**PROCEDURE: Coro Molecular Microbiology Cobas BKV**

1. **PRINCIPLE**
   1. Intended Use
      1. cobas® BKV is an in vitro nucleic acid amplification test for the quantitation of BK virus (BKV) DNA in human EDTA plasma on the cobas® 6800/8800 Systems.
      2. In EDTA plasma, cobas® BKV is intended for use as an aid in the management of BKV in transplant patients. In patients undergoing monitoring of BKV in EDTA plasma, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess viral response to treatment.
      3. The results from cobas® BKV are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Test results must not be the sole basis for patient management decision..
      4. cobas® BKV is not intended for use as a screening test for blood or blood products or human cells, tissues, and cellular and tissue-based products.
   2. Summary and Background
      1. Transplant recipients are at increased risk for many viral and bacterial infections that are more likely to cause severe adverse health outcomes in the transplant patient population compared to the general healthy population. This increased risk is partly attributable to diminished immune system function conferred by the immunosuppressive medications that transplant patients receive in order to reduce their likelihood of graft rejection.
      2. BK virus (BKV) is a small (~5kb), non-enveloped deoxyribonucleic acid (DNA) virus belonging to the polyomavirus family. There are four major BKV subtypes, of which subtype I is the most commonly detected (80%), followed by subtype IV (15%). BKV seroprevalence is > 80% in the general healthy adult population. In immunocompetent persons, BKV is not associated with significant pathology. However, BKV infection may cause severe clinical disease in immunocompromised persons, including transplant recipients.
      3. BKV infections most commonly manifest in the kidneys and urinary tract. After primary infection, the virus remains latent in the renal tubular epithelium and ureteral epithelium, and can be reactivated in immunocompromised individuals. Kidney transplant patients are at higher risk for BKV associated complications compared to recipients of other transplant types, including polyoma virus nephropathy (PVN) and ureteral stenosis. PVN occurs in up to 10% of kidney transplant recipients, and about 50% of PVN affected patients will experience transplant graft failure. In addition, approximately 3% of kidney transplant recipients develop BKV associated ureteral stenosis. Hematopoietic stem cell transplants (HSCT) also experience BKV associated complications at a higher frequency, most commonly in the form of hemorrhagic cystitis (HC). Between 5 to 15% of HSCT patients experience HC.
      4. Guidelines recommend regular monitoring for BKV in kidney transplant patients for up to 5 years post-transplant. This monitoring approach can identify 80-90% of patients at risk for PVN. Plasma testing for BKV viremia is recommended as part of the strategy to identify patients at increased risk for PVN, either as a confirmatory test for patients in whom BKV viruria is detected, or as the primary testing modality for routine screening. There are currently no recommendations for routine BKV monitoring for HSCT patients, and testing is recommended primarily for evaluation of patients with hematuria and clinical symptoms of cystitis. However, elevated BKV DNA levels are associated with a higher risk for HC in transplant patients.
      5. For kidney transplant patients who have a persistent elevation in plasma BKV DNA levels, plasma BKV testing is recommended every 1-2 weeks until the DNA levels returns to an undetectable level on two consecutive measurements.
   3. Sample Preparation
      1. cobas® BKV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800/8800 software which assigns test results for all tests as either target not detected, BKV DNA detected < LLoQ (lower limit of quantitation), BKV DNA detected > ULoQ (upper limit of quantitation), or a value in the linear range LLoQ < x < ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.
      2. Nucleic acid from patient samples and added lambda DNA-QS molecules are simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the glass particles with elution buffer at elevated temperature.
   4. Selective Amplification
      1. Selective amplification of target nucleic acid from the sample is achieved by the use of a dual target virus specific approach from highly-conserved regions of the BKV located in the BKV small t-antigen region and the BKV VP2 region. Selective amplification of DNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the BKV genome. A thermostable DNA polymerase enzyme is used for amplification. The target and DNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs is eliminated by the AmpErase enzyme, which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C
      2. The cobas® BKV master mix contains two detection probes specific for BKV target sequences and one for the DNA-QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of BKV target and DNA-QS in two different target channels. The fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probe to the specific single-stranded DNA templates results in cleavage by the 5'-to-3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Real-time detection and discrimination of PCR products are accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and DNA-QS.
2. **AVAILABILITY**
   1. Specimens may be submitted 7 days a week/ 24 hour a day.
3. **TEST CODE**
   1. BKVLD
4. **SPECIMEN REQUIREMENTS**
   1. Specimen Collection
      1. Handle all specimens as if they are capable of transmitting infectious agents.
      2. Store all samples at specified temperatures.
      3. If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) until completely thawed and then centrifuge to collect all sample volume at the bottom of the tube.
      4. Whole blood should be collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions.
   2. Specimen Transport
      1. Separate plasma from whole blood within by centrifugation at 3000 RPM for 20 minutes at room temperature.
      2. Whole blood collected in BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma preparation.
   3. Specimen Storage
      1. Upon separation plasma samples may be stored for 24 hours at 2-30°C in primary or secondary tubes, followed by:
         1. Storage in primary or secondary tubes for up to 6 days at 2-8 °C.
         2. Storage in secondary tubes for up to 6 months at -15°C to -80°C.
      2. Plasma samples are stable for up to four freeze/thaw cycles when frozen at -15°C to -80°C.
      3. Per Lab Protocol:
         1. RIH: Freeze samples at -80°C immediately after centrifugation and transport to Coro Molecular Microbiology Lab.
         2. TMH: Store plasma at 2-8°C and send to RIH Micro. RIH Micro will freeze plasma and transport to Coro Molecular Microbiology Lab.
         3. Coro Molecular Micro Lab will store all plasma samples at -80°C.
5. **MATERIALS AND REAGENTS**
   1. Reagents
      1. cobas® BKV kit includes the following components that are stored at 2-8°C:
         1. Proteinase Solution
         2. DNA Quantitation Standard
         3. Elution Buffer
         4. Master Mix Reagent 1
         5. BKV Master Mix Reagent 2
      2. Reagents not included in the kit and stored at 2-8°C unless stated otherwise:
         1. MGP Reagent
         2. Specimen Diluent
         3. Lysis Reagent
         4. Wash Reagent, store at 15-30°C
   2. Materials
      1. cobas omni Processing Plate
      2. cobas omni Amplification Plate
      3. cobas omni Pipette Tips
      4. cobas omni Liquid Waste Container
      5. Solid Waste Container and Bag
   3. Reagent Precautions
      1. Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
      2. Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
      3. cobas omni Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
      4. cobas® BKV, cobas omni MGP Reagent, and cobas omni Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry
      5. Do not allow cobas omni Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
      6. Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.
      7. cobas® EBV/BKV Control Kit contains plasma derived from human blood. The source material has been tested by PCR methods and showed acceptable traces of low levels of BKV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
   4. Reagent Storage and Handling
      1. Do not freeze reagents or controls.
      2. Reagents loaded onto the **cobas®** 6800 System are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas®** 6800 Systems allow reagents to be used only if all of the conditions shown in the following table are met. The system automatically prevents use of expired reagents. The following table allows the user to understand the reagent handling conditions enforced by the **cobas®** 6800 System.

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Open-kit stability** | **Number of runs for which this kit can be used** | **On-board stability** |
| **cobas®** BKV | 90 days from first usage | Max 40 runs | Max 40 hours |
| **cobas®** EBV/BKV Control Kit | N/A | N/A | Max 8 hours |
| **cobas®** Buffer Negative Control Kit | N/A | N/A | Max 10 hours |
| **cobas omni** Lysis Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** MGP Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Specimen Diluent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Wash Reagent | 30 days from loading\* | N/A | N/A |

*\*Time is measured from the first time that reagent is loaded onto the* ***cobas®*** *6800 Systems.*

* + 1. Do not use reagents after their expiration dates.
    2. Do not pool reagents.
    3. Gloves must be worn and must be changed between handling specimens and cobas® 6800 reagents to prevent contamination.
    4. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
    5. Handle all reagents with caution and avoid contact with skin, eyes, or mouth. Refer to the package insert for any known toxicity.
       1. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.
       2. If spills occur on the cobas® 6800 System, follow the instructions in the appropriate cobas® 6800 System – System Manual to clean.

1. **QUALITY CONTROL**
   1. Quality Control Information
      1. One Buffer Negative Control (Buff (–) C)
      2. One Low Positive Control (EBV/BKV L(+)C)
      3. One High Positive Control (EBV/BKV H(+)C)
      4. Store all controls at 2-8°C.
      5. Controls are stable until the expiration date indicated.
      6. Record QC results on the sheets provided. Include date of testing, kit lot #, control lot #s, expiration dates, and results.
      7. Batch validity is checked within the cobas 6800 software (monitor) and is printed with the run report.
      8. The batch is valid if no flags appear for all three controls.
      9. Validation of patient results is performed automatically by the cobas® 6800 software based on negative and positive control results.
      10. New lot numbers/shipment of BKV kits are QC’d using control kits that have passed QC.
      11. New lot numbers/ shipments of Positive and Negative Control kits are run using BKV kits that have passed QC.
      12. Verification of Performance is run on new lot numbers of BKV kits and/or every 6 months, after Software Upgrades, and after major System Upgrades.
          1. A purchased Verification Panel is run which tests the linear range of the assay.
          2. Results are plotted and the R² value is determined.
          3. An unacceptable result would be an R² value <0.9900 or 2 panel members with a >0.5 Log difference from the expected result and would warrant an investigation and repeat.
      13. Environmental testing is performed monthly
          1. The hood, instruments, and bench space is swabbed and placed in an aliquot of plasma dilution matrix and placed on the run of patient samples.
          2. For any positive result, clean all areas and retest.
   2. Acceptable Limits
      1. For any run, valid results must be obtained for both the Positive and Negative Controls for the cobas® 6800 Software to display the reportable cobas® BKV results from that run.
      2. QC statistics are calculated monthly to define analytic imprecision and to monitor trends over time.
2. Refer to Viral Load Monitoring Statistics on the M drive Molecular folder.
   1. Corrective Actions
      1. The assay will require repeating if either positive or negative controls are not valid.
      2. If the EBV/BKV L(+) and EBV/BKVC H(+) Control or the BUFF(-) Control results are consistently invalid, contact your local Roche Support Network Customer Support Center for technical assistance.
   2. Control Flags
      1. Control flags for negative and positive controls:

|  |  |  |  |
| --- | --- | --- | --- |
| **Negative Control** | **Flag** | **Result** | **Interpretation** |
| (–) C | Q02  (Control batch failed) | Invalid | An invalid result or the calculated titer result for the negative control is not negative. |
| **Positive Control** | **Flag** | **Result** | **Interpretation** |
| EBV/BKV L(+)C and EBV/BKV H(+)C | Q02  (Control batch failed) | Invalid | An invalid result or the calculated titer result for the positive control is not within the assigned range. |

*If the batch is invalid, repeat testing of the entire batch including samples and controls.*

1. **TEST PROCEDURE**
   1. Clean bench tops with 10% bleach followed by 70% alcohol pre and post running the assay.
   2. Remove frozen plasma samples from freezer to thaw and come to room temperature. They may be placed in DI H2O.
   3. Required sample volume is 350 ul.
   4. Go to the 6800 **Monitor Tab** and check the taskbar and messages at the top left on the monitor screen.
      1. Address any issues or maintenance due.
   5. Refill reagents and consumables as prompted by the system:
      1. Load wash reagent, lysis reagent and diluent.
      2. Load tip racks, processing plates and amplification plates.
      3. Load Magnetic Glass Particles.
      4. Load test specific reagents.
      5. Load control cassettes.
      6. Replace rack for clotted tips.
   6. Set the system to “Ready”.
      1. In the task overview, ensure that there is no maintenance overdue.
      2. On the **Monitoring** tab, Choose the **Start** button.
         1. The system changes to **Preparing** status.
      3. Wait for the system to change to **Ready** status before you start loading. This may take 15 minutes.
   7. Organize the Viral Load runs for the day and make Tasklists.
   8. Load sample racks onto rack trays.
      1. Cobas 6800 has LIS Order Download
      2. For any samples without LIS barcode use Rack Based Ordering, i.e. environmental samples.
         1. Designated BKV racks have Orange labels on them
         2. Sample ID must be entered in Manual Barcode Entry tab
   9. Bring racks and samples to hood for loading racks with samples.
      1. BKV, CMV, HIV-1 and HCV viral loads can be performed at the same time on the same processing plates.
      2. Vortex samples and discard caps.
      3. Check for bubbles and remove if needed.
      4. Samples can be centrifuged to collect all sample volume at bottom of tube if needed.
      5. Add sample plasma tubes to sample racks.
   10. Load trays with sample racks onto the Sample Supply module and go to the “Batches” tab.
       1. Monitor the “error lane” for any problems.
       2. After the sample barcodes are read it will make the “batch” and list the number of BKV’s, CMV’s, HIV’s and HCV’s in the batch.
          1. **Make sure that number matches the expected number of tests**.
          2. Resolve any discrepancies.
   11. Hit the “**Start Manually**” button to begin processing.
       1. At this point you may go to the Routine Tab> Test Order Status to see the finish time.
   12. Monitor the instrument during processing in the Transfer Module.
       1. Address any errors or issues.

**Note: Do not walk away from instrument until samples are moved to the Processing module.**

* 1. Unload racks and samples when finished pipetting.
     1. Recap tubes in the hood with new caps and store in freezer boxes.
  2. Unload consumables at the end of processing:
     1. Remove amplification plates from the analytic module.
     2. Unload empty control cassettes.
     3. Empty solid waste.
     4. Empty liquid waste.

1. **RESULTS INTERPRETATION**
   1. Results
      1. From the **Routine Tab > Control Batch**
      2. Choose the control batch for your run.
      3. From the drop-down list choose Print from both right and left side of screen.
         1. The printout from the left has lot #s of reagents and controls
         2. The printout from the right has the actual results of controls.
         3. View results
   2. Reporting Results- Refer to Appendix A for LIS resulting instructions.
      1. The cobas® 6800 System automatically determines the BKV DNA concentration for the samples and controls. The BKV DNA concentration is expressed in International Units per milliliter (IU/mL).
      2. For a valid batch, check each individual sample for flags in the cobas® 6800 software and/or report. The result interpretation should be as follows:

|  |  |
| --- | --- |
| **Result Read-Out from cobas® System** | **Analytical Interpretation** |
| Target Not Detected | BKV DNA not detected.  **Report results as “Not Detected.”** |
| < Titer Min | BKV DNA detected but not quantified.  Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. **Report results as “<21.5 IU/mL”**  **Titer min = 21.5 IU/mL** |
| Titer | BKV DNA detected and quantified.  Calculated titer is within the Linear Range of the assay – greater than or equal to 21.5 IU/mL and less than 100,000,000 IU/mL.  **Report results as “(Titer) IU/mL”** |
| > Titer Max | BKV DNA detected but not quantified.  Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. **Report results as “>100,000,000**  **Titer max = 1.0E+08 IU/mL** |

* + 1. Release Results
       1. Select all test results to be released
       2. Choose the **Release button**
          1. It is possible to release an Invalid result so use caution when releasing.
       3. Test results are sent to the SOFT Instrument Menu for posting.
    2. Invalid Patient Results
       1. An Invalid sample will be retested on the next run. If it repeats as Invalid report as Indeterminate- Suggest repeat.

1. **LIMITATIONS**
   1. Recommendations regarding monitoring BKV viral load post-transplant and medically relevant BKV DNA thresholds vary among transplant type and transplant institutions.
   2. cobas® BKV test results should be interpreted in the context of other clinical data and should not be the sole basis for treatment decisions.
   3. cobas® BKV has been evaluated only for use in combination with the cobas® EBV/BKV Control Kit, cobas® Buffer Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the cobas® 6800/8800 Systems.
   4. This test has been validated only for use with EDTA plasma. Testing of other sample types with cobas® BKV may result in inaccurate results.
   5. Quantitation of BKV DNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
   6. As with any molecular test, mutations within the target regions of cobas® BKV could affect primer and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
   7. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences.
   8. Due to the potential for variability in BKV DNA measurements across different BKV assays, it is recommended that the same device be used for the serial quantitation of BKV DNA when managing individual patients.
   9. Reliable results depend on proper sample collection, storage and handling procedures.
   10. cobas® BKV is not intended for use as a screening test for the presence of BKV in blood or blood products or human cells, tissues, and cellular and tissue-based products.
2. **INTERFERENCES**
   1. Interfering Substances
      1. Elevated levels of triglycerides (37 mmol/L), conjugated bilirubin (0.2 g/L), unconjugated bilirubin (0.2 g/L), albumin (60 g/L), hemoglobin (2 g/L) and human DNA (2 mg/L) in EDTA plasma samples were tested in the presence (100 IU/mL) and absence of BKV DNA. The tested endogenous interferences were shown not to interfere with the test performance of cobas® BKV.
      2. In addition, drug compounds (listed in Table 21 of the package insert) were tested at three times the Cmax in presence (100 IU/mL) and absence of BKV DNA. The tested interferences were shown not to interfere with the test performance of cobas® BKV.
      3. All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with cobas® BKV for all samples without BKV target and positive results were obtained on all of the samples with BKV target. Furthermore, the mean log10 titer of each of the positive BKV samples containing potentially interfering substances was within ± 0.5 log10 of the mean log10 titer of the respective positive spike control.
3. **TECHNICAL ASSISTANCE**
   1. Roche Support Network Customer Support Center at 1-800-526-1247.
4. **REFERENCES**
   1. Roche Cobas BKV Package Insert Version 2.0
   2. Roche Cobas 6800 Operators Manual

**Appendix A**

**BKV Viral Load SCC Soft Resulting**

Test ID: **BKVLD**

Template: **BKVLD**

Workstation: **RMOLM**

1. Print a “Resulting Worklist” by Template: BKVLD
   1. Status= Pending and Nonverified
   2. Start date= go back 1 month
   3. End date= current date
   4. Received box- unchecked
   5. Check list for any old outstanding orders- investigate and resolve any issues.
   6. List will be in Order sequence number from low to high. Except:
      1. STATs will go to top of list
      2. Add ons or any order that has been changed will stay at the bottom
      3. Print list:
         1. Click Printer icon
         2. Choose Worklist- Layout Horizontal or Vertical
         3. Print to Local Printer J73
   7. Use this list to check against specimens in the freezer
2. Create a Tasklist
   1. Follow procedure for “Creating a Tasklist” in Soft Manual under TASKLIST
      1. Template= BKVLD
   2. Number the specimens according to the tasklist beginning with #1 and ending with #93
   3. Print the worklist and check it against the samples to verify both are in the same order.
3. Posting Results using LIS Interface
   1. View and Review results from the cobas 6800
   2. Click on all results to be released and click the **RELEASE** button
   3. Results will transfer to the Soft Instrument Menu
   4. From SoftLab, go to “Interfaces”, and “Instrument Menu”
   5. Select Cobas 6800 from the Instrument Menu
   6. Select “Loadlist and Today’s Results”, “Not Posted”, “By Sequence”
   7. Each order will be highlighted individually. Verify the result against the instrument printout. Click “Post All” for each order to be verified.
   8. If any Result Comments, i.e. phone reports need to be added:
      1. Do not Post result
      2. Go to “Lab Result” tab.
      3. Open “Comment” box for line BKPCR and add comment, i.e. @CALT, to the box. OK and Save
      4. Go back to “Instrument” tab and “Post” result.
   9. Check Results
      1. Go to “Resulting Worklist” by Tasklist
      2. Choose Tasklist
      3. Enter Tasklist ID
      4. Review Worklist to verify that all results have posted. They should all have “\*” next to them.
      5. Print a new pending worklist and check on any outstanding orders.