HiCrome™ Bacillus Agar

Recommended for isolation and differentiation between various species of *Bacillus* from a mixed culture by chromogenic method.

M1651

Composition **

Ingredients	Grams/Litre
Peptone	10.00
HM extract#	1.00
D-Mannitol	10.00
Sodium chloride	10.00
Chromogenic mixture	3.20
Phenol red	0.025
Agar	15.00

Final pH (at 25°C) 7.1 ± 0.2
** Formula adjusted, standardized to suit performance parameters
#Equivalent to Meat extract

Directions

Suspend 49.22 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of Bacillus Selective Supplement (FD324) if desired. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Majority of *Bacillus* species apparently have little or no pathogenic potential and are rarely associated with disease in humans or lower animals. The principal exception to this are *Bacillus* anthracis, the agent of anthrax, and *Bacillus cereus*. However a number of other species, particularly those of the *B. subtilis* group, have been implicated in food poisoning and other human and animal infections (4). *Bacillus cereus* causes food poisoning due to consumption of contaminated rice (2, 1, 5) other starchy foods such as potato, pasta and cheese have also been implicated, eye infections and a wide range of other clinical conditions like abscess formation, meningitis, septicemia and wound infection.

HiCrome™ *Bacillus* Agar is based on the formulation of MYP Agar formulated by Mossel et al (2) used for enumeration of *Bacillus cereus* and *Bacillus* thuringiensis when present in large number in certain foodstuffs. The medium contains peptone and HM extract, which provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other growth nutrients. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by phenol red. Mannitol fermenting organisms like *B. megaterium* yield yellow coloured colonies. The chromogenic mixture present in the medium is cleaved by the enzyme b-glucosidase found in *B. cereus* resulting in the formation of blue colonies. *B. thuringiensis* also grows as blue/green colonies on this medium as *B. cereus* and *B. thuringiensis* are biochemically identical. If selective isolation of *B. cereus* or *B. thuringiensis* is required aseptically add Bacillus Selective Supplement (FD324).

Type of specimen

Clinical, food samples

Specimen Collection and Handling

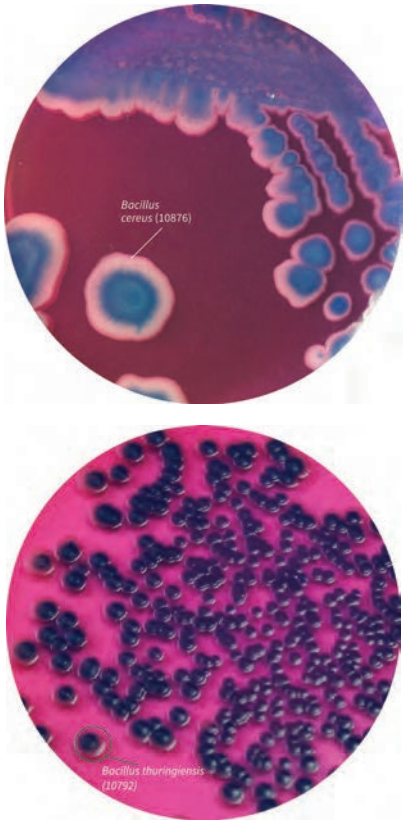
For Clinical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6, 7).

For Food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8)

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets



M1651 HiCrome™ Bacillus Agar

HiCrome™ Single Streak Rapid Differentiation Series | HiCynth | Free from BSE/TSE/GMO

HiCrome™ *Bacillus* Agar (M1651) is also available as HiCrome™ *Bacillus* HiCynth™ Agar (MCD1651) wherein all animal/vegetable based nutrients are substituted with chemically defined nutrients.

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Limitations

1. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.

Quality Control

Appearance of Powder : Light yellow to pink coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium : Red coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 4.92% w/v aqueous solution at 25°C. pH : 7.1 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 30°C for 24-48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth **	Recovery **	Growth ***	Recovery ***	Colour of colony
Bacillus subtilis (6633) (00003*)	50-100	fair	20-30%	inhibited	0%	yellowish green to green
Bacillus cereus (10876)	50-100	good-luxuriant	≥50%	good-luxuriant	≥50%	light blue,# large, flat with blue centre
Bacillus thuringiensis (10792)	50-100	good-luxuriant	≥50%	good-luxuriant	≥50%	light blue, large, flat with irregular margins
Bacillus megaterium (14581)	50-100	good-luxuriant	≥50%	inhibited	0%	yellow, mucoid colonies
Bacillus coagulans (7050) (00002*)	50-100	good-luxuriant	≥50%	inhibited	0%	pink, small, raised colonies
Bacillus pumilis (14884)	50-100	good-luxuriant	≥50%	poor	10-20%	light green to green colonies
Staphylococcus aureus subsp aurreus (25923) (00034*)	50-100	luxuriant	≥50%	inhibited	0%	yellow colonies
Enterococcus faecalis (29212) (00087*)	50-100	luxuriant	≥50%	inhibited	0%	light green to green colonies

Key : * : Corresponds to WDCM number

** : Growth without addition of FD324 *** : Growth with addition of FD324

: Colony surrounded by pink halo

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6, 7).

References

1. Bouza E., Grant S., Jordan C. et al, 1979, Arch. Ophthamol., 97:488.
2. Mortimer P. R. and McCann G., 1974, Lancet, 1043.
3. Mossel D. A. A., Koopman M. J. and Jongerium E., 1967, Appl. Microbiol.,15:650.
4. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed. American Society for Microbiology, Washington, D.C.
5. Wohlgemuth K., Kirkbride C. A., Bicknell E. J. and Ellis R. P., 1972 Am. Vet. Met, Ass. 161:1691.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1
8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Ready Prepared Media

Code	Product Name	Usage	Packing
Category : 90 mm Ready Prepared Petri Plates			
MP1651	Hicrome™ Bacillus Agar Plate	for isolation and differentiation between various species of *Bacillus* from a mixed cultures.	50 plts