissolve the medium completely.

Refer to the monographs of Minerals Modified Glutamate Medium Base (REF 401737) for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Sodium Glutamate	Raw material/supplement	4123642	300 g (46.9 or 23.4 L)

STAA SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Streptomycin sulphate	250 mg
Thallous acetate	25 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water under aseptic conditions. Add to 500 mL of STAA Agar Base (REF 402079), supplemented with 7.5 g of glycerol (REF 421015), autoclaved at 115°C for 15 minutes and cooled to 47-50°. Mix well and pour into sterile Petri dishes.

Refer to the monograph of STAA Agar Base (REF 402079), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
STAA Agar Base	Dehydrated medium	4020792	500 g (28.9 L)
STAA Selective Supplement	Freeze-dried supplement	4240052	10 vials, each for 500 mL of medium

STREPTO B SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Antimicrobial mix	0.04 g
Chromogenic mix	0.05 g

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water under aseptic conditions. Add to 500 mL of Chromogenic Strepto B Agar Base (REF 408010), autoclaved at 121°C for 15 minutes and cooled to 47-50°. Mix well and pour into sterile Petri dishes.

Refer to the monograph of Chromogenic Strepto B Agar Base (REF 408010), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Strepto B Supplement	Freeze-dried supplement	4240053	10 vials, each for 500 mL of medium base

TICARCILLIN IRGASAN ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 250 mL OF MEDIUM

Ticarcillin 0.25 mg
Irgasan 0.25 mg

DIRECTIONS

Dissolve the contents of one vial with 2 mL of sterile purified water. Add to 250 mL Yersinia ITC Broth Base, REF 402265, autoclaved at 121°C for 15 minutes and cooled to approximately 47-50 °C; also add the contents of one vial of Potassium Chlorate Supplement (REF 4240065). Mix well and dispense the medium aseptically in 90 mL amounts into flasks of suitable capacity, so as to obtain the minimum area/volume ratio (relative anaerobiosis).

Refer to the monograph of Yersinia ITC Broth Base (REF402265), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Ticarcillin Irgasan Antimicrobic Supplement	Freeze-dried supplement	4240060	10 vials, each for 250 mL of medium

TTC SOLUTIONS

COMPOSITION

TTC 1% SOLUTION REF 42111801 (BOTTLE CONTENT)

Triphenyl tetrazolium chloride	0.3 g
Purified water	30 mL

TTC 0.05% SOLUTION REF 421510 (BOTTLE CONTENT)

Triphenyl tetrazolium chloride	0.0125 g
Purified water	25 mL

DIRECTIONS

The solutions are ready to use. They can be used for the supplementation of the following media.
To 500 mL of autoclaved and cooled TTC Tergitol Agar Base REF 402160T add 1.25 mL of TTC 1% Solution (REF 42111801) or the content of one bottle (25 mL) of TTC 0.05% Solution (REF 421510).
To 1000 mL of cooled Azide Maltose Agar KF REF 401107, add 10 mL of TTC 1% Solution (REF 42111801)
To 1000 mL of cooled Slanetz Bartley Agar w/o TTC REF 402047 add 10 mL of TTC 1% Solution (REF 42111801)

Refer to the monographs of relevant basal media for the intended use, test procedures and other information.

PACKAGING

Product	Type	REF	Pack
TTC 1% Solution	Liquid supplement	42111801	30 mL
TTC 0.05% Solution	Liquid supplement	421510	10 x 25 mL

TWEEN® 20

COMPOSITION - FLASK CONTENTS

Polysorbate 20	100 mL

DIRECTIONS

Dissolve 40 mL of Tween® 20 in 960 mL of purified water by mixing while heating in a water bath at 49 °C ± 2 °C. Add 25 g of TAT Broth (REF402100). Heat for about 30 min with occasional agitation to obtain solution. Mix and dispense the medium into suitable containers. Sterilize in the autoclave at 121 °C for 15 minutes.

Refer to the monograph of TAT Broth (REF402100) for intended use, test procedure and other information.

®Tween is a trademark of ICI Americas Inc.

PACKAGING

Product	Type	REF	Pack
Tween® 20	Liquid supplement	42120501	100 mL

TWEEN® 80

COMPOSITION - FLASK CONTENTS

Polysorbate 80	100 mL

DIRECTIONS

The product is ready to use. Tween 80 may be used for the supplementation of the following media:
Letheen Broth AOAC REF 401591: 5 g/L,
Rogosa Bios Agar REF 401985: 1 g/L
Rogosa Bios Broth REF 401990: 1 g/L

Refer to the monographs of the relevant basal media for intended use, test procedure and other information.

®Tween is a trademark of ICI Americas Inc.

PACKAGING

Product	Type	REF	Pack
Tween® 80	Liquid supplement	42120502	100 mL

UREA 40 % SOLUTION

COMPOSITION - FLASK OR VIALS CONTENTS

	REF 42211601	**REF 4240096**
Urea	20 g	2 g
Purified water	50 mL	5 mL

DIRECTIONS

Urea 40% Solution is ready-to-use
To 950 ml of Urea Agar Base (Christensen) (REF 402175) or Urea Broth Base (Stuart) (REF 402180), autoclaved at 121°C for 15 minutes and cooled to 47-50°C, add 50 mL of Urea 40% Solution. Mix well and distribute into sterile tubes

Refer to the monographs of the relevant basal media for intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Urea 40% Solution	Liquid supplement	42211601 4240096	50 mL 10 x 5 mL

VANCOMICIN ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS

Vancomycin	25 mg

DIRECTIONS

Suspend 32.3 g of Modified Lauryl Sulfate Tryptose (mLST) Broth Base (REF401476) in 500 mL of cold purified water. Heat to dissolve and sterilize by autoclaving at 121°C for 15 minutes. Cool to room temperature and add 1 mL of Vancomycin Antimicrobic Supplement (REF 4240057), reconstituted with 5 mL of sterile purified water. Final vancomycin concentration in the medium: 10 mg/L. Mix well and distribute into sterile tubes (10 mL/tube) under aseptic conditions. The vancomycin solution may be kept at 0 °C to 5 °C for 15 days.

Refer to the monograph of Modified Lauryl Sulfate Tryptose (mLST) Broth Base (REF401476), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Vancomycin Antimicrobic Supplement	Freeze-dried supplement	4240057	10 vials (25 mg/vial)

VANCOMYCIN CSB SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	5 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water. Add to 500 mL of Cronobacter Screening Broth Base, REF 401355, autoclaved at 121°C for 15 minutes and cooled below 47°C. Mix well and dispense 10 mL into sterile tubes under aseptic conditions.
Vancomycin CSB Supplement may be used for the supplementation of Aeromonas Selective Agar Base (Havelaar) REF 401019, according to USEPA method: 1 mL of Vancomycin CBS Supplement + 500 mL of pre-cooled medium base supplemented with ampicillin (final concentration of vancomycin: 2 mg/L).

Refer to the monographs of Cronobacter Screening Broth Base (REF 401355) and Aeromonas Selective Agar Base (Havelaar) (REF 401019), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Vancomycin CSB Supplement	Freeze-dried supplement	4240057C	10 vials, each for 500 mL of medium base

VCN ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	1.50 mg
Colistin	3.75 mg
Nystatin	6250 IU

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water. Add to 500 mL of GC medium Base, REF 401520, autoclaved at 121°C for 15 minutes and cooled to 47-50°C.

Refer to the monograph of GC Medium Base (REF 401520), for the intended use, the details of Thayer-Martin medium preparation, the test procedure and other information.

PACKAGING

Product	Type	REF	Pack
VCN Antimicrobic Supplement	Selective supplement	4240007	10 vials, each for 500 mL of medium

VCNT ANTIMICROBIC SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Vancomycin	1.50 mg
Colistin	3.75 mg
Nystatin	6250 IU
Trimethoprim	2.50 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water. Add to 500 mL of GC medium Base, REF 401520, autoclaved at 121°C for 15 minutes and cooled to 47-50°C.

Refer to the monograph of GC Medium Base (REF 401520), for the intended use, the details of modified Thayer-Martin medium preparation, the test procedure and other information.

PACKAGING

Product	Type	REF	Pack
VCNT Antimicrobic Supplement	Selective supplement	4240008	10 vials, each for 500 mL of medium

XLT4 SUPPLEMENT

COMPOSITION - BOTTLE CONTENTS

Tergitol 4 (Niaproof 4/Sodium tetradecilsulfate)	100 mL

DIRECTIONS

XLT4 Supplement is ready-to-use.
Suspend 58 g of XLT4 Agar Base (REF 402207) in 1000 mL of cold purified water and add 4.6 mL of XLT4 Supplement. Heat to boiling with frequent agitation, to dissolve completely. Do not overheating, do not autoclave. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

Refer to the monograph of XLT4 Agar Base (REF 402207), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
XLT4 Supplement	Liquid supplement	4240097	100 mL

YERSINIA SELECTIVE SUPPLEMENT

COMPOSITION - VIAL CONTENTS FOR 500 mL OF MEDIUM

Cefsulodin	7.50 mg
Novobiocin	1.25 mg

DIRECTIONS

Dissolve the contents of one vial with 5 mL of sterile purified water. Add to 500 mL CIN Agar Base, REF 401302, autoclaved at 121°C for 15 minutes and cooled to approximately 47-50 °C. Mix well and pour into sterile Petri dishes.

Refer to the monograph of CIN Agar Base (REF 401302), for the intended use, test procedure and other information.

PACKAGING

Product	Type	REF	Pack
Yersinia Selective Supplement	Freeze-dried supplement	4240011	10 vials, each for 500 mL of medium

AGAR, PEPTONES AND OTHER BIOLOGICAL MATERIALS

AGAR

AGAR BIOS SPECIAL LL

DESCRIPTION

Agar Bios LL is a polysaccharide obtained from marine seaweed of the *Rhodophyceae* family through an extraction process that results in a fine homogeneous powder. Agar Bios LL is the gelling agent of choice for the preparation of culture media for microbiology. Agar Bios LL is free of inhibitors that can mask the optimal development of microorganisms and of substances metabolizable by bacteria. It also possesses other properties such as transparency, and batch-to-batch reproducibility. This type of agar undergoes strict controls to guarantee its superior quality and excellent consistent performance. It can be used in culture media at the concentration of 13-15 g/L. Agar Bios LL is of European origin.

CHEMICAL CHARACTERISTICS

Powder appearance	White to pale brown powder
Particle size	> 95% through 60 mesh ASTM
Loss on drying	≤ 12%
Total ash	≤ 5.0 %
Appearance of 1.5 % sol. at 50°c	Yellow to very light yellow; opalescent to limpid
Water absorption (5g/100mL)	>25 mL
Gelling temp. (1.5% sol.)	34 - 38 °C
Melting temp. (1.5% sol.)	85 - 90 °C

Before autoclaving		**After autoclaving**	
Clarity 1.5% sol. before autoclaving	< 8 NTU	Clarity 1.5% sol. after autoclaving	< 8 NTU
Colorimetry 450 nm	< 0.250 nm	Colorimetry 450 nm	< 0.250 nm
pH 1.5% solution at 18-25°c	6.0 - 7.5	pH 1.5% solution at 18-25°c	6.0 - 7.5
Gel strength (Nikan method 1.5% at 20°)	800-1100 g/cm²	Gel strength (Nikan method 1.5% at 20°)	800-1100 g/cm²

MICROBIOLOGICAL TESTS

Total microbial count	< 5000 CFU/g
Thermophilic bacteria	< 100 CFU/g
Yeast and mould	< 100 CFU/g

PACKAGING

Product	Type	REF	Pack
Agar Bios LL	Raw material for culture media	4010302 4010304	500 g 5 kg

AGAR HIGH GEL STRENGTH

DESCRIPTION

Agar High Gel Strength is the gelling agent of choice in the preparation of culture media for microbiology. Is a combination of polysaccharides made up of neutral molecules (agarose) plus others electrically charged (agaropectin). It is obtained from various types of red algae of the *Rhodophyceae* family. Agar High Gel Strength is free of inhibitors that can mask the optimal development of microorganisms and of substances metabolizable by bacteria. Thanks to its high gel strength it can be used in culture media at the concentration of 11-13 g/l. Agar Bios LL is of European origin.

CHEMICAL CHARACTERISTICS

Appearance of 1.3% sol. at 50°C	Yellowish, limpid
Water absorption (5g/100ml)	>25 mL
Powder appearance	Fine homogeneous cream-white powder
Sieve analysis (ASTM) - retained on mesh 80 /	< 5%
Gel strength	>1050 g/cm²
Loss on drying	< 10%
Total ash	< 4%
pH 1.5% solution (20-25°c)	6.0 - 7.5
Nephelos after autoclaving	< 8 NTU (430nm)
Gelling point	30 - 35 °C
Melting point	> 85°C
Calcium	< 4000 ppm
Magnesium	< 1200 ppm
Toxic substances	absence

PACKAGING

Product	Type	REF	Pack
Agar High Gel Strength	Raw material for culture media	4010312 4010314	500 g 5 kg

AGAR TECHNICAL

DESCRIPTION

Agar Technical is a semi-purified gelling agent used for the preparation of culture media for microbiology or for biotechnology applications. Agar Technical is free of inhibitors that can mask the optimal development of microorganisms. It can be used in culture media at the concentration of 15-20 g/L

CHEMICAL CHARACTERISTICS

Powder appearance	Fine homogeneous brown to beige powder
Appearance of 1.3% sol. at 50°C	Dark yellow to very light yellow; almost turbid to limpid
Water absorption (5g/100ml)	>25 ml
sieve analysis (ASTM) under sieve	100%
% H_2O	< 12%
Total ash	< 6.5%
Acid insoluble ashes	< 0.03%
Calcium by atomic absorption	< 1000 ppm
Magnesium by atomic absorption	< 500 ppm
Sulphate	< 3 %
Clarity 1.5% sol.	≤ 8 NTU
pH 1.5% solution	6.0 - 8.0
Gel strength (Nikkan) 1.5%	550-650 g/cm²
Gelling temp. (1.5% sol.)	35 ± 3 °C
Melting temp. (1.5% sol.)	88 ± 5 °C

MICROBIOLOGICAL TESTS

Total plate count /	< 1000 CFU/g
Heat resistant thermophilic	≤ 1 CFU/g
Heat resistant mesophilic	≤ 1 CFU/g

PACKAGING

Product	Type	REF	Pack
Agar Technical	Raw material for culture media	4010302 4010304	500 g 5 kg

AGAR HIGHLY PURIFIED

DESCRIPTION

Agar Highly Purified is manufactured from carefully selected *Gelidium* seaweeds (*Rhodophyceae*). Through purification process the product obtained has an ash content essentially lower than that of standard bacteriological agar. This process also has decreased the small oligosaccharides fraction and increased the agarose content. The physiochemical, biochemical and bacteriological properties of this product are strictly controlled to guarantee its exceptional behaviour in biochemical, bacteriological and mycological applications.

CHEMICAL CHARACTERISTICS

Powder appearance	Fine homogeneous white powder
% H_2O	≤ 10%
Ash	< 1.6%
Before autoclaving	
Turbidity 1.5% sol.	< 15 NTU
pH 1.5% solution	< 7.0
Colorimetry 450 nm 1.5% sol.	< 0.150 NMU
Gel strength	700 - 1200 g/cm2
After autoclaving	
Turbidity 1.5% sol.	< 15 NTU
pH 1.5% solution	< 7.0
Colorimetry 450 nm 1.5% sol.	< 0.150 NTU
Gel strength	700 - 1200 g/cm2
Gelling temp. (1.5% sol.)	32 - 37.5 °C
Melting temp. (1.5% sol.)	80 - 95 °C

PACKAGING

Product	Type	REF	Pack
Agar Highly Purified	Raw material for culture media	4010352	500 g

AGAROSE

DESCRIPTION

A neutral polysaccharide with a very high gelling power. Structurally it is a polymer of high molecular weight (above 120,000 Daltons) formed by repetitive units. Agarose is used in biochemistry and for analytical and preparative separation techniques such as: diffusion and immunodiffusion, electrophoresis, immunoelectrophoretic, agarose beads preparation, cell culture, cell and enzyme immobilisation.

CHEMICAL CHARACTERISTICS

Powder appearance	Fine homogeneous white powder
Moisture	≤ 7%
Ash	≤ 4%
Electroendosmosis	0.05 - 0.13
Sulphates	≤ 0.1%
DNAse -RNAse	Not detected
Clarity 1.5% (NTU)	≤ 3
pH 1.5% solution (20-25°c)	6.5- 7.5
Colorimetry 450 nm 1.5% sol.	< 0.250
Gel strength 1%	≥ 1200 g/cm²
Gel strength 1.5%	≥ 2500 g/cm²
Gelling temperature (1.5% sol.)	34.5 - 37.5 °C
Melting temperature (1,5% sol.)	86.5 - 89.5 °C

PACKAGING

Product	Type	REF	Pack
Agarose	Raw material for culture media	4110381	100 g

ACID DIGEST OF CASEIN

DESCRIPTION

Acid Digest of Casein is obtained by hydrochloric acid hydrolysis of casein, resulting in a high content of free amino acids, with the exception of tryptophan. It is used in diagnostic culture media in which high contents of sodium chloride is tolerated: for the study of bacterial antibiotic resistance, for vitamin tests and to evaluate the metabolism of tryptophan. Casein hydrolysate can also find use in the production of vaccines when it is necessary to use a peptone with a high content of free amino acids. The raw materials are collected in New Zealand.

CHEMICAL CHARACTERISTICS

Solubility in water at 5%	total
pH 5 % sol.	4.7 - 5.7
Stability of an aqueous 5% solution after 15 min. at 121°C	stable
Proteoses	absent
Tryptophane	absent
Indole test	negative
Nitrites test	negative
Total nitrogen	7.0 - 8.5 %
Amino nitrogen	5.0 - 6.5%
Chlorides (as NaCl)	≤ 45%
Sulfuric ashes	≤ 57%
Loss on drying	≤ 6%

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 5,000 CFU/g

PACKAGING

Product	Type	REF	Pack
Acid Digest of Casein	Raw material for culture media	4122652	500 g

BEEF EXTRACT POWDER

DESCRIPTION

Beef Extract Powder is a purified and standardised extract of beef meat. It is also known as meat extract. It is often used in association with casein peptones as a source of nutrients in culture media for microbiology. At a final concentration of 0.3-0.5% it gives clear aqueous solutions, not requiring filtration. This reference has an EDQM certificate to allow its use in biopharmaceuticals. Other applications exist in diagnostics and fermentation (biomass production).

CHEMICAL CHARACTERISTICS

Powder appearance	fine, homogeneous, straw powder
Appearance of 2% solution	dark yellow. limpid
pH 2% solution (20-25°c)	6.8 - 7.4
Absorbance 450 nm	0.160 - 0.460
Ash	0 - 15 %
Loss on drying	0 - 7.5 %
Sodium chloride	0 - 5.0 %
Total nitrogen	11.5 - 15.6%

PACKAGING

Product	Type	REF	Pack
Beef Extract Powder	Raw material for culture media	4111252	500 g
		4111254	5 Kg

BILE SALTS

DESCRIPTION

Bile Salts are produced from pH-adjusted (neutral) bovine bile, filtered and spray-dried. Because it has not undergone a hydrolysis process, the bile acids remain predominantly in their natural conjugated form, so ox bile powder is freely soluble in water. Bile Salts consist mainly of sodium glycocholate and sodium taurocholate. Bile Salts are used in microbiological culture media where bile acids can regulate microbiological growth to enhance media selectivity. The product is also known as Oxgall or Ox bile powder. Bile Salts are of New Zealand origin.

CHEMICAL CHARACTERISTICS

Powder appearance	fine. homogeneous. straw-coloured powder
Total bile acids	≥ 50%
pH 2% sol. (20-25°C)	7.0 - 8.5
Loss on drying	≤ 6.0%
Solubility (2% autoclaved solution)	clear no sediment

PACKAGING

Product	Type	REF	Pack
Bile Salts	Raw material for culture media	41113012	500 g

BILE SALTS N° 3

DESCRIPTION

Bile Salts N°3 are highly purified and standardized bile extracts, consisting mainly of sodium cholate and sodium deoxycholate. They give a clear, colourless aqueous solution at 0.15%. Bile Salts N° 3, due to their inhibitory properties against Gram-positive bacteria, are widely used in culture media for the isolation of Gram-negative bacteria. Bile Salts n° 3 are of New Zealand origin.

CHEMICAL CHARACTERISTICS

Powder appearance	fine. homogeneous. straw-coloured powder
Total bile acids	≥ 50%
pH 2% sol. (20-25°C)	7.0 - 8.5
Loss on drying	≤ 6.0%
Solubility (2% autoclaved solution)	clear no sediment

PACKAGING

Product	Type	REF	Pack
Bile Salts N°3	Raw material for culture media	41113022	500 g

GELATIN BIOS

DESCRIPTION

Gelatin Bios, is produced by partial hydrolysis of collagen rich pork skins. It is used in microbiological media for studying the gelatinolytic activity of bacteria. Gelatin Bios is free from fermentable carbohydrates and preservatives, and is very soluble in water giving a clear, colourless solution. Gelatin Bios quality control file includes a manufacturer declaration for use in food and pharmaceutical industries. Gelatin Bios is conformed to Reg. N° (EU) 853/2004.

CHEMICAL CHARACTERISTICS

Bloom (g)	280 - 310 g
Viscosity	4.5 - 5. 5 mPa.S
Water content	≤ 13 %
pH	4.5 - 5.5
Ash	≤ 2.0 %
Arsenic	≤ 1.0 mg/kg
Lead	≤ 5.0 mg/kg
Cadmium	≤ 0.5 mg/kg
Mercury	≤ 0.15 mg/kg
Chromium	≤ 10 mg/kg
Copper	≤ 30 mg/kg
Zinc	≤ 30 mg/kg
Peroxides	≤ 10 mg/kg
Sulphur dioxide	≤ 40 mg/kg

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 1.000 /g
Escherichia coli	Negative /10 g
Salmonella	Negative /25 g
Anaerobic sulphide reducing bacteria	< 10 /g

PACKAGING

Product	Type	REF	Pack
Gelatin Bios	Raw material for culture media	4115152	500 g

GELATIN PEPTONE

DESCRIPTION

Gelatin Peptone Bios is a pancreatic digest of porcine skin with a low cystine and tryptophan content. It is also free of carbohydrates.
Gelatin Peptone is a pancreatic digest of porcine skin. Its fermentable carbohydrate content is low and it is used in culture media, especially for non-fastidious microorganisms. It is also used in fermentation studies.

CHEMICAL CHARACTERISTICS

Powder appearance	fine, homogeneous, cream powder
Appearance of 2% solution	pale yellow, limpid
pH 2% solution (20-25°c)	6.5 - 7.5
Loss on drying	≤ 6.0%
Total nitrogen (TN)	> 10 %
α-amino nitrogen (AN)	< 2.7 %
Ash	≤ 15%

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 5000/g
Salmonella	absent /25 g
Coliforms	absent /1 g
Yeasts and moulds	< 100/g

PACKAGING

Product	Type	REF	Pack
Gelatin Peptone	Raw material for culture media	4115182	500 g

LECITHIN

DESCRIPTION

Lecithin comes from non-genetically modified soya. The product complies with 1829/2003/EC and 1830/2003/EC. Lecithin can be used as supplement in liquid or solid media for the inactivation of the activity of preservatives contained in cosmetic or pharmaceutical products.

CHEMICAL CHARACTERISTICS

IR identification	conform to standard
Acetone insoluble substances	≥ 96.5%
Toluene insoluble substances	≤ 0.3%
Acid value	≤ 35 mg KOH/g
Loss on drying	< 1.5 %
Peroxide value	≤ 3 meq O_2/kg

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 1,000 CFU/g
Yeasts	≤ 50 CFU/g
Moulds	≤ 50 CFU/g
Enterobacteriaceae	absent / 1 g
Salmonella	absent / 375g

PACKAGING

Product	Type	REF	Pack
Lecithin	Raw material for culture media	41EAT0242	500 g

LIVER EXTRACT

DESCRIPTION

Liver extract is a dehydrated and standardised extract from pork liver used for the preparation of infusion media. It is useful for the culture of *Trichomonas vaginalis* and other fastidious protozoa, for those culture media designed to target pathogenic fungi, anaerobic bacteria and PPLO.

CHEMICAL CHARACTERISTICS

Powder appearance	fine, homogeneous, brown powder
pH 5% solution (20-25°c)	6.5 - 7.5
Total nitrogen (TN)	10.5 - 12.5 %
Amino nitrogen (AN)	5.0 - 6.5 %
Loss on drying	≤ 6.0%
Ash	≤ 18%

PACKAGING

Product	Type	REF	Pack
Liver Extract	Raw material for culture media	41228012	500 g

MALT EXTRACT

DESCRIPTION

Malt Extract is obtained by extracting the soluble products from sprouted grain with a drying process at low temperature that conserves the nitrogen and carbohydrate contents. It is used as an ingredient in culture media for the propagation of yeast and moulds.
Malt extract is generally used in concentrations of 1–10% in mycological culture media, and provides carbon, protein, high concentration of carbohydrates (mainly maltose) and other nutrients for the cultivation of yeasts and moulds.

CHEMICAL CHARACTERISTICS

Appearance of 2% sol.	limpid. yellow
Powder appearance	homogeneous, beige powder
Solubility in water at 3%	total
pH 3% sol (20-25°C)	4.8 - 5.8
Loss on drying	≤ 6.0%
Ash	≤ 4.5%
Chloride (as NaCl)	≤ 1.0%
Maltose	≥ 60%

MICROBIOLOGICAL TESTS

Total aerobic microbial count	< 10,000 CFU/g
Salmonella	absence/25g
Escherichia coli	absence/10g
Yeasts and moulds	< 20 UFC/g

PACKAGING

Product	Type	REF	Pack
Malt Extract	Raw material for culture media	4116502	500 g

MYCOLOGICAL PEPTONE

DESCRIPTION

Mycological Peptone is used for the cultivation of dermatophytes, saprophytic moulds and yeasts. It gives a luxuriant growth with typical morphology and pigmentation of the colonies

CHEMICAL CHARACTERISTICS

Loss on drying	< 5.0 %
Solubility in water (2 % solution)	complete
pH (2 % solution)	5.1 – 5.5
Microbiological performance in SDA	passes test

PACKAGING

Product	Type	REF	Pack
Mycological Peptone	Raw material for culture media	4117102	500 g

PEPTOCOMPLEX

DESCRIPTION

Peptocomplex is a result of a mixture of peptones obtained from pancreatic digestion of casein and enzymatic digestion of meat. Peptocomplex provides the high amino acid content and small polypeptides characteristic of the pancreatic digest of casein and the larger polypeptides characteristic of the enzymatic digestion of meat. It is excellent for the cultivation of many microorganisms and for general microbiological applications.

CHEMICAL CHARACTERISTICS

Total nitrogen (TN)	> 10.0 %
Amino nitrogen (AN)	> 3.5 %
pH 2% solution	6.7 – 7.3
Loss on drying	≤ 6.0%
Solubility in water (2 % solution)	complete
Microbiological performance	passes test

PACKAGING

Product	Type	REF	Pack
Peptocomplex	Raw material for culture media	4123102	500 g

PEPTONE BACTERIOLOGICAL

DESCRIPTION

Petone Bacteriological is a high-quality hydrolysate produced by enzymatic digestion of animal tissues. Petone Bacteriological provides nitrogen, amino acids, and carbon for microbial growth in culture media used for analytical applications, the production of antibiotics, vaccines, and enzymes. It is a general-purpose highly nutritive peptone, with neutral pH, that gives clear solution in water. The country of origin of animal tissues is New Zealand. This reference has an EDQM certificate to allow its use in biopharmaceuticals.

CHEMICAL CHARACTERISTICS

Powder appearance	Fine. homogeneous. ochre powder
Appearance of 2% solution	Gold-yellow. limpid
Appearance of buffered peptone water	Yellow. limpid
Amino nitrogen	≥ 3 %
Total nitrogen (TN)	≥ 10 %
Loss on drying	≤ 5.0%
Ash	≤ 15%
Reducing sugars	Negative
Undigested protein	Negative to trace positive
Proteoses	Slightly positive or positive
Nitrites	Negative to trace positive
Tryptophan	Positive
pH 2% solution (20-25°c)	6.5 - 7.5

MICROBIOLOGICAL TESTS

Standard plate count	< 5,000/g
Coliforms	Negative
Salmonella	Negative
Yeasts and moulds	< 100/g

PACKAGING

Product	Type	REF	Pack
Peptone Bacteriological	Raw material for culture media	4122592 4122594	500 g 5 kg

PEPTONISED MILK

DESCRIPTION

Peptonised Milk is a pancreatic digest of high-grade skimmed milk powder. The product may be used on its own, or with other ingredients in media for isolation of lactobacilli and bacteriological examination of dairy products.

CHEMICAL CHARACTERISTICS

Appearance of 2% solution	pale yellow. limpid
pH, 2% sol.	6.2 - 6.6
Total nitrogen (TN)	≤ 5
α-amino nitrogen (AN)	≤ 1.5
Loss on drying	0-5 %

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 10,000 CFU/ 10g
Enterobacteriaceae	≤ 100 CFU/ 10g
Salmonella	absent/ 25 g

PACKAGING

Product	Type	REF	Pack
Peptonised Milk	Raw material for culture media	4122982	500 g

PROTEOSE PEPTONE

DESCRIPTION

Proteose Peptone is an enzymatic digestion of animal tissues, rich in peptides. It is commonly used in the preparation of culture media for the production of toxins and is utilized in the fermentation industry for starter cultures. It is a highly nutritious source for the growth of a wide range of fastidious and non-fastidious microorganisms. The countries of origin of animal tissues are Australia and USA.

CHEMICAL CHARACTERISTICS

Powder appearance	dense, homogeneous, ochre powder
Appearance of 2% solution	dark yellow. limpid
Total nitrogen (TN)	≥ 10 %
Amino nitrogen (AN)	≥ 3.4 %
Loss on drying	≤ 6.0%
Ash	≤ 10%

MICROBIOLOGICAL TESTS

Standard plate count	< 5,000 CFU/g
Coliforms	negative
Salmonella	negative

PACKAGING

Product	Type	REF	Pack
Proteose Peptone	Raw material for culture media	4123302	500 g

SKIM MILK

DESCRIPTION

Skim Milk is a soluble spray-dried skimmed milk, suitable for bacteriological purposes. It is used in culture media for the isolation and cultivation of lactic-acid bacteria and for the differentiation of microorganisms on the basis of proteolysis and coagulation of casein. Skim milk must be reconstituted by mixing 100 g of powder with 930 mL of water. Skim milk is not intended for human consumption.

CHEMICAL CHARACTERISTICS

Fats	≤ 1%
Moisture	≤ 4%
Proteins	≥ 34%
Evaluation of antimicrobial activity	negative

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 20.000 CFU/g
Enterobacteriaceae	<10 CFU/g
Listeria monocytogenes	absent / 25 g
Yeasts and moulds	≤ 100 CFU/g
Staphylococcus aureus	absent / 1 g
Salmonella	absent/ 250 g

PACKAGING

Product	Type	REF	Pack
Skim Milk	Raw material for culture media	4120402	500 g

SOY PEPTONE

DESCRIPTION

Soy peptone is an enzymatic hydrolysed of GMO safe soy flour. Certified non-animal, it provides a good balance of sugars, nitrogen and trace elements. It promotes the growth of a good variety of strains, including yeasts and moulds.
It is soluble in water and is a plant source of peptones for microbiological use including the commercial manufacture of antibiotics and fermentation products, as well as the preparation of culture media.
The product complies with European Regulations EC 1829/2003 and 1830/2003 regarding GMO.

CHEMICAL CHARACTERISTICS

Powder appearance	fine homogeneous beige powder
Appearance of 2% solution	dark yellow. limpid
Solubility in water 5%	complete
pH 5% sol.	6.6 - 7.6
Loss on drying	≤ 11%
Total nitrogen (TN)	8.8 - 10.1 %
α-amino nitrogen (AN)	3.0 - 3.8 %
AN/AT x 100	30 - 43
Residue on ignition	≤ 18%
Chlorides	≤ 3%

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 10,000 CFU/g
Staphylococcus aureus	absent/ 10 g
Escherichia coli	absent/ 1 g
Salmonella	absent/ 25 g
Coliforms	≤ 10 CFU /g

PACKAGING

Product	Type	REF	Pack
Soy Peptone	Raw material for culture media	4123252	500 g

TRYPTONE

DESCRIPTION

Tryptone is a product obtained by pancreatic digest of milk casein. It is a highly concentrated mixture of amino acids, peptides and larger protein fractions. Tryptone is used as a microbiological nutrient for the preparation of diagnostic culture media and for the production of antibiotics, toxins, enzymes, and other biological products. It can be used for indole production test because of its high tryptophan content. The country of origin of milk casein is New Zealand.

CHEMICAL CHARACTERISTICS

Powder appearance	fine, homogeneous, beige powder
Appearance of 2% solution	pale yellow, limpid
Solubility in water 2%	complete
pH 2% sol.	6.5 - 7.5
Loss on drying	≤ 6.0%
Total nitrogen (TN)	≥ 10%
α-amino nitrogen (AN)	≥ 3.9%
Residue on ignition	≤ 15%

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 5,000 CFU/ g
Salmonella	absent /1 g
Coliforms	absent /1 g
Yeasts and moulds	≤ 100 CFU/g

PACKAGING

Product	Type	REF	Pack
Tryptone	Raw material for culture media	4122902	500 g

TRYPTOSE

DESCRIPTION

Tryptose is a mixture of enzymatically digested proteins with rich nutritional value for microbiological culture media. Tryptose is a source of nitrogen, carbon, amino acids, peptides, vitamins and trace elements necessary for promoting the growth of fastidious microorganisms.

CHEMICAL CHARACTERISTICS

Powder appearance	fine, homogeneous, pale-yellow powder
Appearance of 2% solution	pale yellow, limpid
Solubility in water 2%	complete
pH 2% sol.	6.8 - 7.4
Total nitrogen (TN)	≥ 10%
α-amino nitrogen (AN)	≥ 4.2%

PACKAGING

Product	Type	REF	Pack
Tryptose	Raw material for culture media	4122602	500 g

VEGETABLE PEPTONE P

DESCRIPTION

Vegetable Peptone P is a pea peptone obtained by hydrolysis with bacterial enzymes. It does not contain any component of animal origin, does not present any risk for BSE, is a non-GMO peptone (according to European Regulations CE 1829/2003 and 1830/2003) and does not contain allergens. The product provides clear solutions at the concentrations normally used (2%) and is autoclavable at 121°C for 15 minutes. It is an excellent source of organic nitrogen and growth factors, promotes the growth of a wide variety of microorganisms and can be used in culture media for industrial fermentations and for analytical microbiology.

CHEMICAL-PHYSICAL CHARACTERISTICS

Appearance	cream colour powder
Solubility in water at 5%	complete
pH (5% solution)	7.2 ± 0.2
Loss on drying	≤ 7.5
Total nitrogen TN	10-15%
amino nitrogen	3-5%
Residue on ignition	15-18%
Chloride (NaCl)	2-6%

MICROBIOLOGICAL TESTS

Total aerobic microbial count	≤ 10,000
Staphylococcus aureus	absent/10 g
Salmonella	absent/10 g
Coliforms	≤ 10/ g
Yeasts and moulds	≤ 20/ g

PACKAGING

Product	Type	REF	Pack
Vegetable Peptone P	Raw material for culture media	4124002 4124004	500 g 5 kg

YEAST EXTRACT

DESCRIPTION

Yeast Extract is a water-soluble spry-dried extract resulting from the autolysis of primary grown yeast cells of the species *Saccharomyces cerevisiae*. During the production the process is carefully controlled so that the B-complex vitamins are almost completely conserved. Yeast Extract is an excellent source of B-complex vitamins and it is standardised for bacteriological purposes. It is particularly useful for media containing sodium thioglycollate.

CHEMICAL CHARACTERISTICS

Appearance of 2% solution	yellow, limpid
Dry matters	≥ 94 %
Sodium chloride	≤ 0.5%
pH 8,33 % sol. (20-25°c)	6.8 - 7.2
Total nitrogen (TN)	≥ 10.0
α-amino nitrogen (AN)	≥ 4.5

MICROBIOLOGICAL TESTS

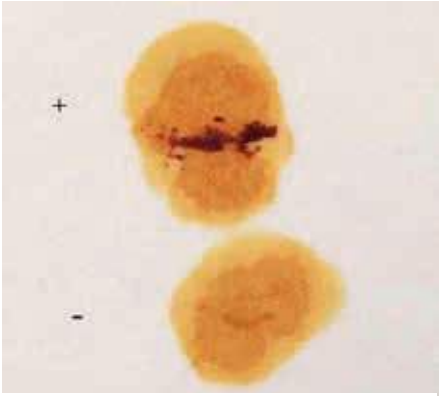
Standard plate count	≤ 5,000 CFU/g
Coliforms	≤ 5 CFU/g
E. coli	0 CFU/g
Yeasts	≤ 50 CFU/g
Moulds	≤ 50 CFU/g

PACKAGING

Product	Type	REF	Pack
Yeast Extract	Raw material for culture media	4122202 4122204	500 g 5 kg

ACID PHOSPHATASE REAGENTS

Biochemical identification reagent



Acid Phosphatase Test: *C. perfringens* (+), *C. bifermentans* (-)

INTENDED USE

For the confirmation test of *Clostridium perfringens* colonies according to ISO 14189.

COMPOSITION – PACK CONTENT

ACID PHOSPHATASE REAGENT -VIAL A	
1- Naftyl phosphate monosodium salt	0.08 g
Acetic acid	0.0012 mL
Sodium acetate	0.0016 g
Purified water	4 mL
ACID PHOSPHATASE REAGENT -VIAL B	
Fast Blue B	0.16 g
Acetic acid	0.0012 mL
Sodium acetate	0.0016 g
Purified water	4 mL

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Acid phosphatase catalyses the hydrolysis of the naphthyl phosphate, releasing alpha-naphthol and phosphate and forming an azo dye with the Fast Blue B which results in the development of a purple colour.
The acid phosphatase test is indicated as confirmation test of *C. perfringens* colonies grown on TSC Agar and sub-cultured on blood agar, according to ISO 14189.[1]

PHYSICAL CHARACTERISTICS

Acid Phosphatase Reagent- Vial A: clear colourless solution
Acid Phosphatase Reagent -Vial B: cloudy brown-orange solution with possible precipitates

SPECIMENS

The test is performed on microbial colonies grown on Columbia Blood Agar or Blood Agar plates or other suitable medium according to ISO 14189.

TEST PROCEDURE

- Obtain the specimen from primary isolation culture streaked on Blood Agar or Columbia Blood Agar or Tryptic Soy Agar plates.
- Incubate at 36 ± 2 ° C for 21 ± 3 hours in anaerobiosis.
- Streak some colonies grown on the incubated medium as described above on filter paper and add 1-2 drops of Acid Phosphatase Reagent- Vial A and immediately afterwards 1-2 drops of Acid Phosphatase Reagent- Vial B.
- Wait 3-4 minutes for reading the results.

READING AND INTERPRETATION

Examine the reaction area for the appearance of a purple colour.
Positive test: the development of a purple colour after 3-4 minutes from the addition of the reagent indicates the positivity to the test of the acid phosphatase.
Negative test: if there is no purple colour development, the test is considered negative.
Clostridium perfringens develops black or grey to brown-yellow colonies on TSC Agar, positive for acid phosphatase testing.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.

Positive control
C. perfringens ATCC 13124 purple colour development
C. perfringens NCTC 8238 purple colour development
Negative control
C. bifermentans NCTC 506 no purple colour development

ATCC is a trademark of American Type Culture Collection; NCTC: National Type Culture Collection

LIMITATIONS OF THE METHOD

- Readings over 4 minutes may give dubious or confusing results.

STORAGE CONDITIONS

Upon receipt, store the product in the original pack at 2-8°C away from direct light. If properly stored, the product may be used up to the expiration date. Do not use the product beyond this date. Opened container can be used up to the expiration date. Repeated openings of the container do not affect the performances of the product. The presence of precipitates in the reagent does not affect performance; however, it can be filtered before use on filter paper.

REFERENCES

1. ISO 14189:2013. Water quality-Enumeration of Clostridium perfringens-Method using membrane filtration.

PACKAGING

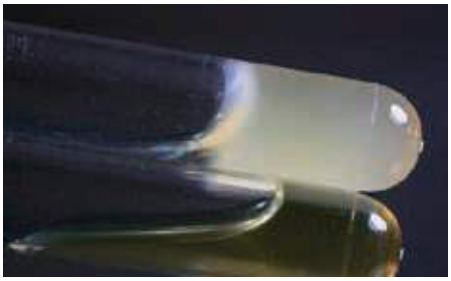
Product	Type	REF	Pack
Acid Phosphatase Reagents	Liquid reagents	192010	4+4 mL

Acid Phosphatase Reagent- Vial A: vial with dropper cap containing 4 ml of solution.
Acid Phosphatase Reagent- Vial B: vial with dropper cap containing 4 ml of solution.
Both reagents are ready-to-use for performing the acid phosphatase test.

IFU rev 3, 2023/03

COAGULASE PLASMA EDTA

Biochemical identification reagent



Coagulase Plasma. From the top to bottom:
S.aureus (+) and *S.epidermidis* (-)

INTENDED USE

In vitro diagnostic. For the qualitative detection of coagulase enzyme in staphylococci.

TYPICAL COMPOSITION – VIAL CONTENTS *

REF 429936: Rabbit Plasma EDTA, 5.0 mL (lyophilized)
REF 429937: Rabbit Plasma EDTA, 2.5 mL (lyophilized)
REF 429938: Rabbit Plasma EDTA, 1.0 mL (lyophilized)

*The vial content may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The coagulase test was developed from observations, in the early 1900s, that certain staphylococci clotted plasma from the goose,[1] man, horse, and sheep[2]. Today, the widely used and generally accepted method of differentiating staphylococci associated with acute infection or food poisoning is the identification of extracellular coagulase production using a tube coagulase test.[3,4] Based on their ability to clot plasma, staphylococci may be divided into coagulase-positive or coagulase-negative ones.
The enzyme coagulase acts on a “coagulase reacting factor” present in rabbit plasma, producing a thrombin-like substance which activates fibrinogen to form fibrin, causing the plasma to clot. Coagulase exists in two forms: “bound coagulase” (or clumping factor) which is bound to the cell wall and “free coagulase” which is liberated by the cell wall. Bound coagulase is detected by the slide coagulase test, whereas both free and bound coagulase are detected by the tube coagulase test.[5]
Coagulase Plasma EDTA, in the different proposed formats, is recommended for performing the tube test. Typically, rabbit plasma is inoculated with growth from isolated colonies and examined for the presence of a gel or clot at 4 hours and, if negative, examined again at 24 hours. In addition to *Staphylococcus aureus* also other species, including *Staphylococcus schleiferi* and *Staphylococcus intermedius* may give positive results in the tube coagulase test but are not common isolates from human infections.[3,5]
For the tube coagulase test, EDTA plasma is superior to citrated plasma because citrate-utilizing organisms such as *Pseudomonas* species, *Serratia marcescens, Enterococcus faecalis* and strains of *Streptococcus* will clot citrated plasma.[5]

METHOD OF PREPARATION

REF 429936: reconstitute the content of one vial with 15 mL of sterile purified water under aseptic conditions.
REF 429937: reconstitute the content of one vial with 7.5 mL of sterile purified water under aseptic conditions.
REF 429938: reconstitute the content of one vial with 3 mL of sterile purified water under aseptic conditions.
Stir for 30 seconds by means of a vortex to dissolve completely. The rabbit plasma results to be diluted 1:3.

PHYSICAL CHARACTERISTICS

Appearance of lyophilized pellet	dense pastille
Appearance of coagulase plasma after reconstitution	yellowish to pink opalescent solution

SPECIMENS

In clinical and non-clinical microbiology, the specimens consist of suspected staphylococcal colonies grown on plating media. Coagulase Test EDTA cannot be used for the direct testing of clinical specimens.

TEST PROCEDURE

From the surface of each selected colony, remove an inoculum with a sterile loop and transfer it to a tube of Brain Heart Infusion Broth.
Incubate the broth at 35-37°C for 18-24 hours.
Aseptically add 0.5 mL of each culture to 0.5 mL of Coagulase Plasma EDTA in small sterile tubes, and incubate at 35-37°C.

READING AND INTERPRETATION

Observe every 60 minutes in the first 4 hours of incubation for clotting by gently slanting the tube. Do not shake.
If no clot is observed by 4 hours, the tube should be read again after 18-24 h of incubation at 35-37°C.
Any degree of clotting represents a positive test. A flocculent or fibrous precipitate is not a true clot and should be recorded as negative.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.
Positive control: *S. aureus* ATCC 25923; negative control: *S. epidermidis* ATCC 12228

LIMITATIONS OF THE METHOD

- Some species of staphylococci other than *S. aureus* (*S. intermedius, S. hycus, S. schleiferi* subsp. *coagulans, S. delphini, S. lutrae, S. pseudointermedius, S. argenteus*) may give positive reaction to coagulase test.[3-6]
- The colony inoculum used for testing must be pure because a contaminant may produce false results after prolonged incubation.[5]
- The tube coagulase test should not be unduly agitated as this can cause the clot to shrink also giving a false negative result.[5]
- Care should be taken when using tube coagulase test directly on presumptive positive coagulase blood culture broth while recent reports have indicated no loss of sensitivity when the tube coagulase test is performed directly on uncentrifuged blood culture broths.[7,8]
- Observation for clotting should be made within the first 4 h since some staphylococci produce fibrinolysin, which may lyse clots early in the incubation period.[9,10] If no clotting is observed, however, the tube should be incubated overnight and observed again for delayed clotting.
- False-negative coagulase reactions may occur if the test isolate is older than 18-24 hours or if there is scant growth.
- Slide coagulase test has a sensitivity lower than tube test since it detects only bound coagulase enzyme.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the isolates. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Upon receipt, store the product in the original pack at 2-8°C away from direct light. Opened and reconstituted vial under aseptic conditions can be stored at 2-8°C for up to 5 days or aliquot into 0.5 ml amounts and stored at -20ºC for 14 days. Allow plasma to equilibrate to room temperature before use. Do not use the plasma if is clotted upon rehydration, if it is contaminated or if there are other signs of deterioration (precipitate, atypical colour).

REFERENCES

1. Loeb L. The influence of certain bacteria on the coagulation of the blood. J med Res 1903; 10: 407
2. Much H. Uber eine Vorstufe des Fibrinfermentes in Kulturen von Staphylokokkus aureus. Biochem. Z.1908;14: 143
3. Becker K, Skov R, VonEiff C. Staphylococcus, Micrococcus, and other catalase-positive cocci: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology, 12th ed. Washington, DC: American Society for Microbiology; 2019
4. ISO 6888-1, 2021 - Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) —Part 1: Method using Baird-Parker agar medium. Second edition, 2021-08
5. Public Health England. (2018). UK Standards for Microbiology Investigations: Coagulase test test Procedures TP 10, Issue no: 6, Issue date: 01.08.18
6. Staphylococcus argenteus: Another Coagulase Positive Staphylococcus. May 28, 202, Podcast. American Society for Microbiology.
7. Davis TE, Fuller DD, Aeschleman EC. Rapid, direct identification of Staphylococcus aureus and Streptococcus pneumoniae from blood cultures using commercial immunologic kits and modified conventional tests. Diagn Microbiol Infect Dis 1992; 15:295-300. B, II
8. McDonald CL, Chapin K. Rapid identification of Staphylococcus aureus from blood culture bottles by a classic 2-hour tube coagulase test. J Clin Microbiol 1995; 33:50-2. B, II
9. Yrios JW. Comparison of rabbit and pig plasma in the tube coagulase test. J Clin Microbiol 1977; 5:221-4. B, II
10. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019

PACKAGING

Product	Type	REF	Pack
Coagulase Plasma EDTA	Identification reagent	429936	4 vials with 5 ml of rabbit plasma (4 x 15 mL: 120 tests)
Coagulase Plasma EDTA	Identification reagent	429937	4 vials with 2,5 ml of rabbit plasma (4 x 7,5 mL: 60 tests)
	Identification reagent	429938	10 vials with 1 ml of rabbit plasma (10 x 3 mL: 60 tests)

IFU rev 1, 2021/10

INDOLE SPOT REAGENT

Biochemical identification reagent

INTENDED USE

Liquid reagent for indole spot test, as an aid in the differentiation of the *Enterobacteriaceae* and other genera.

COMPOSITION – BOTTLE CONTENTS

p-dimethylamino cinnamaldeide	0.2 g
HCl 10%	10 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Bacteria that possess the enzyme tryptophanase are capable of hydrolysing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia.[1] The chief requirement for culturing an organism prior to perform the indole test is that the medium contains a sufficient quantity of tryptophan. Typically, indole test can be performed by tube method and by spot test.[1-3] Indole Spot Reagent has been devised for spot test. The indole spot test is based on the formation of a green-blue colour complex when indole reacts with aldehyde group of p-dimethylaminocinnamaldeide Indole production is an important characteristic in the identification of many microorganisms, being particularly useful in separating *E. coli* (positive) from members of the *Klebsiella-Enterobacter-Hafnia-Serratia* group (mostly negative). It is used as part of the IMViC procedures, a battery of tests designed to distinguish among members of the family *Enterobacteriaceae*.

PHYSICAL CHARACTERISTICS

Appearance of the reagent	limpid, yellow solution

SPECIMENS

In clinical and non-clinical microbiology, the specimens consist of colonies grown on plated media. Kovács' Reagent cannot be used for the direct testing of clinical specimens.

TEST PROCEDURE

Inoculate the strain to be tested on an agar medium that contains tryptophan. Tryptic Soy Agar (REF 402150) or Blood Agar Sheep plates (REF 541136) can be used. Incubate for 18 to 24 hours at the appropriate temperature to allow the growth.

Touch the colonies to be tested with the tip of a swab or place a piece of filter paper into a sterile Petri dish and smear an isolated pure colony using a sterile loop. Squeeze 2 drops of Indole Spot Reagent onto the swab or the filter paper.

READING AND INTERPRETATION

Examine the reaction area and record the colour.
Production of a distinct green-blue colour within 30 seconds: indole positive strain.
No colour production or orange or violet colour production within 30 seconds: indole negative strain.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.

Positive control: *Escherichia coli* ATCC 25922; negative control: *Enterobacter aerogenes* ATCC 13048

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- This test should be used only on colonies from media containing sufficient tryptophan and no glucose.[4]
- Colonies from mixed culture should not be used, because indole-positive colonies can cause indole negative colonies to appear weakly positive.[4]
- Organisms to be tested by the spot indole method must be taken from a tryptophan-containing medium (for example blood agar) and never from MacConkey agar media as they have pH indicators and pigmentation of lactose-positive colonies which will make interpretation of colour reaction difficult.[2]
- Indole is a diffusible product. To mitigate indole diffusion, select a well isolated colony for the spot indole test.[2]
- Change in colour of the reagent from yellow to brown indicates improper storage, which may cause weaker reactions.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the colonies. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Upon receipt, store the product in the original pack with the cap tightly closed, at 10-30°C away from direct light. Opened container can be used up to the expiration date. Repeated openings of the container do not affect the performances of the product. Do not use the reagent with atypical brown colour.

REFERENCES

1. Maria P. MacWilliams. Indole Test Protocol. ASM, 08 December 2009, American Society for Microbiology © 2016.
2. Public Health England. UK Standards for Microbiology Investigation, Indole test. TP 19, Issue 4, 2018.
3. Cowan and Steel's Manual for identification of Medical Bacteria, 3rd ed. 1993.
4. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

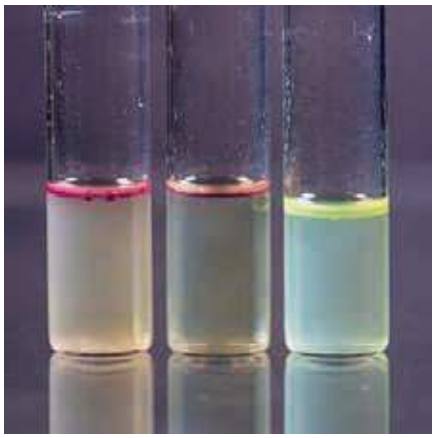
PACKAGING

Product	Type	REF	Pack
Indole Spot Reagent	Liquid reagent	19171003	10 mL, primary packaging: bottle with dropper

IFU rev 3, 2023/03

KOVACS' REAGENT

Biochemical identification reagent



Kovacs' Reagent - from left: *E. coli* (+), *H. influenzae* (+), *S.* Typhimurium (-)

INTENDED USE

In vitro diagnostic device. Reagent for indole test, used for the detection of tryptophanase production, as an aid in the differentiation of the *Enterobacteriaceae* and other genera.

COMPOSITION – TYPICAL FORMULA (100 mL)

p-dimethylaminobenzaldehyde	5 g
N-amyl alcohol	75 mL
Hydrochloric acid (concentrated)	25 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Bacteria that possess the enzyme tryptophanase are capable of hydrolysing and deaminating tryptophan with the production of indole, pyruvic acid, and ammonia.[1] The chief requirement for culturing an organism prior to perform the indole test is that the medium contains a sufficient quantity of tryptophan. The indole test is based on the formation of a red colour complex when indole reacts with aldehyde group of p-dimethylaminobenzaldehyde, under acidic conditions. Typically, indole test can be performed by tube method and by spot test.[1-3] Indole production is an important characteristic in the identification of many microorganisms, being particularly useful in separating *E. coli* (positive) from members of the *Klebsiella-Enterobacter-Hafnia-Serratia* group (mostly negative). It is used as part of the IMViC procedures, a battery of tests designed to distinguish among members of the family *Enterobacteriaceae*.[1] Kovács' Reagent for indole test is included in several ISO Standards[4-8] as an aid in the identification of *E. coli*, *E. coli* O157, *Salmonella*, *Vibrio*, *Yersinia*, isolated from the food chain.

PHYSICAL CHARACTERISTICS

Appearance of the reagent	limpid, yellow solution

SPECIMENS

In clinical and non-clinical microbiology, the specimens consist of colonies grown on plated media. Kovács' Reagent cannot be used for the direct testing of clinical specimens.

TEST PROCEDURE

Indole tube test

Inoculate the Tryptone Tryptophan Broth (REF 402165) or Peptone-Tryptone Water (REF 401891) with the test organism and incubate at 37°C for 24 to 48 h.

• add several drops of the Kovács' Reagent and shake gently.

• examine the upper layer of liquid after about 1 min for the appearance of a red-pink colour.

Indole spot test

Inoculate the bacterium to be tested on an agar medium that contains tryptophan. Tryptic Soy Agar (REF 402150) or Blood Agar Sheep plates (REF 541136) can be used. Incubate for 18 to 24 hours at the appropriate temperature to allow the growth.

Place a piece of filter paper (Whatman no.1) into a sterile Petri dish and moisten with 1 -1.5 mL of Kovács' Reagent.

Smear an isolated pure colony onto the saturated surface of the filter paper using a sterile loop and examine for the appearance of a red-pink colour within 1-3 minutes.

READING AND INTERPRETATION

Indole tube test

Positive result: formation of a pink to red colour within 1 minute (occurring normally within a few seconds)

Negative result: no colour change, the reagent layer remains yellow or slightly cloudy

Indole spot test

If indole is present, a red-pink colour will develop within 1 to 3 minutes.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.

Positive control: *Escherichia coli* ATCC 25922; negative control: *Enterobacter aerogenes* ATCC 13048

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- This test should be used only on colonies from media containing sufficient tryptophan and no glucose.[9]
- Colonies from mixed culture should not be used, because indole-positive colonies can cause indole negative colonies to appear weakly positive.[9]
- If Peptone Broth, other than the suggested reference, is used instead of Tryptophan broth, the batch should be checked with a positive control to ensure the peptone is adequate for indole production. This is because there are varieties of peptone broth media on the market, and some are unsuitable for indole production because they contain too little tryptophan.[2]
- Organisms to be tested by the spot indole method must be taken from a tryptophan-containing medium (for example blood agar) and never from MacConkey agar media as they have pH indicators and pigmentation of lactose-positive colonies which will make interpretation of colour reaction difficult.[2]
- Indole is a diffusible product. To mitigate indole diffusion, select a well isolated colony for the spot indole test.[2]
- The tube test is a more sensitive method of detecting indole than the spot test.
- Kovacs Indole Reagent is not recommended for use with anaerobic bacteria.
- Change in colour of the reagent from yellow to brown indicates improper storage, which may cause weaker reactions.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the colonies. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Upon receipt, store the product in the original pack with the cap tightly closed, at 10-30°C away from direct light. Opened container can be used up to the expiration date. Repeated openings of the container do not affect the performances of the product. Do not use the reagent with atypical brown colour.

REFERENCES

1. Maria P. MacWilliams. Indole Test Protocol. ASM, 08 December 2009, American Society for Microbiology © 2016.
2. Public Health England. UK Standards for Microbiology Investigation, Indole test. TP 19, Issue 4, 2018.
3. Cowan and Steel's Manual for identification of Medical Bacteria, 3rd ed. 1993.
4. ISO 7251:2005. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of presumptive Escherichia coli. Most probable number technique.
5. ISO 16654:2001Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Escherichia coli O157.
6. ISO 6579-1:2017. Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella. Part 1: Detection of Salmonella spp.
7. ISO 21872-1:2017 Microbiology of the food chain. Horizontal method for the determination of Vibrio spp. — Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus.
8. ISO 10273:2017 -Microbiology of the food chain. Horizontal method for the detection of pathogenic Yersinia enterocolitica.
9. Atlas R, Snyder J. Reagents, Stains and Media: Bacteriology. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.

MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Kovacs' Reagent	Liquid reagent	19171000 19171001	50 mL 500 mL

IFU rev 1, 2022/04

MONO CONFIRM TEST

INTENDED USE
For the confirmation of *Listeria monocytogenes* and *Listeria* spp.

COMPOSITION – KIT CONTENTS
- 12 strips with 8 microwells each for carry out 24 tests.

The following tests are performed into the microwells with the following dried substrates:

WELLS	TESTS	SUBSTRATES
A or E	Acid production from arabitol (ARA)	D-arabitol
B or F	Acid production from α-methyl- D-glucoside (MEGLU)	α -methyl-D-glucoside
C or G	Beta-glucosidase (X-GLUPY)	5-bromo-4-chloro-3-indolyl-beta-D-glucopyranoside
D or H	D-aminopeptidase (AMP)	aminoacil β- naphtylamide

- 1 frame with the lid
- p-dimethylaminobenzaldehyde 5% solution in HCl 10%, 4 mL
- 24 plastic pipettes
- Standard Mac Farland n. 2
- 1 Product Information and result reporting paper

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Mono Confirm Test is a micro method based on four biochemical reactions for confirmation of *Listeria* spp. and differentiation of *Listeria monocytogenes* from other non-*L. monocytogenes* species. Substrates are dried in plastic microwells; the kit provides 12 strips with 8 microwells; 2 strains can be tested with each strip. *Listeria* spp. is differentiated from other genera by β-glucosidase test and acid production from arabitol and α-methyl D-glucoside. *L. monocytogenes* is identified by detection of a new specific enzyme D-aminopeptidase, which is present in all non-pathogenic *Listeria* species and absent in *L. monocytogenes* strains.

PHYSICAL CHARACTERISTICS
Appearance of p-dimethylaminobenzaldehyde 5% solution limpid, very pale-yellow solution

SPECIMENS
The specimens consist of pure colonies grown on plated media.

TEST PROCEDURE
1. For confirmation of presumptive *L. monocytogenes* or Listeria spp., take at least one colony from selective medium (up to a maximum of five colonies from each plate of each selective medium).
2. Streak the selected colonies onto the surface of pre-dried plates of a non-selective agar, for example TSYEA (Tryptic Soy Yeast Extract Agar REF 402166) for obtaining well isolated colonies.
3. If required check for Gram staining, motility, catalase and oxidase. *Listeria* strains are short Gram-positive rods, motile at 25°C but not at 37°C, catalase positive, oxidase negative.
4. With the colonies cultivated on TSYEA, prepare 1 mL of bacterial suspension in sterile saline solution with a turbidity equal to Mc Farland n° 2 (between 6 and 8 colonies.)
5. Transfer the needed strips onto the frame
6. Distribute 3 drops of bacterial suspension into the wells A-B-C-D or E-F-G-H by means of the supplied plastic pipettes.
7. Cover the inoculated strips with the lid and incubate at 37°C for 18 - 24 hours
8. Read the reactions which do not need the additional reagent in the wells A-B-C (or E-F-G): ARA, MEGLU, X-GLUPY
9. Add one drop of dimethylaminobenzaldehyde reagents to the well D (or H) and read the reaction AMP
10. Record the positive and negative results and identify the strains according to the below schemes
11. Wash with a disinfectant the frame and the lid after the use.

READING AND INTERPRETATION
READING

TEST	POSITIVE REACTION	NEGATIVE REACTION
Arabitol (ARA)	straw colour	blue or blue-green colour
Methyl-glucoside (MEGLU)	straw colour	blue or blue-green colour
Beta-glucosidase(X-GLUPY)	blue - clear blue colour	colourless
D-aminopeptidase (AMP)	yellow colour (after dimethylaminobenzaldehyde addition)	Colourless (after dimethylaminobenzaldehyde addition)

RESULTS INTERPRETATION

ARA	MEGLU	X-GLUPY	D-AMP	IDENTIFICATION
-	-	-	*	Not-*Listeria* sp.
-	+	+	*	Not-*Listeria* sp.
-	-	+	*	Not-*Listeria* sp.
-	+	-	*	Not-*Listeria* sp.
+	-	-	*	Not-*Listeria* sp.
+	-	+	*	Not-*Listeria* sp.
+	+	-	*	Not-*Listeria* sp.
+	+	+	+	*Listeria* sp. not-*monocytogenes*
+	+	+	-	*Listeria monocytogenes*

* When even one of the tests ARA, MEGLU, X - GLUPY is negative, do not read the AMP reaction

USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.

Positive control: *L. monocytogenes* ATCC 13932 or ATCC 19111
Negative control: *L. innocua* ATCC 33090

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD
- According to ISO 11290, isolates which are considered to be *L. monocytogenes* may be sent for further characterization to a recognized national or regional Listeria Reference Laboratory.[1]

STORAGE
Upon receipt, store the product in the original pack with the cap tightly closed, at +2/+8°C away from direct light. If properly stored, the product may be used up to the expiration date. Do not use the product beyond this date.

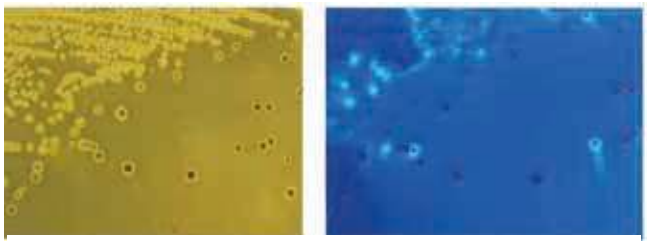
REFERENCES

1. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.

PACKAGING
Product	Type	REF	Pack
Mono Confirm Test	Kit	193000	24 tests

IFU rev 3, 2023/03

MUCAP TEST
Biochemical identification reagent



Salmonella sp. and *Proteus mirabilis* mixed culture on Hektoen Enteric Agar, flooded with MUCAP Test reagent.
At left: normal light, at right under Wood's lamp

INTENDED USE
In vitro diagnostic. Liquid reagent for rapid differentiation of *Salmonella* spp. colonies directly on selective enteric plating media.

COMPOSITION – BOTTLE CONTENT
4- methylumbellyferil caprylate dissolved in heptane 8 mL

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Gastrointestinal infections are still a global public health problem, being a leading cause of medical consultation. Among the bacteria responsible for these syndromes, *Salmonella* spp. are one of the most common causes of bacterial diarrheal diseases.
The fluorogenic substrates, derived from methylumbelliferone and coumarins, have been widely tested for the rapid identification of various microbial species and have demonstrated high sensitivity.[1,2]
The MUCAP Test reagent, developed and synthesized by Biolife and earlier described in some scientific papers,[3,4] is an easy and rapid screening test for presumptive identification of *Salmonella* colonies, directly on selective enteric plating media,[5,6] using an eight-carbon-atom ester conjugated with methylumbelliferone.
The MUCAP Test reagent detects the C_8 esterase enzyme, encoded by the apeE gene, present in *Salmonella* spp.[9] The MUCAP Test reagent consists of 4-methylumbellyferil caprylate dissolved in heptane; this substrate interacts with the *Salmonella* C_8 esterase in a specific manner, leading to the rapid release on the medium of umbelliferone, strongly fluorescent under Wood's lamp at 366 nm.

PHYSICAL CHARACTERISTICS
Reagent appearance colourless, limpid

SPECIMENS
In clinical and non-clinical microbiology, the specimens consist of suspected *Salmonella* colonies grown on selective enteric plating media. MUCAP Test reagent cannot be used for the direct testing of clinical specimens.

TEST PROCEDURE
The test is carried-out by flooding with one drop of MUCAP reagent all the suspect *Salmonella* colonies (lactose negative and H_2S positive or negative colonies) cultivated on a selective enteric plating medium.
The following scheme should be used:
1. Observe the colonies under a Wood's lamp (366nm) before adding the reagent to ensure that no spontaneous fluorescence occurs.
2. Add a drop of reagent to each isolated colony or to a group of colonies.
3. After 3-5 minutes observe the plates under the Wood's lamp (wavelength 366 nm) in semi-darkness.

READING AND INTERPRETATION
Positive result: appearance of a blue fluorescence over the whole colony or on the edge of black centred colonies.
Negative result: no development of fluorescence
The fluorescent colonies can be presumptively identified as *Salmonella* and subjected to a complete identification with appropriate tests.
Fluorescence negative colonies can be considered non-*Salmonella* and therefore plates are discarded.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.
Positive control: S. Typhimurium ATCC 14028
Negative control: *P. mirabilis* ATCC 25933

ATCC is a trademark of American Type Culture Collection

PERFORMANCES CHARACTERISTICS

Several experimental works have been published for the evaluation of the specificity and sensitivity of MUCAP Test in identifying *Salmonella* colonies. The sensitivity values are almost constantly close to 100%;[7-19] the specificity values are more variable and sometimes dependent on the isolation and enrichment media combination and vary between 80% and 100%.[7-19]
The specificity and sensitivity of the detection lactose negative, H_2S positive and MUCAP positive colonies are respectively of 100% and 99.8%.[15]

LIMITATIONS OF THE METHOD

- Before carrying out the test it is advisable to examine the plates under Wood's lamp for the presence of natural fluorescence developed by *Pseudomonas* spp. Strains with natural fluorescence should not be subjected to the MUCAP Test
- Lactose positive *Salmonella* strains, with atypical colonies on conventional lactose-containing media such as SS Agar, Hektoen Enteric Agar etc, may not be detected with MUCAP Test; however, these strains can be detected with the reagent on lactose-free media.
- Since most of the false positive strains are oxidase positive,[6] the H_2S negative and fluorescence positive colonies can be tested with the oxidase reagent or strips. The combination of MUCAP and oxidase tests raises the specificity of C_8 esterase detection from 94.1% to 98.2%.[16]
- The reagent does not affect the viability of the flooded colonies.
- Do not take any reading after 5 minutes because the reagent is susceptible to autolysis due to the watery nature of the medium and so false positive results might occur.
- The MUCAP Test can be carried-out on the most commonly used culture media (e.g. Mac Conkey Agar, SS Agar, Hektoen Enteric Agar, Brilliant Green Agar, Desoxycholate Agar, XLD Agar etc.). MUCAP Test cannot be performed on colonies cultivated on Bismuth Sulphite Agar.
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the MUCAP positive colonies. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the reagent in the original pack at 2-8°C away from direct light. If properly stored, the reagent may be used up to the expiration date. Do not use the reagent beyond this date. Opened bottle can be used up to the expiration date. Repeated openings of the bottle do not affect the performances and do not cause contamination of the reagent. Do not use the reagent with signs of deterioration (turbidity, precipitate, atypical colour).

REFERENCES

1. Godsey JH, Matteo MR, Shen D,Tolman G,Gohlke JH. Rapid identification of Enterobacteriaceae with microbial enzyme activity profiles. J Clin Microbiol 1981; 13:483-490.
2. Malfa S. Potenza D. Metodi rapidi enzimatico-fluorescenti per l'identificazione di batteri patogeni di frequente isolamento: E. coli, P. aeruginosa, Salmonella spp. XV Congresso Nazionale AMCLI, 1986, Milano
3. Pontello M, Russolo S, Carozzi F, Bottiroli U. Evaluation of a new rapid method (MUCAP Test) for the presumptive identification of Salmonella on primary isolation media. 5th Int. Simp. on Rapid Method and Aut. in Microb. and Immunol. Florence 4-6 Nov. 1987.
4. F. Ottaviani. Esperienze su una tecnica fluoro-enzimatica per lo screening rapido di Salmonella Industrie Alimentari – XXVII, Novembre 1988.
5. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. By Gary W. Procop, Deirdre L. Church, Geraldine S. Hall, William M. Janda. Jones & Bartlett Learning, 2020
6. Manual of Environmental Microbiology edited by Marylynn V. Yates (Editor-in-Chief), Cindy H. Nakatsu (Editor), Robert V. Miller (Editor), Suresh D. Pillai (Editor). ASM Press, 2020
7. Agostinelli A. Agostinelli C. Damiani AM. Gambella O. Valutazione di un reattivo specifico per l'identificazione di Salmonella spp. sulla piastra di primo isolamento. Microbiologia Medica. 1990 Vol. 5 n° 3, 129.
8. Anichini P. Lavacchi A. Pecile P. Nicoletti P. Valutazione di un nuovo metodo basato sulla fluorescenza nello screening delle coprocolture per la ricerca di Salmonella. Microbiologia Medica 1990; Vol. 6, n° 4, 117
9. Carinato ME, Collin-Osdoby P, Yang X, Knox TM, Conlin CA, Miller CG. The apeE gene of Salmonella typhimurium encodes an outer membrane esterase not present in Escherichia coli. J Bacteriol 1998 Jul;180(14):3517-21.
10. Humbert F. Salvat G. Colin P. Lahellec C. Bennejean G. Rapid identification of Salmonella from poultry meat products by using 'Mucap Test'. Int J Food Microbiol. 1989 Feb;8(1):79-83.
11. Aguirre, P.M., Cacho, J.B., Folgueira, L., Lopez, M., Garcia, J., Velasco, A.C. Rapid fluorescence method for screening Salmonella spp. from enteric differential agars. J. Clin. Microbiol. 1990; 28:148
12. Munoz P. Diaz D. Carcemado E. Rodriguez M. Rodeno P. Bonza E. Evaluation of a rapid method of detection of Salmonella in differential enteric media. 30th ICAAC, Atlanta, Ga, 1990
13. Manafi M. Sommer R. Comparision of three rapid screening methods for Salmonella spp.: MUCAP Test, MicroScreen Latex and Rambach Agar. Lett. App. Microbiol. 1992; 14: 163
14. Olsson. M. Syk A. Wollin R. Identification of Salmonella with the 4-methylumbelliferyl caprylate fluorescence test. J. Clin. Microbiol. 1991; 29: 2631
15. Ruiz, J., Sempere, M.A., Varela, M.C., Gomez, J. Modification of the methodology of stool culture. J. Clin. Microbiol. 1992; 30: 525
16. Ruiz J. Varela M.C. Sempere M.A. Lopez M.L. Gomez J. Oliva J. Presumptive identifiaction of Salmonella enterica using two rapid tests. Eur. J. Clin. Microbiol. Infect. Dis. 1991; 10: 649
17. Wei Zhang, Zongke Sun, Bo Lu, Youbin Wang, Pei Ding, Ping Zheng, Xiping Chen. Detection of Salmonella in water by using MUCAP test. Wei Sheng Yan Jiu . 2010 May;39(3):381-3
18. Chu Tianxin. Rapid Identification of Salmonella spp. using MUCAP Fluorescence Test. Chinese Journal of Public Health, 2000 16(1): 69-70.
19. S Abdalla, J Vila, M T Jimenez de Anta. Identification of Salmonella spp. with Rambach agar in conjunction with the 4-methylumbelliferyl caprylate (MUCAP) fluorescence test. Br J Biomed Sci .1994 Mar;51(1):5-8.

PACKAGING

Product	Type	REF	Pack
MUCAP Test	Identification reagent	191500	8 mL (160 tests)

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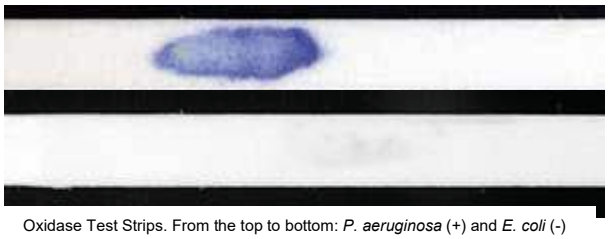
OXIDASE TEST STRIPS



Oxidase Test Strips. From the top to bottom: *P. aeruginosa* (+) and *E. coli* (-)

INTENDED USE

In vitro diagnostic device. For the detection of the cytochrome oxidase enzyme in bacteria isolated on solid culture media.

COMPOSITION – PACK CONTENT

30 paper strips impregnated with
N,N,N',N' tetramethyl-p-phenylenediamine dihydrochloride in a preservative solution.

PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The final stage of bacterial respiration may involve the use of the enzyme cytochrome oxidase, which catalyses the oxidation of cytochrome c while reducing oxygen to form water.[1] The cytochrome system is usually only present in aerobic organisms which are capable of utilising oxygen as the final hydrogen receptor.[2]
The test reagent, N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) acts as an artificial electron acceptor for the enzyme oxidase. When the reagent is oxidized by cytochrome c, it changes from colourless to a dark indophenol blue.
The oxidase test is used as an aid for the differentiation of *Neisseria*, *Moraxella*, *Campylobacter* and *Pasteurella* species (oxidase positive).[2] It is used to differentiate pseudomonads from related species; all *Pseudomonas* species are oxidase positive except a few *Pseudomonas* species that are oxidase negative; *P. luteola, P. oryzihabitans, P. syringae* and *P. viridiflava* are all oxidase negative.[2]
The performance of the oxidase test is essential for colonies suspected to belong to *Neisseria*; both *N. gonorrhoeae* and *N. meningitidis* give a positive reaction.[3]
Within *Bordetella* genus, *B. pertussis* and *B. bronchiseptica* are oxidase positive, *B. parapertussis* is oxidase negative.[4]
Within the *Burkholderia cepacia* complex all the species are oxidase positive with the exception of *B. contaminas* (variable) and *B. pyrrocinia* (variable)[5]
The oxidase test with the compound TMPD is recommended by ISO 16266,[6] ISO 13720,[7] ISO 11059[8] for the confirmation test of *P. aeruginosa* or *Pseudomonas* spp. in water, meat and milk products and by ISO 9308[9] for differentiating coliform bacteria (oxidase negative) from *Aeromonas* and other oxidase positive Gram-negative bacteria.
Oxidase Test Strips are paper strips impregnated with Kovacs' oxidase reagent[10,] N,N,N,N'-tetramethyl-p-phenylenediamine dihydrochloride in a preservative solution and dried.

PHYSICAL CHARACTERISTICS

Paper strips appearance	colourless

SPECIMENS

In clinical and non-clinical microbiology, the specimens consist of colonies grown on plated or tubed media. Oxidase Test Strips cannot be used for the direct testing of clinical specimens.

TEST PROCEDURE

Observe the culture for purity and sufficient growth on media such as blood agar, chocolate agar, Tryptic Soy Agar, Nutrient Agar.
Moist a portion of the paper strip with 2 drops of purified water.
Use a platinum or plastic sterile loop and pick a well-isolated colony from a fresh (18 to 24-hour culture) bacterial plate and rub onto the moistened filter paper.

READING AND INTERPRETATION

Examine the reaction area for the appearance of a colour ranging from blue to grey-blue.
Oxidase positive organisms produce a colour ranging from blue to grey-blue within 30 seconds.
Oxidase negative organisms produce no colour change within 30 seconds test period.

USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed the test strains useful for the quality control.
Positive control: *P. aeruginosa* ATCC 27853; negative control: *E. coli* ATCC 25922

ATCC is a trademark of American Type Culture Collection

LIMITATIONS OF THE METHOD

- The oxidase test may be used in the presumptive identification of *Neisseria* spp. and in the differentiation and identification of Gram-negative bacilli. All oxidase-positive organisms should be examined by Gram stain to determine cellular morphology and Gram reaction
- Weak oxidase producers (e.g., *Pasteurella*) may appear negative within the time limits of the test.
- The test should not be performed on cultures from media containing tellurite and fermentable carbohydrates such as glucose, as these may prevent the reaction from occurring and give false negative results.[2]
- Bacteria grown on media containing dyes may give aberrant results.[1,2]
- *Candida albicans* will occasionally give positive result with oxidase test when grown on chocolate agar but give negative reactions when grown on Sabouraud dextrose agar.[2]
- Use a young culture growing on an agar plate or agar slant, preferably less than 24 hr old. Older cultures are less metabolically active and results from these are unreliable. [1,2]
- Using nickel, steel and other wire loops may give false-positive results and this may occur due to surface oxidation products formed during flame sterilisation. It is important to use only platinum or inert transfer loops, sterile wooden sticks, sterile plastic loops, sterile swabs, etc.[1,2]
- It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed for complete identification of the colonies. If relevant, perform antimicrobial susceptibility testing.

STORAGE CONDITIONS

Upon receipt, store the product in the original pack at 2-8°C away from direct light. If properly stored, the product may be used up to the expiration date. Do not use the product beyond this date. Opened container can be used up to the expiration date. Repeated openings of the container do not affect the performances of the product. Do not use the strips with signs of deterioration (atypical colour).

REFERENCES

1. Shields P, Cathcart L. Oxidase Test Protocol. ASM 11-11-2010. B, American Society for Microbiology © 2016.
2. Public Health England. (2019). Oxidase test. UK Standards for Microbiology Investigations. TP 26 Issue 4.
3. Elias J, Frosh M, Vogel U. Neisseria. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
4. Kilgore PE, Coenye T. Bordertella and related genera. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
5. LiPuma JJ, Currie BJ, Peacock SJ, Vandamme PAR. Miscellaneous Gram-negative bacteria. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019
6. ISO 16266:2006 Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration
7. ISO 13720:2010 Meat and meat products — Enumeration of presumptive Pseudomonas spp.
8. ISO/TS 11059:2009 [IDF/RM 225:2009] Milk and milk products — Method for the enumeration of Pseudomonas spp.
9. ISO 9308-1:2014 Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora.
10. Kovács, N. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature (London) 1956; 178:703

PACKAGING

Product	Type	REF	Pack
Oxidase Test Strips	Paper strips for oxidase test	191040ST	30 paper strips 7.5 cm x 0.5 cm

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APPENDIX TO THE MANUAL: GHS HAZARD PICTOGRAMS

The GHS hazard pictograms in this table are provided as generic warning information when using Biolife products. As legislation and classification are subject to frequent revision, for more precise, complete and up-to-date information on hazards and risks, consult product labels and safety data sheets at www.biolifeitaliana.it.

SIGNIFICANCE OF PICTOGRAMS

NA: NOT APPLICABLE


ACUTE TOXICITY


SERIOUS HEALTH HAZARD


HEALTH HAZARD/HAZARDOUS TO THE OZONE LAYER


CORROSIVE


HAZARDOUS TO THE ENVIRONMENT


FLAMMABLE

DEHYDRATED CULTURE MEDIA

ACETAMIDE BROTH	40101012	
AEROMONAS SELECTIVE AGAR BASE (HAVELAAR)	4010192	NA
AESCULIN BILE AZIDE AGAR	4010142	NA
AESCULIN BILE AZIDE BROTH	40101412	
AGAR LISTERIA ACC.TO OTTAVIANI & AGOSTI (ALOA®)	4016052	
ALKALINE PEPTONE WATER	4010322	NA
AMIES TRANSPORT MEDIUM	4010342	NA
ANTIBIOGRAMMA BASE AGAR	4010492	NA
ANTIBIOTIC BASE AGAR A2	4010502	NA
ANTIBIOTIC BROTH A3	4010652	NA
ANTIBIOTIC SEED AGAR A1	4010752	NA
APT AGAR	4010852	NA
APT BROTH	4010902	NA
ASPARAGINE ENRICHMENT BROTH	40109512	NA
AZIDE DEXTROSE BROTH	4011052	
AZIDE DEXTROSE BROTH -ROTHE	4011062	
AZIDE MALTOSE AGAR KF	4011072	
BACILLUS CEREUS AGAR BASE - MYP	4011112	NA

BACILLUS CEREUS AGAR BASE - PEMBA	4011122	NA
BAIRD PARKER AGAR BASE	4011162	NA
BCSA BURKHOLDERIA CEPACIA SELECTIVE AGAR BASE	4011532	NA
BILE AESCULIN AGAR	4010172	NA
BILE ESCULIN AZIDE AGAR ISO FORMULATION	4010182	NA
BIOTONE (TRYPTOSE) AGAR	4011452	NA
BIOTONE (TRYPTOSE) BROTH	4011462	NA
BISMUTH SULPHITE AGAR U.S.P.	40121022	NA
BLOOD AGAR BASE	4011552	NA
BLOOD AGAR BASE N° 2	4011562	NA
BRAIN HEART INFUSION AGAR	4012352	NA
BRAIN HEART INFUSION BROTH	4012302	NA
BRILLIANT GREEN AGAR	4012552	NA
BRILLIANT GREEN AGAR MODIFIED	4012562	NA
BRILLIANT GREEN BILE BROTH 2%	4012652	NA
BRILLIANT GREEN BILE BROTH 2% MUG	4012661	NA
BROMOCRESOL PURPLE GLUCOSE AGAR	4012732	NA
BRUCELLA BROTH	4012742	NA
BRUCELLA MEDIUM BASE	4012752	NA
BRYANT BURKEY BROTH BASE W/RESAZURIN	4012692	NA
BUFFERED PEPTONE WATER	4012782	NA
BUFFERED PEPTONE WATER (MEAT)	401278B2	NA
BUFFERED PEPTONE WATER (CASEIN)	401278C2	NA
BUFFERED PEPTONE WATER pH 7.2	401278S2	NA
CAMPYLOBACTER BLOOD AGAR BASE	4012852	NA
CAMPYLOBACTER BLOOD FREE MEDIUM BASE BOLTON (mCCDA)	4012822	NA
CAMPYLOBACTER BLOOD FREE MEDIUM BASE KARMALI	4012832	NA
CAMPYLOBACTER BOLTON ENRICHMENT BROTH BASE	401286B2	NA
CAMPYLOBACTER ENRICHMENT BROTH BASE	4012862	NA
CANDIDA AGAR (NICKERSON)	4012802	NA
CARY-BLAIR TRANSPORT MEDIUM	4012872	NA
C-EC AGAR	4012982	NA

CHAPMAN-STONE MEDIUM	4013002	NA
CHLORAMPHENICOL GLUCOSE YEAST EXTRACT AGAR	4012892	NA
CHROMOGENIC B. CEREUS AGAR BASE	4080202	NA
CHROMALBICANS AGAR	4080002	NA
CHROMART CRE-ESBL AGAR BASE	4080252	NA
CHROMOGENIC CANDIDA AGAR	4080052	
CHROMOGENIC COLIFORM AGAR	4012992	NA
CHROMOGENIC COLIFORM AGAR ISO FORMULATION	4012972	NA
CHROMOGENIC E.COLI O157 AGAR	4055812	NA
CHROMOGENIC LACTOBACILLUS ACIDOPHILUS AGAR (CLAA)	4015682	NA
CHROMOGENIC SALMONELLA AGAR BASE	4053502	NA
CHROMOGENIC STREPTO B AGAR BASE	4080102	NA
CHROMOGENIC URINE AGAR IV	409810G2	NA
CHROMOGENIC URINE AGAR IV CLEAR	409810C2	NA
CHROMOGENIC CRONOBACTER ISOLATION AGAR	4080302	NA
CHROMOGENIC YERSINIA AGAR BASE	4080502	NA
CIN AGAR BASE	4013022	NA
CLED MEDIUM	40129012	NA
CLOSTRIDIUM AGAR	4013032	NA
CLOSTRIDIUM BROTH	4013042	NA
CLOSTRIDIUM BOTULINUM AGAR BASE	4013062	NA
CLOSTRIDIUM PERFRINGENS AGAR BASE	4013072	NA
COLUMBIA AGAR EP	4011342	NA
COLUMBIA AGAR BASE	4011362	NA
COLUMBIA CNA AGAR BASE	40113612	NA
m-CP AGAR BASE	4013202	NA
CPLM TRICHOMONAS BROTH	4013312	NA
CRONOBACTER SCREEN BR BASE	4013552	NA
CZAPEK DOX AGAR	4013602	NA
CZAPEK DOX BROTH	4013652	NA
DECARBOXYLASE MOELLER BASE BROTH	4013662	NA
DERMATOPHYTE SELECTIVE MEDIUM - DTM - (TAPLIN)	40136912	

DESOXYCHOLATE AGAR	4013702	NA
DESOXYCHOLATE CITRATE AGAR	4013752	NA
DESOXYCHOLATE LACTOSE AGAR	4013802	NA
DESOXYRIBONUCLEASE TEST MEDIUM	4013682	NA
DEXTROSE AGAR	4013852	NA
DEXTROSE BROTH	4013862	NA
DG 18 AGAR BASE	4013942	NA
DG 18 CHLORAMPHENICOL AGAR	401394C	
DIFFERENTIAL CLOSTRIDIAL AGAR (DCA)	4013122	NA
DOUBLE MODIFIED LYSINE IRON AGAR (DMLIA)	4013252	NA
DRBC AGAR BASE	4013932	NA
DRBC CHLORAMPHENICOL AGAR	401393C2	
DRIGALSKY LACTOSE AGAR	4013302	NA
EC BROTH	4014252	NA
EC BROTH MUG	4014261	NA
E.C.O.A.GAR	4014302	NA
EC X-GLUC AGAR	4019682	NA
EDWARD'S AESCULIN MEDIUM	4014312	
EE BROTH MOSSEL	4014662	NA
ENDO AGAR	4014602	
m - ENDO BROTH	4014612	
ENTEROBACTERIA ENRICHMENT BROTH MOSSEL EP	4014672	NA
ENTEROBACTER SAKAZAKII ISOLATION AGAR (ESIA)	4014782	NA
EO.ME.BLUE AGAR W/LACTOSE AND SUCROSE	40145012	NA
ETHYL VIOLET AZIDE BROTH (EVA BROTH) -LITSKY	4014852	
m - FAECAL COLIFORM AGAR	4014872	NA
m - FAECAL COLIFORM BROTH	4014862	NA
FERMENTATION BROTH BASE	4014882	NA
FRASER BROTH BASE	4014952	NA
GASSNER MEDIUM	4015012	NA
GC MEDIUM BASE	4015202	NA
GELATIN PEPTONE BIOS AGAR	4015102	NA

GIOLITTI-CANTONI BROTH	4015162	NA
GLUCOSE OF MEDIUM	4015252	NA
GN BROTH HAJNA	4015242	NA
HEART INFUSION AGAR	4015352	NA
HEART INFUSION BROTH	4015402	NA
HEKTOEN ENTERIC AGAR	4015412	NA
HERELLEA AGAR	4015432	NA
HHD BROTH	4015292	NA
IRON SULFITE AGAR	4015652	
KANAMYCIN AESCULIN AZIDE AGAR BASE	4015522	NA
KLIGLER IRON AGAR	4015602	NA
LACTOSE BROTH	4015752	NA
LACTOSE GELATIN MEDIUM	4015762	NA
LACTOSE SULFITE MEDIUM	4015792	NA
LAURYL PEPTO BIOS BROTH	4015802	NA
LAURYL SULFATE BROTH MUG IDF FORMULATION	401580F1	NA
LEGIONELLA BCYE AGAR BASE	4015822	NA
m - LES ENDO AGAR	4015932	
LETHEEN BROTH AOAC	4015912	NA
LEVINE EMB BLUE AGAR	4015952	NA
LINDEN GRAIN BROTH	4015874	NA
LISTERIA BUFFERED ENRICHMENT BROTH	401601B2	
LISTERIA ENRICHMENT BROTH	4016012	
LISTERIA ENRICHMENT BROTH UVM 1	4015982	NA
LISTERIA FRASER BROTH BASE	4015962	NA
LISTERIA FRASER BROTH BASE HALF CONCENTRATION	4015942	NA
LISTERIA OXFORD AGAR BASE	4016002	
LISTERIA PALCAM AGAR BASE	4016042	
LITMUS MILK	4016112	NA
LOWENSTEIN JENSEN MEDIUM BASE	4016352	
LPT DILUTION BROTH	4016392	NA
EUGON BROTH	4016432	NA

LYSINE IRON AGAR	4016362	NA
M 17 AGAR	4017192	NA
M 17 AGAR W/O LACTOSE	401719W2	NA
M 17 BROTH	4017202	NA
m-GREEN AGAR	4015272	NA
MAC CONKEY AGAR	4016702	NA
MAC CONKEY AGAR MUG	4016722	NA
MAC CONKEY AGAR OMS W/O CRYSTAL VIOLET	4016712	NA
MAC CONKEY AGAR N. 2	4016732	NA
MAC CONKEY BROTH (PURPLE)	4016752	NA
MAC CONKEY BROTH EP	4016792	NA
MAC CONKEY SORBITOL AGAR	401669S2	NA
MAC CONKEY SORBITOL MUG AGAR	4016692	NA
MALONATE BROTH	4016852	NA
MALT AGAR	4016452	NA
MALT EXTRACT AGAR	4016552	NA
MALT EXTRACT BROTH	4016602	NA
MANNITOL SALT AGAR	4016652	NA
MANNITOL SALT BROTH	4016662	NA
MAXIMUM RECOVERY DILUENT	4016912	NA
MEAT LIVER SR AGAR	4016892	NA
MICROBIAL CONTENT TEST AGAR	4016992	NA
MINERALS MODIFIED GLUTAMATE MEDIUM	4017372	NA
MODIFIED LAURYL SULFATE TRYPTOSE (mLST) BROTH BASE	4014762	NA
MOTILTY MEDIUM	4017142	NA
MOTILITY NITRATE CP MEDIUM	4017262	NA
MRS AGAR ISO FORMULATION	401728S2	NA
MRS AGAR WITH TWEEN 80	4017282	NA
MRS BROTH WITH TWEEN 80	4017292	NA
MRVP MEDIUM	4017352	NA
MSE AGAR	4017382	NA
MUELLER HINTON AGAR II	4017402	NA

MUELLER HINTON BROTH	4017412	NA
MULLER KAUFFMANN TETRATHIONATE BROTH BASE	4017432	NA
MULLER KAUFFMANN TETRATHIONATE BROTH BASE ISO FORM.	4017452	NA
NEOMYCIN ASSAY AGAR A 11	4017752	NA
NUTRIENT AGAR	4018102	NA
NUTRIENT AGAR N° 2	4018132	NA
NUTRIENT AGAR n° 3	4018142	NA
NUTRIENT AGAR WITH NaCl	4018112	NA
NUTRIENT BROTH	4018152	NA
NUTRIENT BROTH N° 2	4018122	NA
NUTRIENT BROTH 13 g/L	4018182	NA
NUTRIENT BROTH AOAC	4018162	NA
NUTRIENT GELATIN	4018202	NA
O/F HUGH LEIFSON BASE	4018362	NA
OGYE AGAR BASE	4018382	NA
PEPTONE (TRYPTONE) WATER	4018912	NA
PEPTONE YEAST EXTRACT AGAR	4018952	NA
PHARMACOPOEIA DILUENT	4013952	NA
PHENOL RED AGAR BASE	4019052	NA
PHENOL RED BROTH BASE	4019102	NA
PHENYLALANINE AGAR	4019162	NA
PLATE COUNT AGAR WITH SKIM MILK	4019182	NA
POLYMYXIN BASE AGAR A 9	4019202	NA
POLYMYXIN SEED AGAR A 10	4019252	NA
POTATO DEXTROSE AGAR	4019352	NA
PSEUDOMONAS AGAR F	4019612	NA
PSEUDOMONAS AGAR P	4019622	NA
PSEUDOMONAS AGAR BASE	4019602	NA
PSEUDOMONAS SELECTIVE AGAR	4019632	NA
PSEUDOMONAS SELECTIVE BROTH	4019642	NA
PURPLE GLUCOSE AGAR	4019702	NA
R2A AGAR	4019962	NA

RAPPAPORT VASSILIADIS SALMONELLA ENRICHMENT BROTH EP	4019792	NA
RAPPAPORT VASSILIADIS (RV) BROTH	4019802	NA
RAPPAPORT VASSILIADIS SEMISOLID MEDIUM BASE MOD (MSRV)	4019822	NA
RAPPAPORT VASSILIADIS SOY (RVS) BROTH	4019812	NA
ROGOSA BIOS AGAR	4019852	NA
ROGOSA BIOS BROTH	4019902	NA
ROSE BENGAL AGAR BASE	4019912	NA
ROSE BENGAL AGAR WITH CHLORAMPHENICOL	4019922	
SABOURAUD BROTH	4020002	NA
SABOURAUD DEXTROSE AGAR	4020052	NA
SABOURAUD DEXTROSE AGAR W/ CAF 50 mg	4020062	
SABOURAUD DEXTROSE AGAR W/ CAF 500 mg	4020072	NA
SABOURAUD EMMONS DEXTROSE AGAR	4020022	NA
SABOURAUD MALTOSE AGAR	4020102	NA
SELENITE BROTH	4020252	
SELENITE BROTH BASE	402025B2	NA
SELENITE CYSTINE BROTH	4020262	
SELENITE CYSTINE BROTH BASE	402026B2	NA
SENECA BASE	405582S2	NA
SHIGELLA BROTH BASE	4020402	NA
SIM BIOS MEDIUM	4020362	NA
SIMMONS CITRATE AGAR	4020452	NA
SLANETZ BARTLEY AGAR	4020462	
SLANETZ BARTLEY AGAR W/O TTC	4020472	
SPORULATION AGAR AK	4020702	NA
SPORULATION BROTH	4020712	NA
SS AGAR	4020752	NA
STAA AGAR BASE	4020792	
STAPHYLOCOCCI 110 MEDIUM	4020852	NA
STREPTOCOCCUS SELECTIVE AGAR	4020872	NA
STREPTOCOCCUS SELECTIVE BROTH	4020882	

STUART TRANSPORT MEDIUM	4020912	NA
SUGAR FREE AGAR BASE	4020982	NA
SULPHITE POLYMIXIN SULFADIAZINE (SPS) AGAR	4020942	NA
TAYLOR LYSINE DECARBOXYLASE BROTH	401367L2	NA
TCBS KOBAYASHI AGAR	4021062	NA
TAT BROTH	4021000	NA
TETRATHIONATE BROTH BASE	4021252	NA
THIOGLYCOLLATE MEDIUM	4021372	NA
THIOGLYCOLLATE MEDIUM ALTERNATIVE	4021352	NA
TODD-HEWITT BROTH	4021342	NA
TRIPLE SUGAR IRON AGAR U.S.P.	4021412	NA
TRIPLE SUGAR IRON AGAR ISO	402141S2	NA
TRYPTIC GLUCOSE EXTRACT AGAR	4021442	NA
TRYPTIC GLUCOSE YEAST AGAR	4021452	NA
TRYPTIC SOY AGAR	4021502	NA
TRYPTIC SOY BLOOD AGAR BASE	4021512	NA
TRYPTIC SOY BROTH	4021552	NA
TRYPTIC SOY BROTH Y IRRADIATED	402155G2	NA
TRYPTIC SOY BROTH MODIFIED (mTSB)	402155M2	NA
TRYPTIC SOY YEAST EXTRACT AGAR (TSYEA)	4021662	NA
TRYPTIC SOY YEAST EXTRACT BROTH (TSYEB)	4021672	NA
TRYPTIC SOY BROTH NaCl 10%	40215512	NA
TRYPTONE BILE X-GLUC AGAR (TBX)	4021562	NA
TRYPTONE SULFITE NEOMYCIN (TSN) AGAR	4021592	NA
TRYPTONE TRYPTOPHAN MEDIUM	4021652	NA
TRYPTOSE AGAR	4011452	NA
TRYPTOSE BROTH	4011462	NA
TRYPTOSE PHOSPHATE BROTH	4011502	NA
TSC AGAR BASE	4021582	NA
TTC TERGITOL 7 AGAR BASE	402160T2	NA
UREA AGAR BASE-CHRISTENSEN	4021752	NA
UREA BROTH BASE - STUART	4021802	NA

VIOLET RED BILE AGAR (VRBL)	4021852	NA
VIOLET RED BILE AGAR MUG	4021861	NA
VIOLET RED BILE GLUCOSE AGAR (VRBG)	4021882	NA
VIOLET RED BILE GLUCOSE AGAR (VRBGA) EP	4021892	NA
VOGEL-JOHNSON AGAR	4021922	NA
WL NUTRIENT MEDIUM	4021952	NA
WORT AGAR BASE	4022032	NA
WORT BROTH BASE	4022022	NA
XLD AGAR	4022062	NA
XLD AGAR ISO FORMULATION	4022082	NA
XLT4 AGAR BASE	4022072	NA
YEAST EXTRACT AGAR	4022752	NA
YEAST NITROGEN BASE	4022552	NA
YERSINIA ITC BROTH BASE	4022652	NA
YERSINIA PSB BROTH	4022702	NA

SELECTIVE SUPPLEMENTS AND ENRICHEMENTS

AEROMONAS SELECTIVE SUPPLEMENT (AMPICILLIN)	4240012	
ALOA ENRICHMENT-SELECTIVE SUPPLEMENTS	423501	
ALOA ENRICHMENT-SELECTIVE SUPPLEMENTS	423505	
BACILLUS CEREUS ANTIMICROBIC SUPPLEMENT	4240001	NA
BCSA SELECTIVE SUPPLEMENT	42400073	
BIOVITEX -RESTORING FLUID	4240009	
BIOVITEX -RESTORING FLUID	42185011	
BOLTON BROTH SELECTIVE SUPPLEMENT	4240025	
BOLTON CCDA ANTIMICROBIC SUPPLEMENT	4240020	
BRILLIANT GREEN 0,1% SOLUTION	421505	NA
CAMPYLOBACTER GROWTH SUPPLEMENT	4240021	
CEFIXIME TELLURITE O157 SUPPLEMENT	42ISEC	
CFC PSEUDOMONAS SUPPLEMENT	4240075	
CHLORAMPHENICOL ANTIMICROBIC SUPPLEMENT	4240003	

CHROMOGENIC B. CEREUS SUPPLEMENTS	4240090	
CHROMOGENIC YERSINIA SUPPLEMENTT	4240095	
CLOSTRIDIUM BOTULINUM ANTIMICROBIC SUPPLEMENT	4240066	
CN PSEUDOMONAS SUPPLEMENT	4240046	
CNA ANTIMICROBIC SUPPLEMENT	4240018	
COAGULASE PLASMA EDTA	429938	NA
COAGULASE PLASMA EDTA	429937	NA
COAGULASE PLASMA EDTA	429936	NA
CRE SUPPLEMENT	4240082	
m-CP ANTIMICROBIC SUPPLEMENT	4240070	NA
m-CP SUPPLEMENT A	4240070A	NA
m-CP SUPPLEMENT B	4240070B	
m-CP SUPPLEMENT C	4240070C	NA
D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT	4240002	NA
D-CYCLOSERINE 4-MUP SUPPLEMENT	4240049	NA
DERMATOPHYTE ANTIMICROBIC SUPPLEMENT	4240024	
DMLIA NOVOBIOCIN SUPPLEMENT	4240029	
EGG YOLK EMULSION	42111601	NA
EGG YOLK EMULSION	42111605	NA
EGG YOLK EMULSION	42111600	NA
EGG YOLK TELLURITE EMULSION 20%	423700	NA
EGG YOLK TELLURITE EMULSION 20%	423701	NA
EGG YOLK TELLURITE EMULSION 20%	423702	NA
EGG YOLK TELLURITE EMULSION 50%	42111602	NA
EGG YOLK TELLURITE EMULSION 50%	42111604	NA
EGG YOLK TELLURITE EMULSION 50%	42111603	NA
ESBL SUPPLEMENT	4240080	
FRASER SELECTIVE SUPPLEMENT	4240043	
FRASER HALF SELECTIVE SUPPLEMENT	4240044	
GARDNERELLA ANTIMICROBIC SUPPLEMENT	4240019	
GENTAMICIN ANTIMICROBIC SUPPLEMENT	4240004	
GLYCEROL	421015	NA

IODINE SOLUTION	421501	
KANAMYCIN POLYMYXIN B ANTIMICROBIC SUPPLEMENT (SFP)	4240005	
KANAMYCIN SELECTIVE SUPPLEMENT	4240055	
KARMALI ANTIMICROBIC SUPPLEMENT	4240035	
LEGIONELLA AB SELECTIVE SUPPLEMENT	423225	
LEGIONELLA BCYE α-GROWTH SUPPLEMENT	423210	
LEGIONELLA BCYE α-GROWTH SUPPLEMENT w/o CYSTEINE	423212	
LEGIONELLA MWY SEL SUPPLEMENT (ISO)	423220	NA
LEGIONELLA GVPC SELECTIVE SUPPLEMENT	423215	
LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)	4240056	
LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)	42185056	
LISTERIA FRASER SUPPLEMENT (FE AMMONIUM CITRATE)	42185056A	
LISTERIA MOX - COL SUPPLEMENT	4240039	
LISTERIA OXFORD ANTIMICROBIC SUPPLEMENT	4240038	
LISTERIA PALCAM ANTIMICROBIC SUPPLEMENT	4240042	
NOVOBIOCIN ANTIMICROBIC SUPPLEMENT	4240045	
NOVOBIOCIN MKTT SELECTIVE SUPPLEMENT	4240047	
OXYTETRACYCLINE ANTIMICROBIC SUPPLEMENT	4240000	
PENICILLIN 500 IU SELECTIVE SUPPLEMENT	4240050	
POTASSIUM CHLORATE SUPPLEMENT	4240065	NA
POTASSIUM TELLURITE 1% SOLUTION	42211501	NA
POTASSIUM TELLURITE 3,5% SOLUTION	42211502	NA
PP PSEUDOMONAS SUPPLEMENT	4240048	
PRESTON ANTIMICROBIC SUPPLEMENT	4240017	
RPF SUPPLEMENT	423101	NA
RPF SUPPLEMENT II	423102	NA
RPF SUPPLEMENT II	423102D	NA
ROSOLIC ACID	4211901	
SALMONELLA SELECTIVE SUPPLEMENT VIAL A	4240013A	
SALMONELLA SELECTIVE SUPPLEMENT VIAL B	4240013B	
SENECA EE-EC SUPPLEMENT	4240023	

STREPTO B SUPPLEMENT	4240053	NA
SKIRROW ANTIMICROBIC SUPPLEMENT	4240016	
SODIUM BISELENITE	4123651	
STAA SELECTIVE SUPPLEMENT	4240052	
TICARCILLIN IRGASAN ANTIMICROBIC SUPPLEMENT	4240060	
TTC 0.05% SOLUTION	421510	NA
TTC 1% SOLUTION	42111801	NA
TWEEN 20	42120501	NA
TWEEN 80	42120502	NA
UREA 40% SOLUTION	42211601	NA
UREA 40% SOLUTION	4240096	NA
VANCOMICIN ANTIMICROBIC SUPPLEMENT	4240057	
VANCOMICIN ANTIMICROBIC SUPPLEMENT	4240057C	
VCN ANTIMICROBIC SUPPLEMENT	4240007	
VCNT ANTIMICROBIC SUPPLEMENT	4240008	
XLT4 SUPPLEMENT	4240097	
YERSINIA SELECTIVE SUPPLEMENT	4240011	

AGAR, PEPTONES AND OTHER BIOLOGICAL MATERIALS

ACID DIGEST OF CASEIN	4122652	NA
AGAR BACTERIOLOGICAL	4110406	NA
AGAR BIOS SPECIAL LL	4110302	NA
AGAR HIGH GEL STRENGTH	4110312	NA
AGAR TECHNICAL	4110252	NA
AGAR HIGHLY PURIFIED	4110352	NA
AGAROSE	4110381	NA
BEEF EXTRACT POWDER	4111252	NA
BILE SALTS	41113012	
BILE SALTS N°3	41113022	
GELATIN BIOS	4115152	NA
GELATIN PEPTONE	4115182	NA
LECITHIN	41EAT0242	NA

LIVER EXTRACT	41228012	NA
MALT EXTRACT	4116502	NA
MYCOLOGICAL PEPTONE	4117102	NA
PEPTOCOMPLEX	4123102	NA
PEPTONE BACTERIOLOGICAL	4122592	NA
PEPTONISED MILK	4122982	NA
PROTEOSE PEPTONE	4123302	NA
SKIM MILK	4120402	NA
SODIUM BISELENITE	4123651	
SODIUM GLUTAMATE	4123642	NA
SOY PEPTONE	4123252	NA
TRYPTONE	4122902	NA
TRYPTOSE	4122602	NA
YEAST EXTRACT	4122202	NA

MISCELLANEOUS PRODUCTS

ACID PHOSPHATASE REAGENT	192010	
INDOLE SPOT REGENT	19171003	
KOVACS' REAGENT	19171000	
KOVACS' REAGENT	19171001	
MONO CONFIRM TEST	193000	
MUCAP TEST	191500	
OXIDASE TEST STRIPS	191040ST	

READY-TO-USE CULTURE MEDIA IN PLATES TUBES AND BOTTLES

None of the ready-to-use products are classified as hazardous

ALPHABETICAL PRODUCT INDEX

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