Introduction:

BK virus is a Polyomavirus, first isolated in 1970 from kidney transplant receipient. The virus is commonly reactivated in immunocompromised transplant patients. BK virus in renal transplant recipients is associated with nephropathy, in bone marrow transplant with haemorrhagic cystitis. Monitoring and treatment of BK virus reactivation in transplant patients is vital for graft survival.

The AltoStar® BKV PCR Kit 1.5 is an in vitro diagnostic test, based on real-time PCR technology, for the detection and quantification of BK virus specific DNA in human plasma. The AltoStar® BKV PCR Kit 1.5 is configured for use with the CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad) in combination with the AltoStar® Automation System AM16, the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5. The results generated with the AltoStar® BKV PCR Kit 1.5 have to be interpreted in conjunction with other clinical and laboratory findings.

Specimen Collection, Transport & Storage

Urine is collected in a sterile container, store at 4^{o}C after collection, if processed within 24 hours; store at -20^{o}C if processing >24 hours.

EDTA blood: plasma should be removed from red cells 4-6 hours after collection. Centrifuge EDTA blood at ≥10,000 RCF (Relative Centrifugal Force). Store centrifuged tube at 4^{o}C if processing within 5 days; store at -20^{o}C if processing >5 days.

The minium volume for the plasma and Urine is 500uL.

Materials, Equipments and Facilities:

- Clean Room: Biosafety Cabinet (MIBCT3), freezer (MIFTG)
- Clean Room: Biosafety Cabinet (MIBCT3), freezer (MIFTG)
- Specimen Preparation area: Biosafety Cabinet (MIBCT7 or MIBCT8)
- AltoStar® AM16
- BIO-RAD CFX96™ Deep Well Real-Time PCR Detection System
- BIO-RAD Hard-Shel®PCR Plates 96-Well WHT/CLR
- BIO-RAD Microseal ®'B' seal Seals
- BIO-RAD Optical Flat 8-Cap Strips for 0.2ml tube strips
- BIO-RAD Low-Profile PCR Tubes 8-tube strip, white
- AltoStar® Processing Plate
- AltoStar® Eluate Plate
- AltoStar® Eluate Plate Sealing Foil
- PCR Plate
- Plate Sealer

- AltoStar® PCR Plate Sealing Foil
- 1000 µl CO-RE Tips
- 300 µl CO-RE Tips
- 300 µl CO-RE Tips
- Waste Bag
- AltoStar® Container Re-Sealing Foil
- Screw Cap – blue/orange/violet
- Variable volume Rainin pipettes: 1 to 20 uL, 10 to 200 uL, 100 to 1000 uL
- Reagent:
  AltoStar® Purification Kit 1.5 - lysis buffer, buffer 1,buffer 2, buffer 3 (Stored at Room Temperature)
  AltoStar® Internal Control 1.5 - Store at -20°C
  Altona AltoStar BKV-PCR Kit 1.0: MasterA, MasterB, Quantitative Standards 1, 2, 3, 4 (QS1, QS2, QS3, and QS4), PCR Grade Water -Stored at -20°C

External Control: BKV High Positive, BKV Negative, BKV Low Positive, to be extracted and run in this order every BKV PCR run or if QC failure occurs.

Procedure:

General Precautions:

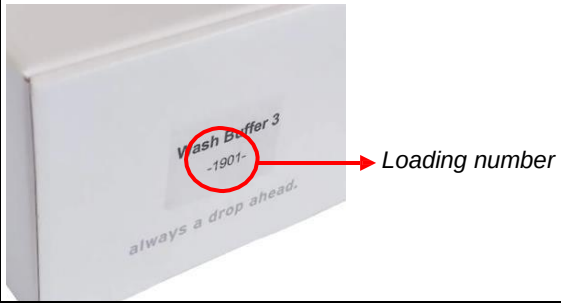
- There must be separate PCR work areas:
  Clean room
  Specimen preparation room
- Powder-free Gloves should only be in use in PCR areas. Change gloves frequently and keep tubes closed whenever possible.
- Prepare Working 1% sodium hypochloride daily.
- Specimen Preparation Supplies and equipment must be dedicated to Specimen Prep Area and not used for other activities and never used in Clean Room.
- Change lab coats and gloves between work areas.
- Use only Aerosol Resistant Tips (ART)
- Use only sterile RNase-free, DNAse-free microtubes
- Thaw components thoroughly at room temperature.
- PCR work areas (Clean Room and Specimen Preparation Area) benchtops and equipment after each shift.

I. Preparing Reagents

1. Ensure to prepare sufficient amounts of non-expired reagents which all have to have the same Loading Number.
2. The Loading Number consists of the last four lot number digits of the Lysis Buffer and Wash Buffer containers and the Magnetic Bead, Enhancer and Elution Buffer tubes.

NOTE

For your convenience, these four digits are displayed as Loading Number on the outside of each component box.



NOTE

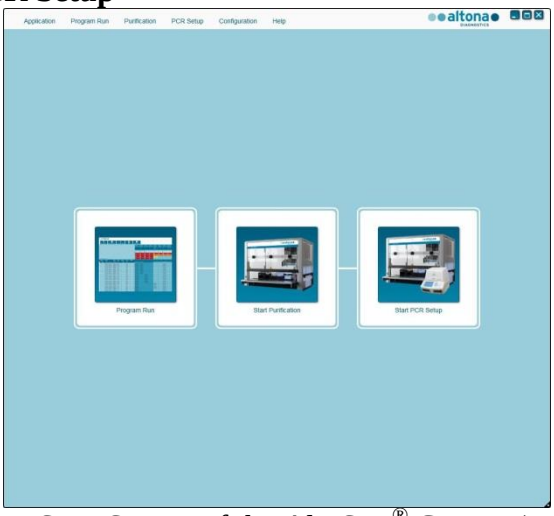
Before processing starts the AltoStar® AM16 automatically verifies :

- If sufficient reagent volume of the AltoStar® Purification Kit 1.5 components and of the AltoStar® Internal Control 1.5 is present.
- If the Loading numbers of the loaded AltoStar® Purification Kit 1.5 components are congruent.

3. Visibly inspect the Lysis Buffer for precipitates. In case precipitates are visible, heat it to below 50 °C. Intermittently pivot the container gently without wetting the seal until precipitates are completely dissolved. Slight color changes may occur to the Lysis Buffer. These slight changes in color do not indicate a change in the quality of the buffer.
4. Vortex the Magnetic Bead tubes for five seconds. Avoid wetting the lid. Do not centrifuge the Magnetic Beads.
5. Thaw the required number of IC tubes (AltoStar® Internal Control 1.5) completely and vortex for five seconds. One IC tube contains sufficient volume for 48 samples.

II. Starting the AltoStar® AM16

1. Turn on the AltoStar® AM16 with the front left green switch and start the computer by pressing the power button.
2. Wait until Windows has booted.
3. Start the AltoStar® Connect software using the a* icon on the Windows desktop, the Windows task bar or in the Windows start men
4. The Start Screen of the AltoStar® Connect software is displayed (see Figure 1) showing three buttons representing the AltoStar® Workflow steps to be performed on the AltoStar® AM16
   - **Program Run**
   - **Start Purification**
   - **Start PCR Setup**



(Figure 1: Start Screen of the AltoStar® Connect)

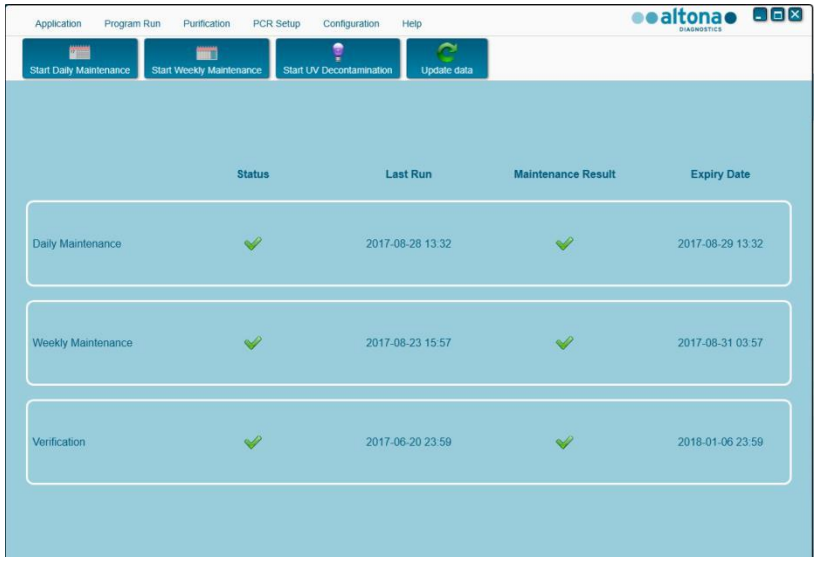
III. Performing Maintenance

Select **Instrument Maintenance** from **Application** drop down menu

A valid status of the Daily Maintenance and Weekly Maintenance is depicted by a green check mark (✔) in the column Status (see Figure 2). If the ⃠ sign is displayed, the respective maintenance procedure has to be performed.

If the Daily or Weekly Maintenance has to be performed:

1. Click the corresponding button in the tool bar.
2. Follow the on screen instructions to complete the maintenance procedure.



(Figure 2: Maintenance Screen with valid maintenance status)

NOTE	
i	**Verification** refers to the semi-annual maintenance procedure that is performed by Hamilton trained field service engineers. The **Verification** row must show a green check mark (✔) in the column **Status** as well. Otherwise the instrument will not process any samples or reagents.

IV. Programming an AltoStar® Run

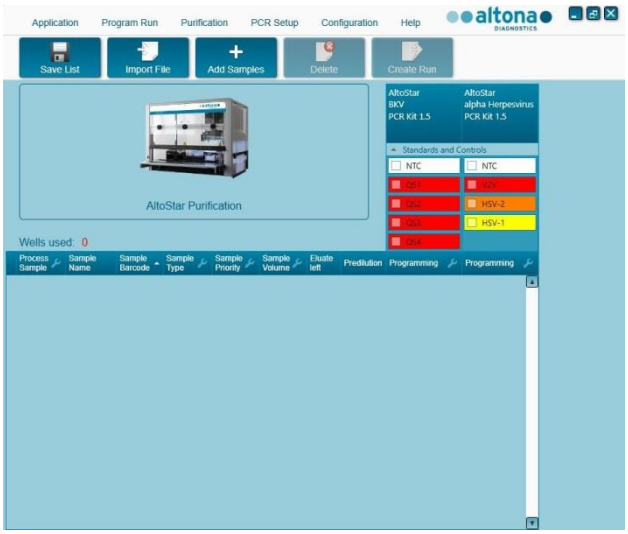
a. Manual Programming

1. Click **Program Run → Program Run (AltoStar® Purification)** in the menu bar; Alternatively, go back to the Start Screen of the AltoStar® Connect software and select the icon **Program Run**.

   The Programming Screen is displayed (see Figure 3) showing the sample table at the bottom of the screen with columns for:

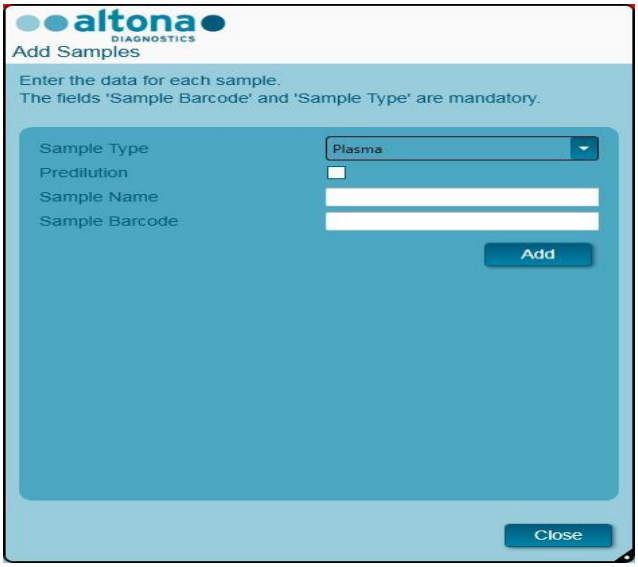
   - Sample properties: **Sample name** (optional), **Sample Barcode**, **Sample Type** and **Predilution**

- Sample settings: **Process Sample**, **Sample Priority**
- Sample information: required **Sample Volume** for the Purification Run (dead volume not factored in), **Eluate left** (determined by assay assignment)
- Assay assignment to the samples: **Programming**



(Figure 3: Programming Screen)

2. Click **the Add Samples** button to manually add samples to the sample table. The **Add Samples** dialog will appear (see Figure 4).



(Figure 4: Add Samples dialog)

3. Select the sample type Plasma or Urine in the **Sample Type** field.

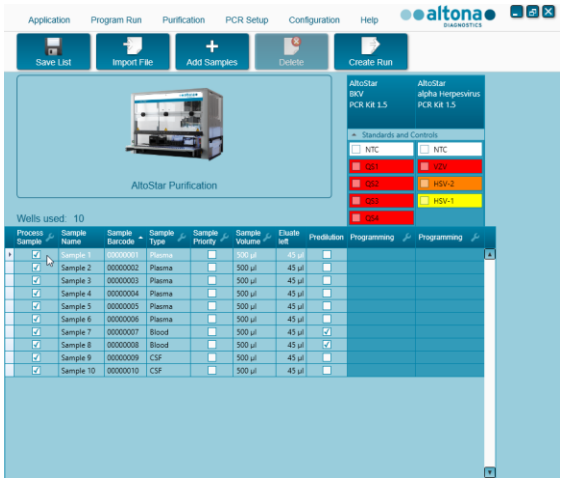
   NOTE: Make sure to select the correct sample type for each sample in the **Sample Type** field of the **Add Samples** dialog. Otherwise the sample may not be processed or the product performance may be compromised.

4. Optional: Enter a sample name (e.g. Patient ID) in the **Sample Name** field.
5. Enter a barcode via the handheld barcode scanner in the **Sample Barcode** field. A unique barcode for each sample tube is required.
6. Check for each sample if the required sample volume of **500 µl** plus dead volume of the sample tube used is available.

   NOTE: Insufficient sample volume (e.g. due to lack of the required dead volume of the sample tube) will lead to the exclusion of the sample in the Purification Run.

7. Click the **Add** button to add the sample to the sample table.
8. Repeat the steps above until all samples are added to the sample table.
9. When all samples are added, click the **Close** button to close the **Add Samples window**. The added samples are displayed in the sample table of the **Programming**

Screen (see Figure 5)

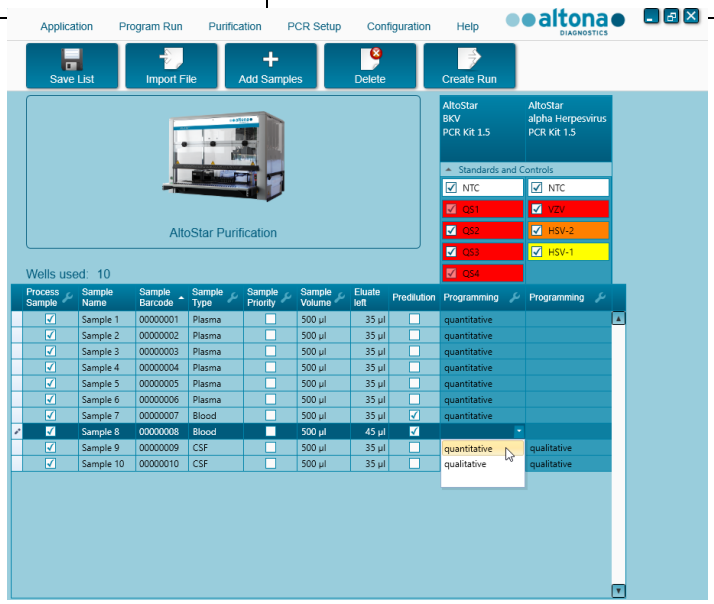


(Figure 5: Programming Screen with added samples)

NOTE: The sample list can be sorted by individual columns by clicking the column header. Multiple samples can be selected by holding down the **Shift-Key** or Ctrl-Key while clicking on sample lines.
The selected samples can be modified collectively by clicking the wrench symbol in the appropriate column header.
Samples can be removed from the list by selecting them and clicking the **Delete** button in the tool bar.

10. Assign the AltoStar® BKV PCR Kit 1.5 assay to specific samples by clicking in the cell which is in the row of the respective sample and in the column of the AltoStar® BKV PCR Kit 1.5 (see 6).

11. Select quantitative or qualitative in the appearing menu.

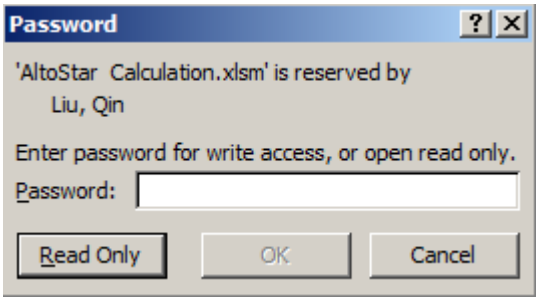


(Figure 6: Programming Screen: PCR Assay Assignment)

NOTE: The correct set of Standards and Controls is automatically selected for the quantitative assay application.
Additionally, the required sample volume for the Purification Run (dead volume not factored in) and the eluate volume that remains available for assignment to other assays are automatically adjusted in the sample list columns **Sample Volume** and **Eluate Left**, respectively.

b. Importing Worklist

1. Open folder **T:\microbiology\Virology\AltoStar\Mast Worklist**
2. Open **Bk_Plasma_Urine_Workllist.xlsm**
3. The Password window pops up, click **Ready Only** button (See Figure 7)



(Figure 7: Password Window)

4. Scan sample ID in column B
5. If sample is urine, change "**Plasma**" to "**Urine**" in column C (See Figure 8)
   - Type operator's initial and Date (dd-mm-yyyy) at the designated cells
   - Click **Export To File**
   - Save the worklist in the following folder:

T:\microbiology\Virology\AltoStar\Mast Work list\Saved Worklist
e.g. for worklist neame: 20181112_QL_BK

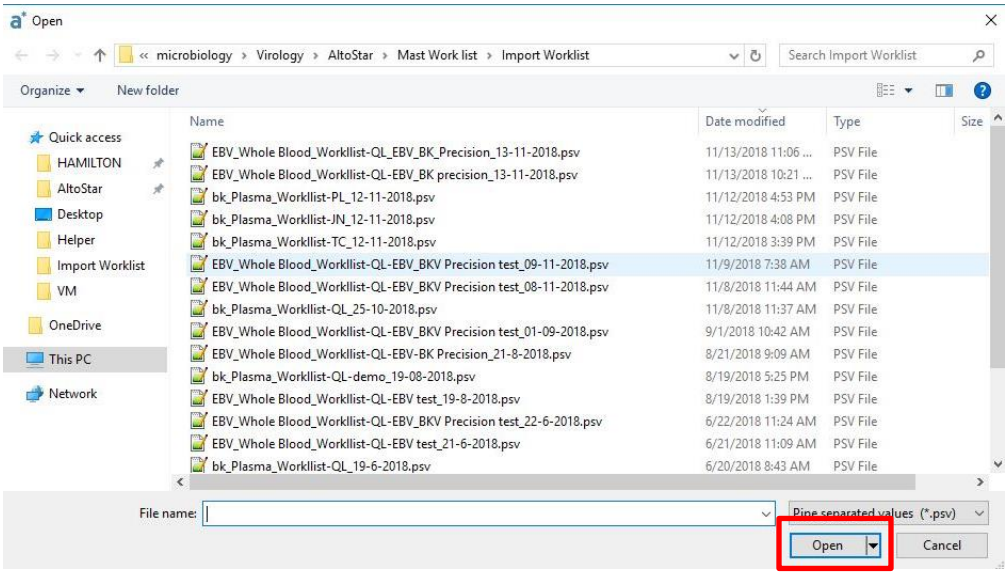
	A	B	C	D	E	F	G	H	I	J
1	*	TC1	Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative		User:	QL	
2	*	TC2	Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative		Date:	12-11-2018	
3	*	TC3	Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative				
4	*	TC4	Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative			Export To File	
5	*	TC5	Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative				
6	*	TC6	Urine	1	AltoStar BKV PCR Kit 1.5	quantitative				
7	*	TC7	Urine	1	AltoStar BKV PCR Kit 1.5	quantitative				
8	*		Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative				
9	*		Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative				
10	*		Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative				
11	*		Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative				
12	*		Plasma	1	AltoStar BKV PCR Kit 1.5	quantitative				

(Figure 8: Worklist Screen)

6. Click **Program Run → Program Run (AltoStar® Purification)** in the menu bar ;
7. Click **Import File** button (See Figure 9)

(Figure 9: Programming Screen)

8. Select the the worklist you created and click **Open** button (See Figure 10)

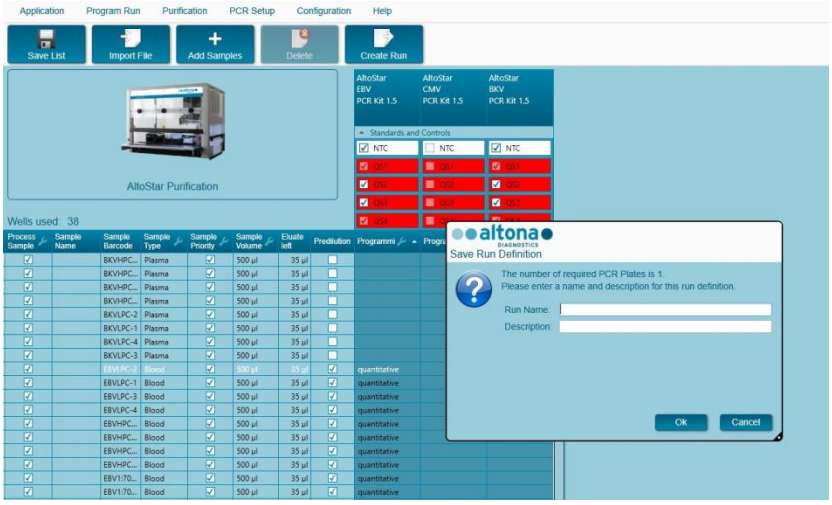


(Figure 10: Worklist Screen)

9. Once samples are all imported, the completion screen pops up.
10. Click **Ok** button (See Figure 11)

(Figure 11: Import complete screen)

11. Click **Create Run** button
12. Enter a unique **Run Name** (e.g. **BKV20181115_QL)** and optionally a Description for identification of the AltoStar® Run later on
13. Click **Ok** button **(**See Figure 12**)**



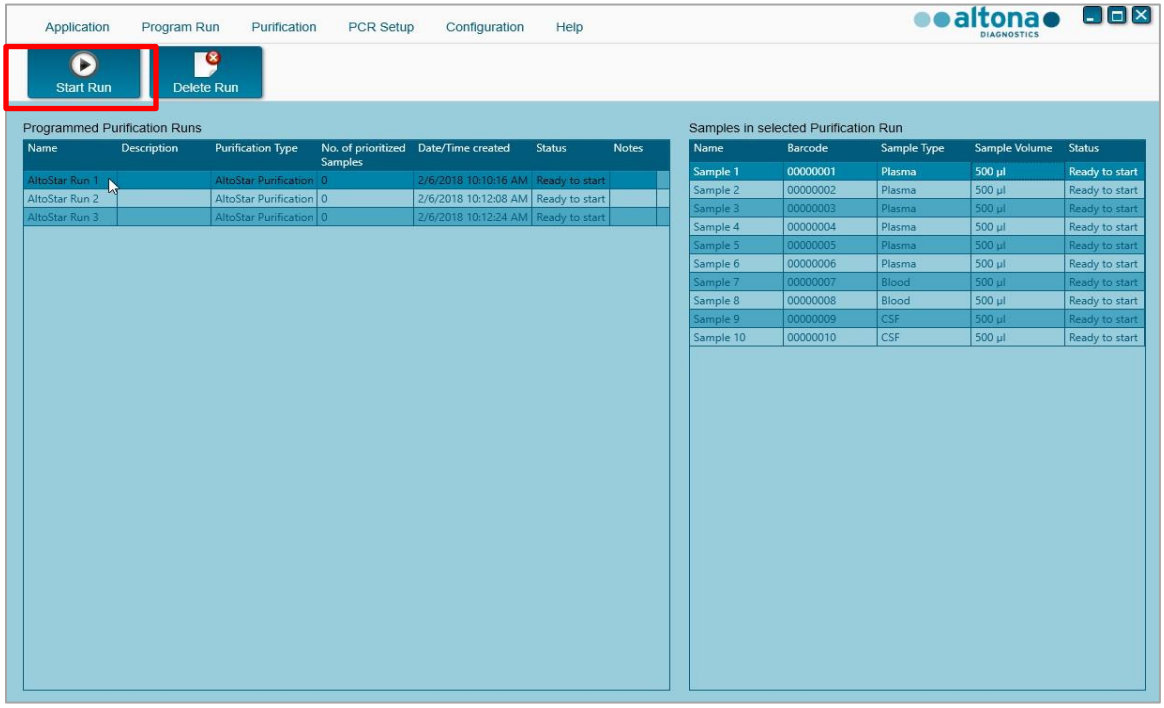
(Figure 12: Save Run Definition dialog)

V. AltoStar Purification

1. Select **Purification → Start Purification** in the menu bar. Alternatively, go back to the Start

Screen of the AltoStar® Connect software and select the icon **Start Purification**.
The Start Purification Run Screen is displayed (See Figure 13). Each programmed AltoStar® Run includes one Purification Run.
The pending Purification Runs are displayed in the **Programmed Purification Runs** table on the left side of the screen.

2. Select the Purification Run to be started in the **Programmed Purification Runs** table.
3. The samples included in the selected Purification Run are displayed in the table on the right side of the screen (**Samples in selected Purification Run**).



(Figure 13: Start Purification Run Screen)

4. Click **Start run** button
5. Purification run starts and a loading dialogue will appear (See Figure 14)

(Figure 14: Purification Loading Dialogue Screen)

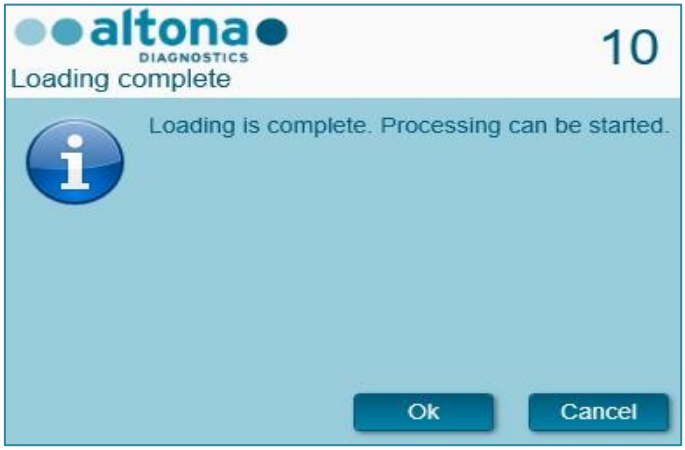
6. All needed items are listed. You can select the needed labware line by line and load the deck accordingly

Note: All reagents must be from the same lot (except for the internal Control)

7. Scan the barcode of the tip park plate twice (See Figure 15)
8. Click **OK** button to confirm the input

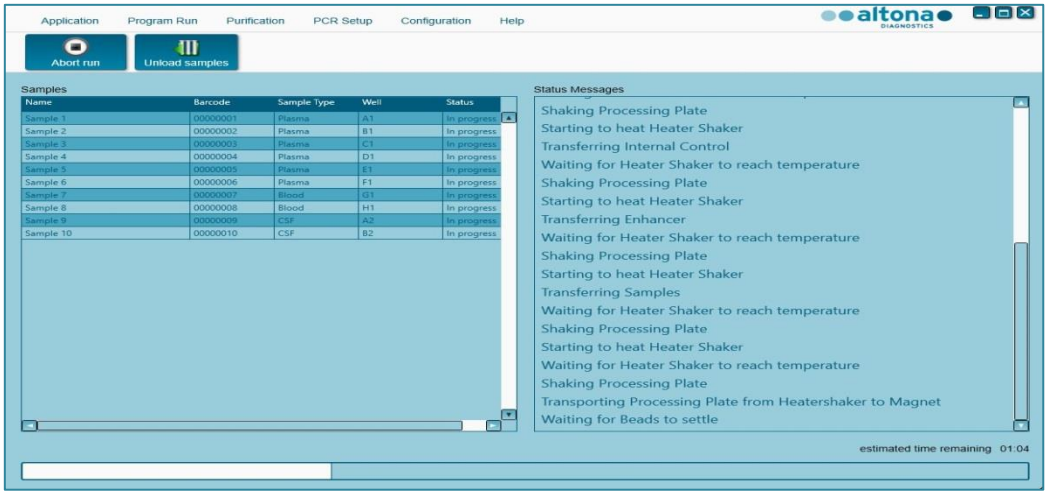
(Figure 15: Tip Park Plate Screen)

9. When the loading is completed, click **OK** button
10. Or wait for the countdown to finish and the run will start automatically (See Figure 16)



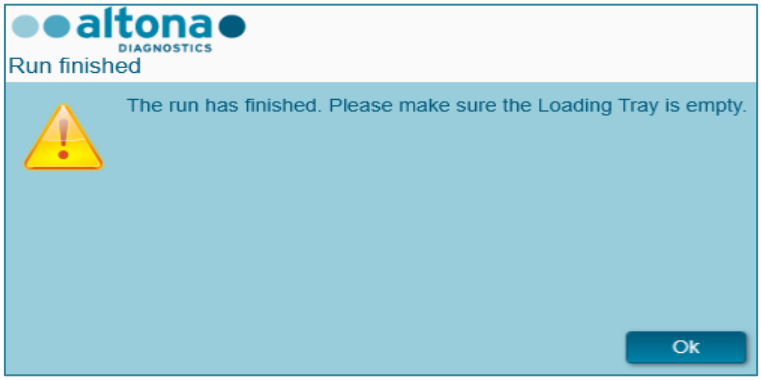
(Figure 16: Loading Complete Screen)

11. After the purification starts, there is no further interaction is required until the purification run has finished.
12. The Processing Status Screen is dsplayed showing the status of the Purification Run and the estimated time remaining (See Figure 17)



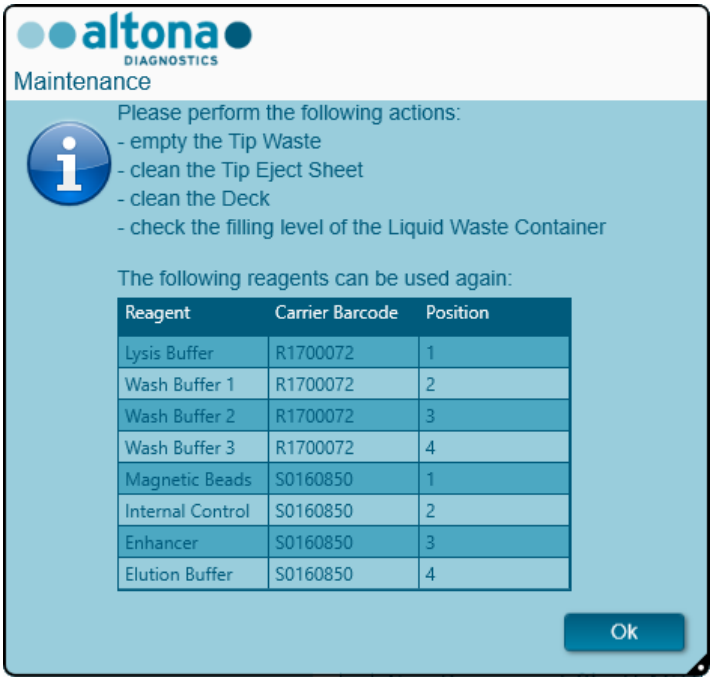
(Figure 17: Processing Status Screen)

13. When purification is completed, the **Run finished** window will pop up. Make sure the loading deck is empty and click "**OK**" button to continue (See Figure 18)



(Figure 18: Run Finished dialog)

14. Follow the instructions of the Maintenance dialog (See Figure 19)



(Figure 19: Maintenance Dialog)

15. If a PCR Setup Run using the currently loaded Eluate Plate is to be started directly after the Purification Run, the AltoStar® Eluate Plate can remain on the carrier position. If the PCR Setup Run is not started directly after the Purification Run, seal and store the AltoStar®

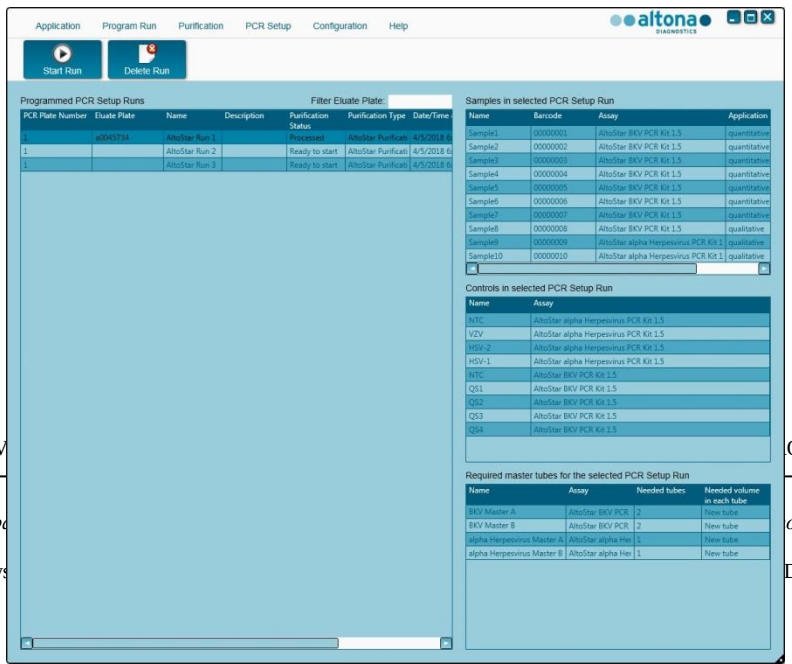
Eluate at 2 - 8°C for up to 24 hours before the start of a PCR Setup.

16. Close tubes with the appropriate tube caps.
17. Close containers with unused AltoStar® Container Re-Sealing Foils
18. Store reagents for reuse as described in Materials, Equipments and Facilities section for the AltoStar® Purification Kit 1.5 and AltoStar® Internal Control 1.5.
19. Dispose of the components of the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 not listed in the table.
20. Confirm the Maintenance dialog by clicking **Ok**

> **CAUTION:** Do not add bleach or acidic solutions to the liquid waste and any liquids containing Lysis Buffer or Wash Buffer 1. These liquids contain guanidine thiocyanate, which can form toxic, highly reactive and volatile compounds when combined with bleach or strong acids. Dispose of hazardous and biological waste in compliance with national, state or local regulations.

VI. AltoStar PCR Setup

Select **PCR Setup → Start PCR** Setup in the menu bar. Alternatively, go back to the Start Screen of the AltoStar® Connect software and select the icon **Start PCR Setup** (See Figure 20)
The pending PCR Setup Runs are displayed in the **Programmed PCR Setup Runs** table on the left side of the screen



(Figure 20: Star PCR Setup Run Screen)

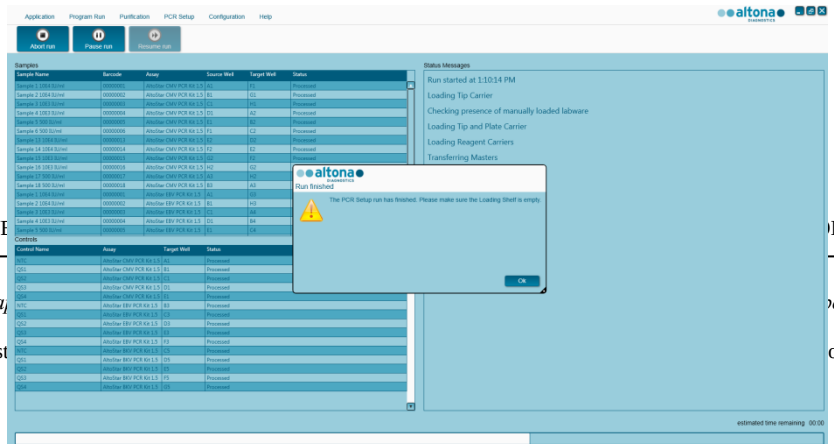
- Select your PCR Setup Run
- Click **Start run** button
- Run starts and a loading dialogue will appear (See Figure 21). All needed items are listed
- Select needed labware line by line and load the deck accordingly
- Note: All reagents must be from the same lot
- Press **OK** button to continue

(Figure 21: Loading dialog)

- When the loading is completed, press "OK" button to confirm (See Figure 22)
  Or wait for the countdown to finish and the run will start automatically

(Figure 22: Loading complete dialog)

- When PCR Setup is completed please make sure the Loading Shelf is empty and select "OK" to continue(See Figure 22);



(Figure 22: Run finished dialog)

- Record remaining tests# correctly for each master mix;
  Note: Only pool the master mix with the same lot#
  The minium volume of the pooled mastermixes:
  Master A: 100 uL
  Master B: 250 uL
- Heat seal PCR plate using **Clear Weld Seal** and heat seal elution plate using **Peel Seal**
- Please follow the instruction and save all re-usable reagents
- Record remaining tests# correctly for each master mix;
- Click "OK" button

VII. Sealing of the AltoStar® Eluate Plate

In case the eluates in the AltoStar® Eluate Plate are to be stored, the plate must be sealed with AltoStar® Eluate Plate Sealing Foil. It is recommended to use the AltoStar® Plate Sealer. The suitability of plate sealers other than the AltoStar® Plate Sealer has to be evaluated by the user.

If the AltoStar® Plate Sealer is used for sealing, proceed as follows:

1. Turn on the AltoStar® Plate Sealer and make sure that the plate adapter is not in the drawer.
   Note: Ensure that the settings of the AltoStar® Plate Sealer are as follows: 170 °C and two seconds.
2. Wait until the set temperature of 170°C is reached. This may take several minutes.
3. Place the AltoStar® Elution Plate on the plate adapter
4. Place one AltoStar® Eluate Plate Sealing Foil on the AltoStar® Eluate Plate. Align the cut corner of the sealing foil with the cut corner of the AltoStar® Eluate Plate. Make sure that all wells of the AltoStar® Elution Plate are covered with foil. Please take special care that the well at the cut corner is covered properly

Note: Do not operate the AltoStar® Plate Sealer without the plate adapter placed in the drawer, as this may render the sealer nonfunctional. In this case contact altona Diagnostics Technical Support for assistance

If the AltoStar® Eluate Plate Sealing Foil or the frame is placed incorrectly, the foil may stick to the heating plate within the AltoStar® Plate Sealer during sealing. This will render the sealer nonfunctional. In this case let the AltoStar® Plate Sealer cool down to room temperature and contact altona Diagnostics Technical Support for. Do not try to remove foils while the AltoStar® Plate Sealer is above room temperature.

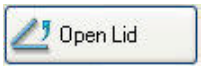
5. Assemble the sealing frame on top to hold down the sealing foil
6. Open the drawer via the **Operate** button.
7. Place the assembly consisting of the plate adapter, the AltoStar® Eluate Plate, the AltoStar® Eluate Plate Sealing Foil and the sealing frame into the AltoStar® Plate Sealer and press the **Operate** button.
8. The drawer closes automatically, seals for two seconds and reopens automatically.
9. Take the sealed AltoStar® Eluate Plate and the plate adapter out of the AltoStar® Plate Sealer and close the AltoStar® Plate Sealer by pressing the **Close** button.

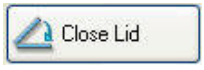
VIII. Unsealing of the AltoStar® Eluate Plate

Remove the AltoStar® Eluate Plate Sealing Foil from the AltoStar® Eluate Plate as follows:

1. Briefly centrifuge the AltoStar® Eluate Plate in a plate centrifuge to remove any liquid from the inside of the sealing foil.
2. Press the AltoStar® Eluate Plate onto a table to avoid sudden plate movements during the removal of the sealing foil.
3. Start peeling in one corner and slowly and steadily pull the sealing foil towards the diagonally opposite corner until it is removed.

IX. Biorad Real Time PCR System

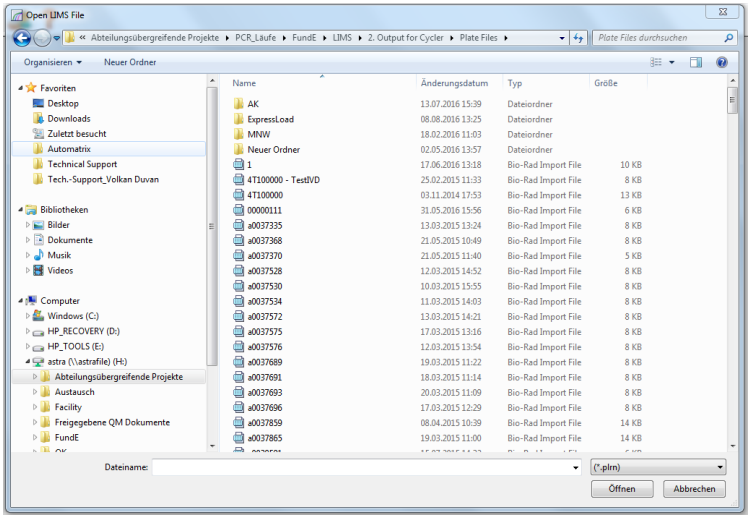
- Click Open Lid button 

to open the lid
- Load sealed plate to the block
- Click Close Lid button 

to close the lid

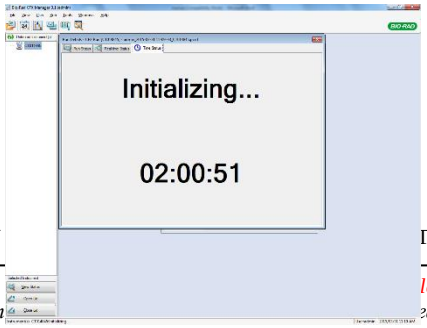
- Select “File”
- Click “Open”
- Choose “LIMS File”

- Click into **Dateinname** field and scan the barcode of the PCR plate
- Click "OK" or press enter key to continue



- Open the lid
- Place the PCR plate in the BioRad system
- Close the lid
- Click "Start Run"
- Save the run in the designated folder
- Click **Time Status** tab 

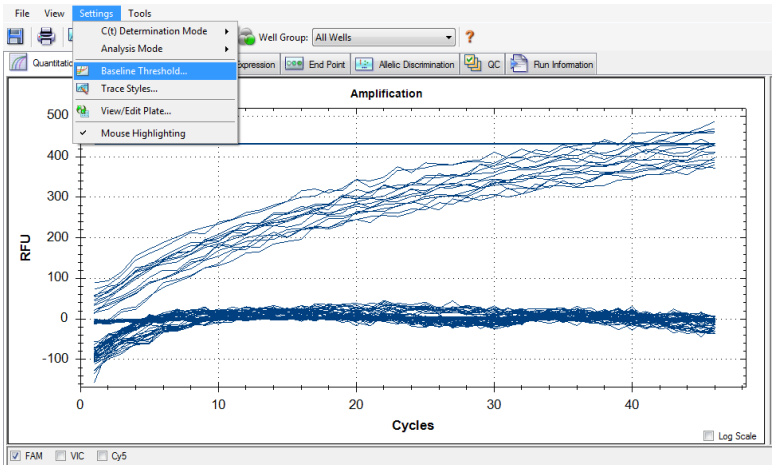
from the Run Details window



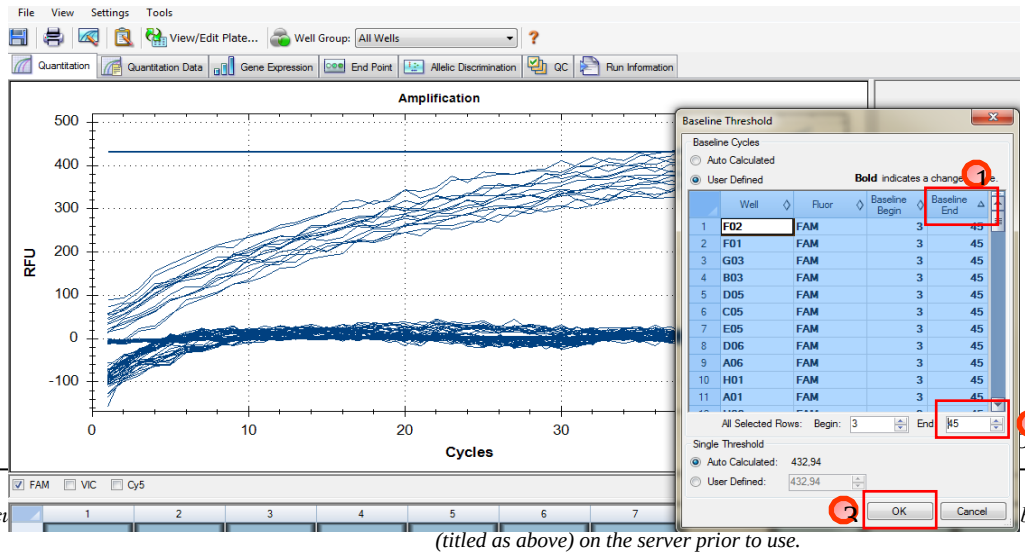
Analysis

Base Line Correction

- Choose BKV from Well Groups pull-down menu
- Change the channel names from Fluorophore to Target
- Select **Settings**
- Choose "**Baseline Threshold**"



- Click "Baseline End" to sort all wells with baseline end less than 10 on the top
- Set the End to 45 for all baselines are less than 10
- Click "OK" button



PCR curve

- Change the channel names **from Fluorophore to Target**
- Check each samples graph and mark on worksheet if positive or negative
- Set the threshold line for each target channel (BKV & IC).
- Click off the ic leaving bkv on. Drag the threshold over all the flat (negative) reaction. Repeat on the ic channel.
  A positive PCR is observed if there is a rise or amplification in the Target channel e.g. BKV. The graph should be exponential and sigmoidal in shape. Conversely a negative PCR is a flat line or no signal.

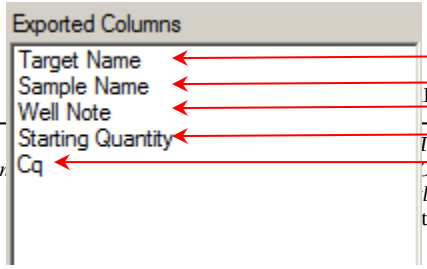
- Check if the QC parameters meet the requirements of a **VALID PCR** run. Print these data and attach with the result

Control Parameter	Valid Values
M Slope	-3.00 to -3.74
PCR Efficiency	85% to 115%
R square (R^2)	>0.98

Export Results

A. Excel Export

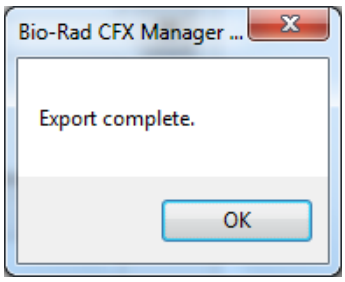
- **Open AltoStar Calculation.xlm (**T:\microbiology\Virology\AltoStar**)**
- Click **Read Only** when Password window pops up
- Minimize **AltoStar Calculation.xlm**
- Maximize **Data Analysis window**
- Click **Export**
- Select **Custom Export…**
- Select Export Format (at top left) to **".xls"**
- Make sure the sequence of the items in Export Columns matches exactly the following:



- Select **Export**
- Save as Window pops up
- Press **Ctrl+Shift+D** at the same time, the final results are calculated automatically
- Print the excel sheet and attach it to the BK worksheet
- The final results with Ct values are exported in Excel sheet
- Change the File name according to the target and save in the following folder:

  T:\microbiology\Virology\AltoStar\LIS Exports\LIS Excel Report

- Click **Save** button
- Close excel
- Click OK button

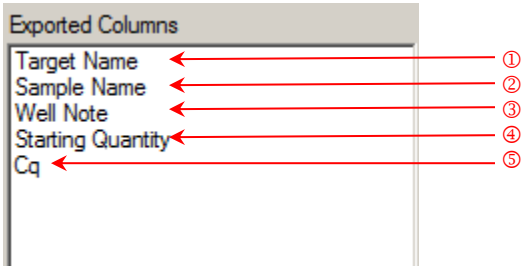


- Click Close button

B. FOR SOFT LAB TESTS:

- Click **Export**
- Select **Custom Export…**
- Change Export Format (at top left) to **".xml"**

- Make sure the sequence of the items in Export Columns matches exactly the following:



- as Window pops up
- Change the File name according to the target and save in the following folder:

  T:\microbiology\Virology\AltoStar\LIS Exports\LIS XLM Report

- Click **Save** button
- Click OK button on pop up indicating "Export complete"
- Click Close button

*If a sample is to be repeated or results are NOT to be auto-released to LIS, see "Appendix 1: Excluding samples from autorelease of results to LIS" before proceeding to step "***To Transfer SoftLab results to LIS.**

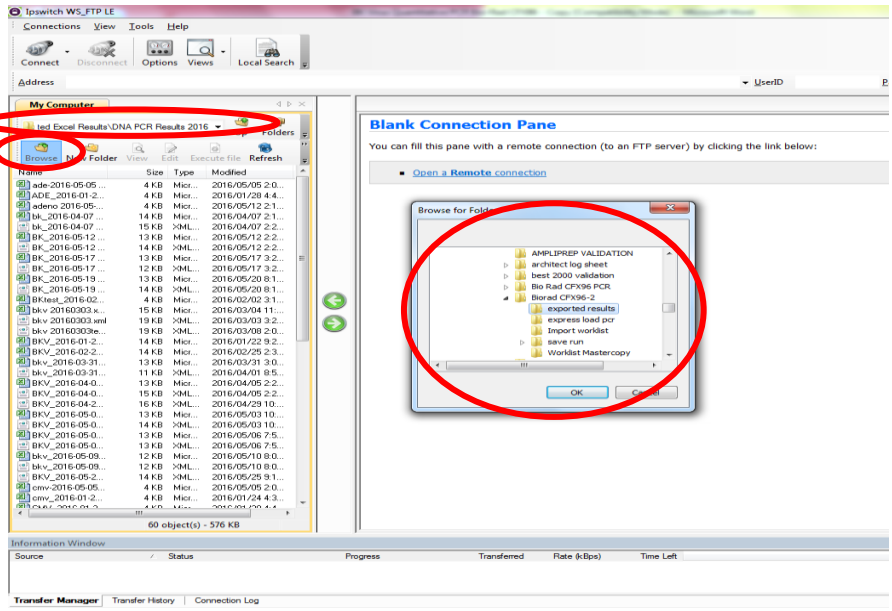
- Close CFX96 Deep Well Real-Time System
- Shut down computer

To Transfer SoftLab result to LIS

- Log on to Desktop Comupter beside BIORAD 1
- Open (double click) on Desktop icon Ipswitch 

- Select "My computer" tab, select "Browse" and select the **folder** you filed is saved in:

(see below)



- Select your **file** from the list generated
  (ensure it is the **.xml** file)
- Click the green arrow pointing to the right
- Done! "Finished" will be displayed at the bottom of the screen.
- Close program (click red "x" at top right of screen)

Interpretation:

Sample ID	FAM (BKV)	HEX (ic)	Interpretation
A	positive	positive	BKV detected
B	Positive	Negative	BKV detected*
C	Negative	Positive	BKV not detected
D	Negative	negative	PCR failure; re extract and repeat

*Negative Internal Control readings in HEX (IC) may occur in strongly positive FAM (bkv) viral loads in the sample often deplete the amplification/detection materials (eg. nucleotides), leading to reduced or absent Internal Control signals.

Reporting

Soft LAB Order (BKU/8BKB)

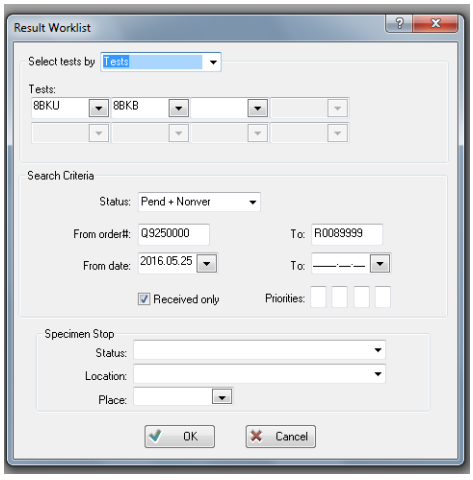
A. Negatives (Not detected) are autoverified as: "Negative" under 8BKU/8BKB Result.

The LIS report is setup to show:

PCR for BK Virus (Blood/Urine): Negative IU/mL
(BKV viral load conversion factor:
1.0 IU/mL = 1.1 copies/mL)
This is a research test.
AltoStar BKV PCR Kit 1.5, altona Diagnostics.

B. For Non-Negative results:

- In softlab "**resulting worklist"**, generate a query by **"tests"** (Select tests by field) and under "Tests" type in 8BKU, 8BKB. Select an approparite date range.



- Click OK. This will generate a list of pending results.

- Reviews results one at a time and verify each result.

For results < 2.00E+2 IU/mL all results will show "<2.00E+2" under 8BKU/8BKB Result

The LIS report is setup to show:

PCR for BK Virus (Blood/Urine): "<2.00E+2 IU/mL
(BK DNA Detected below the linear range of the assay which is 2.00E+2 IU/mL and thus the exact value cannotbe calculated.)
(BKV viral load conversion factor:1.0 IU/mL = 1.1 copies/mL)
This is a research test.
AltoStar BKV PCR Kit 1.5, altona Diagnostics.

For results ≥ 2.00E+2 IU/mL the value will appear under 8BKU/8BKB Result

The LIS report is setup to show:

Example

PCR for BK Virus (Blood/Urine): #.##E+# IU/mL
(BKV viral load conversion factor:1.0 IU/mL = 1.1 copies/mL)
This is a research test.
AltoStar BKV PCR Kit 1.5, altona Diagnostics.

Cleaning

Clean Room: Wipe down with RNAse Away or NucleoClean on paper towel, followed by distilled water, and then 70% alcohol

- Biological Safety Cabinet
- Pipettes
- Bench tops

Specimen Preparation Area: Wipe down with Working 1% hypochloride (made daily), followed by distilled water, and then 70% alcohol

- Biological Safety Cabinet (BSC), pipettes, centrifuge, and bench top.
- Seal and discard BSC waste
- Wash racks.

Amplification Area: Wipe down surfaces with RNAse Away or NucleoClean on KimWipe, followed by UltraPure water, and then 70% alcohol Wipe

- Seal & discard reaction microtubes into biohazard waste after each run.
- Perform the cleaning procedure according the daily maintenance sheet.

Quality Control

Reagent QCs:

- **The run is valid if all the QC parameters (M slope, PCR efficiency and R squares<R2>) are within range.**
- An External Control (external to altona Diagnostics) is used to monitor the isolation, amplification and detection procedures. The result must correspond to expected value supplied by the manufacturer.
- External controls for BKV (high pos, negative and lowpos) should be extracted in the EasyMag and run every time a BKV PCR is run and/or with a PCR kit new lot or shipment.

Daily QCs: Every Run

- Each patient specimen must have an Internal Control (IC) added to monitor both isolation and PCR inhibition.
- Quantitative standards 1-4 are included and shows a positive reading in FAM channel (BKV)
- A Negative Control usually water is included and shows a negative reading in FAM channel and a positive reading in HEX (IC) channel.
- Report all failed QCs to senior/charge technologist.

Failed QC:

Test is invalid without satisfactory QC results.

a. Do not release results pending resolution of QC failure.
b. Inform charge/senior technologist.
c. Record in Reagent Log Chart, Instrument Maintenance Log or Incident Report where appropriate.
d. If the QC failure was due to a simple matter of position reversal or misplacement, the run can be released (positive QC material yielded positive result, negative yielded negative result).
e. If positive QC material yielded negative result, repeat the entire run.
f. If negative QC material yielded positive result, it may be due to cross-contamination from adjacent positive sample within the run or carry-over contamination from previous runs via equipment or the environment. Review procedure and equipment to establish and eliminate potential sources of contamination.
g. The extent and nature of contamination can also be evaluated by comparing the positive rate of the run with its expected positive rate.
h. If the contamination is extensive, it is necessary to quarantine/discard potentially contaminated reagents and consumables and disinfect equipment and environment before repeating the run.
i. If a carry-over contamination is suspected (e.g. two or more runs with negative QC being positive or patient samples have higher than expected positive rate and these samples are often non-repeatable positives), it is necessary to have a thorough environmental disinfection followed by swabbing to monitor.

j. Successful ending to a carry-over contamination may be indicated by QC results and patient positivity rate falling back to the expected normal range and three negative environmental swabs.

Related Documents

Virology Accessioning Manual	
Training Checklist	
BK PCR External Control Log	T:\microbiology\Virology\QC statistics\EXTERNAL QC and INVENTORY Logs\Astra BK PCR

References:

Altona RealStar BKV RT-PCR Kit v1.0, altona Diagnostics

Record of Edited Revisions

Manual Section Name: BK Virus Quantitative PCR Bio-Rad CFX96

Page Number / Item	Date of Revision	Signature of Approval
Manual Transferred from Molecular Diagnostics Manual Policy # MI/MD/v51 archived 2015.12.02	December 2, 2015	Dr. T. Mazzulli
Addition of procedure for MSH + non UHN clients regarding exporting and resulting procedures for softlab 8bku and 8bkb tests Live January 1st, 2016	December 29, 2015	Dr. T. Mazzulli
Updated Reagent and Daily QC to qc specific for BK Added BK Altona reference Updated MSH logo in header.	February 17, 2016	Dr. T. Mazzulli
No revisions - All clients live wih 8bku/8bku pending standing softmic orders.	May 1, 2016	Dr. T. Mazzulli
Annual Review Updated PCR set up from Added per shipment and lot to external QC Number of Test Samples + 5 Standards (*4QS&NTC*) + one TO Number of Test Samples + 4 Standards +1 NTC (*4QS&NTC*) + one extra Updated procedure to Load plate into the centrifuge carrier and spin at 3000 rpm for 2 minutes in the Thermo Scientific Centrifuge before loading into biorad. Under Analysis added steps: • Set the threshold line for each target channel (BKV & IC). Click off the ic leaving bkv on. Drag the threshold over all the flat (negative) reaction. Repeat on the ic channel. And • Check if the QC parameters meet the requirements of a **VALID** PCR run. Print these data and attach with the results Addition of Interpretation Table Section	August 24, 2016	Dr. T. Mazzulli
Addition of Appendix 1: Excluding samples from autorelease of results to LIS	October 19, 2016	Dr. T. Mazzulli
Annual Review	August 30, 2017	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	August 15, 2018	Dr. T. Mazzulli
Annual Review Minor formatting change	April 12, 2019	Dr. T. Mazzulli
Annual review	May 15, 2020	Dr. T. Mazzulli

Full document review included in all updates. Biennial review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Minor formatting change	April 13, 2021	Jessica Bourke
Biennial review	May 05, 2023	Qin Liu