Simplexa™ BKV

A real-time PCR assay intended for the in vitro quantitation of BK virus (BKV).

For in vitro diagnostic use

INTENDED USE
The Focus Diagnostics Simplexa BKV assay is intended for the in vitro quantitation of BK virus (BKV) nucleic acids in urine and/or plasma specimens using the 3M Integrated Cycler.

This assay is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the clinical management of BKV infected patients.

The assay is not intended for donor screening. The assay is for professional use only.

SUMMARY AND EXPLANATION
Human polyomavirus type BK virus (BKV) is a non-enveloped virus with a circular, double stranded DNA genome of 5,300 bp. BKV was first recognized as a member of the polyomavirus family in 1971, after isolation from the urine of a renal-transplant recipient. Worldwide seroprevalence rate is 70 to 90% in adults. Seroconversion typically occurs in childhood by age 4–7. Viremia in primary infection seeds the kidney, where the virus can establish a clinically latent infection. The virus typically remains dormant but intermittent reactivation may occur in certain populations, such as immunosuppressed patients or pregnant women. Reactivation may result in a variety of manifestations, ranging from subclinical viremia or viruria, to nephropathy and ureteral stenosis seen in transplant patients. Overt clinical disease from BKV infection is uncommon, but correlates with the degree of immunosuppression.

Currently, other than PCR, there are no reliable detection and quantification methods for BKV. Viral culture is rarely helpful in providing timely information for patient management because of slow growth and a requirement for specialized cell lines. Serological assays are of minimal use as the majority of the population is seropositive with BKV-related disease resulting from reactivation of latent infection.

PRINCIPLES OF THE PROCEDURE
The test is a real-time PCR amplification and detection system that utilizes a bi-functional fluorescent probe-primer for the detection of BKV DNA in urine and plasma specimens. The assay is composed of two principal steps: (1) extraction of DNA from patient specimens, (2) a bi-functional fluorescent probe-primer is used together with a reverse primer to amplify a specific target (for each analyte and the internal control). The assay provides one result: a well conserved region of the VP2 gene of the BKV genome, is targeted to identify the viral DNA in the specimen. An internal control is used to monitor the extraction process and to detect PCR inhibition. The amplification signal obtained for each specimen is compared to a calibration curve and quantified.

MATERIALS PROVIDED
The Focus Diagnostics Simplexa™ BKV kit contains sufficient reagents for 100 reactions.

Kit Description

<table>
<thead>
<tr>
<th>Component Name</th>
<th>REF</th>
<th>EC SYMBOL ON LABEL</th>
<th>Abbreviated Name</th>
<th>Cap Color</th>
<th>Number of Vials</th>
<th>Reactions per Vial/Kit</th>
<th>Volume per Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplexa™ BKV Primer Mix</td>
<td>MOL2301</td>
<td>REAG A</td>
<td>PM</td>
<td>Brown</td>
<td>2</td>
<td>50/100</td>
<td>50 µL</td>
</tr>
<tr>
<td>Simplexa™ Master Mix</td>
<td>MOL2000</td>
<td>REAG B</td>
<td>MM</td>
<td>Green</td>
<td>2</td>
<td>50/100</td>
<td>200 µL</td>
</tr>
<tr>
<td>Simplexa Extraction &amp; Amplification Control DNA</td>
<td>MOL9001</td>
<td>CONTROL IC</td>
<td>IC</td>
<td>Blue</td>
<td>3</td>
<td>50/150</td>
<td>250 µL</td>
</tr>
<tr>
<td>Simplexa™ BKV Low Positive Control</td>
<td>MOL2302</td>
<td>CONTROL +</td>
<td>LPC</td>
<td>White</td>
<td>6</td>
<td>1/6</td>
<td>200 µL</td>
</tr>
<tr>
<td>Simplexa™ BKV High Positive Control</td>
<td>MOL2303</td>
<td>CONTROL ++</td>
<td>HPC</td>
<td>Red</td>
<td>6</td>
<td>1/6</td>
<td>200 µL</td>
</tr>
</tbody>
</table>
**Component Description**

<table>
<thead>
<tr>
<th>Kit Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplexa™ BKV Primer Mix (PM)</td>
<td>Dye-labeled fluorescent primers specific for detection quantitation of BKV and for the Internal Control.</td>
</tr>
<tr>
<td></td>
<td><strong>Target</strong></td>
</tr>
<tr>
<td>BKV</td>
<td></td>
</tr>
<tr>
<td>Internal Control</td>
<td></td>
</tr>
<tr>
<td>Simplexa™ Master Mix (MM)</td>
<td>DNA polymerase, buffer and dNTPs.</td>
</tr>
<tr>
<td>Simplexa™ Extraction &amp; Amplification Control DNA (IC)</td>
<td>A 577 base pair DNA fragment derived from the gene encoding ribulose-1,5-bisphosphate carboxylase oxygenase large unit N-methyltransferase of the plant <em>Arabidopsis thaliana</em>.</td>
</tr>
<tr>
<td>Simplexa™ BKV Low Positive Control (LPC)</td>
<td>BKV amplicon in human base matrix.</td>
</tr>
<tr>
<td>Simplexa™ BKV High Positive Control (HPC)</td>
<td>BKV amplicon in human base matrix.</td>
</tr>
<tr>
<td>Simplexa™ BKV Barcode Card</td>
<td>Assay specific parameters.</td>
</tr>
</tbody>
</table>

**MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Simplexa™ BKV Quantitation Standard Set (REF MOL2310)
2. 3M Integrated Cycler with Integrated Cycler Studio Software version 3.0 or higher
3. Universal Discs for use on the Integrated Cycler
4. Universal Disc Cover Tape
5. a Roche MagNA Pure LC System and associated consumables.
6. b Roche MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Cat. No 3038505001)
7. b bioMérieux NucliSENS® easyMAG™ instrument and associated consumables and reagents
8. b Biohit/bioMerieux multi-channel pipette
9. b ELISA strip plate
10. Single, multi-channel and/or repeater micropipette(s) with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
11. Freezer (manual defrost) at -10 to -30 °C (for kit component frozen storage)
12. Freezer (manual defrost) at -10 to -30 °C (for specimen frozen storage)
13. Refrigerator at 2 to 8 °C (for specimens and thawed kit components)
14. Biosafety cabinet (laminar flow hood) for extractions
15. Microcentrifuge
16. Vortex mixer
17. Sterile, RNase/DNase-free disposable aerosol-barrier micropipettor tips
18. 1.5 mL polypropylene microcentrifuge tubes and racks (RNase/DNase-free tubes are recommended but not required)
19. Disposable, powder-free gloves
20. Nuclease-Free Water (Used during extraction and as the No-Template Control (NTC))
21. Cooler racks for 1.5 mL microcentrifuge tubes
   a For use with Roche MagNA Pure LC extraction method
   b For use with bioMérieux easyMAG extraction method

**SHELF LIFE AND HANDLING**

1. Store reagents at -10 to -30 °C (do not use a frost-free freezer).
2. Allow reagents to thaw at room temperature (approximate range 18 to 25 °C) before use.
3. Do not use kits or reagents beyond their expiration dates.
4. After addition of Master Mix, use the reaction mix within one hour. Store Reaction Mix at 2 to 8 °C until ready to proceed with PCR Setup.
5. Once thawed, store the Primer Mix, Master Mix, Extraction & Amplification Control DNA, and No Template Control at 2 to 8 °C for no more than 30 days.
6. Do not refreeze Primer Mix, Master Mix, Extraction & Amplification Control DNA or Positive Controls.
7. Do not combine reagents from different kit lots.
WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
2. All human origin materials should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.11,12
3. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
4. Do not pipette by mouth.
5. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used.
6. Dispose of unused kit reagents and human specimens according to local, state and federal regulations.
7. Workflow in the laboratory should proceed in a uni-directional manner, beginning in the Pre-Amplification areas and moving to the Amplification/Detection area: below is the sequence of events that takes place from specimen extraction to Real-Time PCR amplification:
   • Begin with specimen extraction, followed by Real-Time PCR instrument set-up, reagent preparation, and finally Real-Time PCR amplification.
   • Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement is recommended between the different areas.
   • Supplies and equipment used for specimen preparation should not be used for reagent preparation activities or for processing amplified DNA or other sources of target nucleic acid.
   • All amplification supplies and equipment should be kept in the Real Time PCR Instrument Area at all times.
   • Personal Protective Equipment, such as laboratory coats and disposable gloves, should be area-specific.
8. Contamination of patient specimens or reagents can produce erroneous results. Use aseptic techniques.
9. Pipette and handle reagents carefully to avoid mixing of samples from adjacent wells.
10. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
11. Do not substitute or mix reagent from different kit lots or from other manufacturers.
12. Do not interchange the reagent tube caps. This may cause contamination and compromise the test results.
13. Only use the protocol described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results.
14. Assay setup should be performed at room temperature (approximate range 18 to 25 °C). While mixing the reagents, keep the enzymes cold by utilizing a cooler block.
15. Do not re-use Universal Discs that have already been exposed to patient samples or reagents.
16. Dispose of used disc without detaching or removing cover tape.
17. If different Simplexa™ kits or lots are set up on the same disc, Positive and No Template Controls from each kit need to be tested.
18. Master Mix contains > 1% glycerol, which may cause irritation upon inhalation or skin contact. Upon inhalation or skin contact, first aid measures should be taken.
19. Extended storage of extracted specimens at 2 to 8 °C is not recommended; performance has not been established.
20. If kit packaging or contents appear to be broken or damaged do not use and contact Focus Diagnostics. Contact information is on the last page of this document.

INSTRUCTIONS FOR USE

A. SPECIMEN COLLECTION
   Acceptable specimen types include urine and plasma. For Plasma, do not use collection tubes with heparin as an anticoagulant. Heparin inhibits PCR.

B. SPECIMEN EXTRACTION AREA
   Perform in a dedicated area for specimen and control extraction. Sample preparation for extraction should be performed in a biosafety cabinet.

 Extraction using Roche MagNA Pure LC extraction method
   1. Extract patient specimens and assay controls using the Roche MagNA Pure Total Nucleic Acid kit and the Roche MagNA Pure LC Automated Nucleic Acid Extractor instrument. Refer to the manufacturer’s Instructions for Use for nucleic acid extraction using this kit.
   2. Under the “Protocol” drop-down menu on the MagNA Pure LC System, select “Total NA”, and then “Total NA Variable_elution_volume.blk” from the list. This will load the appropriate settings for the run.
   3. The Sample Protocol should be “Total NA Variable_elution_volume”.
   4. 200 µL should be set for the Sample Volume, and the elution volume should be set at 50 µL.
   5. The dilution volume should be set at zero for all specimens.
6. Ensure that the Post Elution Protocol is set to “None”.
7. Ensure that specimens and controls are in the correct position on the Sample Cartridge.
8. Vortex each specimen, LPC and HPC for 2 to 4 seconds and briefly centrifuge to pull contents down to bottom of tube.
9. Pipette 200 µL of each specimen, LPC, HPC and NTC into the corresponding position in the sample cartridge.
10. Visualy check the level of specimen and controls in the Sample Cartridge to ensure specimen(s) were added.
11. Pulse vortex Extraction & Amplification Control DNA (IC) 2 times and briefly centrifuge to pull contents down to bottom of tube.
12. For each set of 16 specimens (1-16 specimens), pipette 100 µL of the (IC) into 6 mL lysis buffer in a conical tube. Mix by vortexing briefly. Add to the appropriate tray on the MagNA Pure extraction instrument.
   o For example, if greater than 16 specimens (17-32 specimens) are extracted, pipette 200 µL of the IC into 12mL lysis buffer in a conical tube. Mix by vortexing briefly. Add to the appropriate tray on the MagNA Pure extraction instrument.
13. Transfer the sample cartridge to the MagNA Pure LC Automated Nucleic Acid extractor and begin the extraction run.
14. After nucleic acid extraction is complete, the cartridge containing the extracted controls and patient specimens can be removed from the MagNA Pure and sealed. Store the extracted DNA at 2 to 8 °C prior to use. Long-term storage of extracted specimens at this temperature is not recommended. Keep extracted DNA specimens on a cooler block while loading disc.

**Extraction using bioMérieux NucliSENS® easyMAG™ extraction method**

1. Refer to the NucliSENS® easyMAG™ User Manual for operation of the instrument and software.
2. Choose the Generic template on the NucliSENS® easyMAG™ software with the following settings;
   - **Default Request:** Generic 2.0.1 (or equivalent)
   - **Run Name Prefix:** (as appropriate)
   - **Sample ID prefix:** (as appropriate)
   - **Sample Type:** Primary
   - **Workflow Defaults:**
     - On-board lysis Incubation
     - On-board Silica Incubation
     - Sample Addition Guidance Off
   - **Reagent Tracking:** Lysis, Silica, Internal Control reagent tracking disabled
3. Enter individual specimen information into Extraction Request screen as below.
   - **Sample ID:** (Enter sample name)
   - **Request:** Generic 2.0.1 (or equivalent)
   - **Volume (mL):** 0.200
   - **Eluate (µL):** 50
   - **Type:** Primary
   - **Priority:** Normal
   - **Matrix:** Other
5. Vortex each specimen, LPC and HPC for 2 to 4 seconds and briefly centrifuge to pull contents down to bottom of tube.
6. Pipette 200µL of specimen, LPC, HPC or NTC to sample vessel(s).
7. Pulse vortex IC two (2) times and briefly centrifuge to pull contents down to bottom of tube.
8. Pipette 5µL of IC to each specimen and all control wells. Change tips in between wells.
9. Load sample vessel(s), new aspirator disposables, and reagents onto the easyMAG™ instrument per User Manual.
10. Initiate the on-board lysis and incubate the lysed specimens for 10 minutes before addition of magnetic silica mixture.
11. During lysis incubation period, prepare magnetic silica mixture. Mix silica and dilute in nuclease-free water by adding 1 part magnetic silica to 2 part nuclease-free water (e.g., 270µL of magnetic silica + 810µL nuclease-free water). Prepare minimally 135µL of magnetic silica mixture per specimen.
12. To transfer silica mixture into ELISA strip wells, mix magnetic silica mixture and use 1 tip and operating mode P2 of the Biohit pipette. Press **Start** to aspirate 1050µL of the magnetic silica mixture and press **Start** again to dispense the first shot back into silica mixture tube. Press **Start** to dispense 125µL of the magnetic silica mixture into 8 individual wells of the ELISA strip. Repeat as necessary for additional ELISA strips.
13. After the 10 minute lysis incubation, use 8 tips (per ELISA strip) and operating mode P3 of the Biohit pipette to transfer 100µL of magnetic silica mixture to each specimen in the sample vessel. Place tips into the ELISA strip wells and press **Start** to mix and aspirate magnetic silica mixture.
14. Transfer magnetic silica mixture to appropriate sample vessel and place pipette tip(s) into specimens below the liquid level. Press **Start** to aspirate, dispense and mix (x3) the magnetic silica and specimens. Ensure pipette tips remain below the liquid level to ensure proper mixing.
15. Repeat steps 13 and 14 for additional sample vessels.
16. After addition of magnetic silica mixture to all sample vessels, start the extraction run.
17. Upon completion of run, remove sample vessel(s) from the instrument. If specimens are not going to be used immediately, transfer into individual tubes to minimize chance of magnetic silica falling back into specimen. Store the extracted DNA at 2 to 8°C prior to use. Long-term storage of extracted specimens at this temperature is not recommended. Keep extracted DNA on a cooler block while loading disc.
C. REAL-TIME PCR INSTRUMENT SETUP


Note: A valid standard curve (calibration run) must be established prior to performing a prediction run.

D. REAGENT PREPARATION AREA

Dedicated area for preparation of Simplexa™ BKV assay reaction mix.

1. Thaw the Primer Mix and the Master Mix at room temperature (approximate range 18 to 25 °C). Each kit component vial contains sufficient reagents for 50 reactions. Prior to each use, gently mix the Primer Mix and Master Mix kit components by inverting 6 to 8 times and briefly centrifuge to pull contents down to bottom of tube.

2. Prepare the required volume of the Reaction Mix in an appropriately sized polypropylene microcentrifuge tube by pipetting the volume of each component as indicated in the table below.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reaction Mix Volume / 1 reaction</th>
<th>Reaction Mix Volume / 10 reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplexa™ Master Mix</td>
<td>4.0 µL</td>
<td>40 µL</td>
</tr>
<tr>
<td>Simplexa™ BKV Primer Mix</td>
<td>1.0 µL</td>
<td>10 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>5.0 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

3. Gently mix the Reaction Mix by inversion or by pipetting 8 to 10 times.

4. Briefly centrifuge to pull contents down to bottom of tube.

5. Proceed to PCR Setup.

6. Use the Reaction Mix within one hour of preparation. Store Reaction Mix at 2 to 8 °C if PCR setup will not be performed immediately after the Reaction Mix is prepared.

E. REAL TIME PCR AMPLIFICATION AREA

Perform in a dedicated area for preparation of the 96-well Universal Disc for Simplexa™ BKV assay.

Refer to example disc layout in section C while performing the following setup:

1. Add 5.0 µL of the Reaction Mix to each well.

2. Add 5.0 µL of the extracted Positive Controls to the “HPC and LPC” well.

3. Add 5.0 µL of extracted patient specimen to the appropriate “S” well.

4. Add 5.0 µL of extracted No-Template Control to the “NTC” well.

5. Cover the disc with the Universal Disc Cover Tape.

6. Open the lid of the Integrated Cycler.

7. Place the sealed Universal Disc onto the platen.

8. Close the lid gently.

9. Click Run.

10. Click Start.

F. DATA ANALYSIS

1. When the run finishes, click Analyze.

2. Review dyes one at a time or select all of the dyes (channels) by marking the check box next to each dye.

3. Press the Print Preview button (bottom right) to review a summary of the predictive values. Check the Include Graphs checkbox to preview the amplification plots. To include the calibration report associated with the prediction run select the Include Calibration Result checkbox. Scroll from page to page using the arrow buttons in the top left corner of the Print preview window.

4. Print or Save the report as needed.

5. Export the predicted values if needed.

QUALITY CONTROL

Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and standard good laboratory practice.

REPORTING RESULTS

1. Run Validity

Determine if the run is valid by reviewing the BKV and Internal Control (IC) results for the Low Positive Control (LPC), High Positive Control (HPC), and No-Template Control (NTC). All three controls must meet acceptance criteria for a run to be valid. If a run is invalid, then all patient specimens must be re-tested.
Acceptance Criteria

<table>
<thead>
<tr>
<th>Control</th>
<th>BKV</th>
<th>Extraction &amp; Amplification Control DNA (IC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-Template Control (NTC)</td>
<td>Not Detected</td>
<td>Detected</td>
</tr>
<tr>
<td>Low Positive Control (LPC)</td>
<td>Within tolerance value on lot specific label</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>High Positive Control (HPC)</td>
<td>Within tolerance value on lot specific label</td>
<td>Not Applicable</td>
</tr>
</tbody>
</table>

- The NTC meets the acceptance criteria if BKV is Not Detected and the IC is Detected. Detecting BKV in the NTC indicates that samples may have been contaminated during processing.
- The LPC meets the acceptance criteria if BKV is Detected in the LPC within the tolerance limits (as indicated on the lot specific label), and the IC should be Detected, but is not required to be Detected.
- The HPC meets the acceptance criteria if BKV is detected in the HPC within the tolerance limits (as indicated on the lot specific label), and the IC should be Detected, but is not required to be Detected.

2. Interpretation of Results

<table>
<thead>
<tr>
<th>Example</th>
<th>BKV value</th>
<th>IC value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not Detected</td>
<td>Detected</td>
<td>BKV Not Detected</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 510 copies/mL</td>
<td>N/A</td>
<td>BKV Detected, below the LLoQ (Limit of Quantitation).</td>
</tr>
<tr>
<td>3</td>
<td>X copies/mL</td>
<td>N/A</td>
<td>BKV Detected at specific concentration.</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 1 x 10^8 copies/mL</td>
<td>N/A</td>
<td>BKV Detected above the ULoQ (Limit of Quantitation).</td>
</tr>
<tr>
<td>5</td>
<td>Not Detected</td>
<td>Not Detected</td>
<td>Invalid, re-extract and repeat.</td>
</tr>
</tbody>
</table>

3. Specimen Result Validity

A specimen is valid if either
1. BKV is Not Detected and the IC is Detected.
2. BKV is Detected. The IC does not need to be detected for BKV positive results.
3. Amplification curves shall be reviewed for every result, especially when a “Data Quality” message is reported. A valid amplification curve shows a smooth, exponential increase. Refer to the operator manual for recommended actions.

LIMITATIONS

1. Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.
2. The 3M Integrated Cycler Studio retains the last valid calibration file to quantify unknown patient specimens. The Quantitation Standards and the patient specimens must be extracted using the same extraction methodology or you may receive erroneous results.
3. When monitoring a patient the extraction method used to prepare the specimen initial determination must be used for all subsequent determinations.
4. All results from this and other tests must be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
5. The prevalence of infection will affect the test’s predictive value.
6. As with other tests, negative results do not rule out BKV infection.
7. False-negative results may occur when the infecting organism has genomic mutations, insertions, deletions, or rearrangements or when performed very early in the course of illness.
8. False-negative results may occur if inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling.
9. As with other tests, false-positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.
10. This test cannot rule out diseases caused by other bacterial or viral pathogens.
11. The performance of this test has not been established for screening of urine or plasma for the presence of BKV.
12. This test is not intended for use as a screening or diagnostic test for the presence of BKV in urine or plasma.
13. The performance of this test has not been established with potentially interfering endogenous or exogenous substances.
PERFORMANCE CHARACTERISTICS

METHOD COMPARISON

One internal testing site participated in the Clinical Agreement Study. Reference results were generated using a high performance laboratory developed test (LDT). 150 Plasma and 125 Urine samples submitted for BKV detection or quantitation were tested in the study. Specimens were extracted using the MagNA Pure extraction method. The specimens included in the analysis (75 Plasma and 55 Urine) were within the combined reportable range of the reference LDT and the Simplexa BKV assay. The Passing-Bablok regression yielded a slope of 1.05 and an intercept of -0.83 for Plasma and a slope of 1.05 and an intercept of -0.82 for Urine.

Clinical samples with results in the linear range of the Simplexa BKV assay were also tested with easyMAG extraction. These samples performed equivalently to samples extracted with MagNA Pure extraction (slope of 0.98 and an intercept of -0.03 for Plasma and a slope of 1.06 and an intercept of -0.4 for Urine).
ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) was calculated by creating contrived samples from a strain of quantified BKV stock spiked into clinical negative plasma and urine matrices. The panel included negatives (unspiked urine and plasma matrices) and samples of varying concentrations, serially diluted, around the approximate LoD (obtained in an earlier phase of testing). For each combination of extraction system (easyMAG, MagNA Pure) and sample type (Plasma, Urine); twenty four (24) replicates from three (3) different extractions and PCR runs at each level were analyzed with Probit Analysis to determine the lowest concentration which could accurately be detected with 95% probability. The LoD protocol was run for each of the two extraction methods and sample types. The individual LoD values are shown in the table below. The LoD was determined to be 510 copies/mL, based on the highest LoD observed across all extraction methods and sample types.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MagNA Pure</td>
<td>easyMag</td>
</tr>
<tr>
<td>Copies/mL</td>
<td>510</td>
<td>385</td>
</tr>
</tbody>
</table>

ANALYTICAL REACTIVITY / CROSS REACTIVITY

Studies have indicated that the primers are specific for BKV and did not cross react with other microorganisms including HIV-1, HIV-2, HSV-1, HSV-2, HHV-6, JCV, VZV, EBV, CMV, Staphylococcus saprophyticus, Enterococcus, Candida, Enterobacter, Citrobacter, E.coli, Klebsiella and Proteus mirabilis. Furthermore, nucleic acid sequence databases indicated that the primers were specific for BKV, and that they did not have significant homology with other pathogens or with human DNA.

INTERFERENCE

The performance of this assay has not been evaluated with potentially interfering substances. The automated nucleic acid extraction process; using either the MagNA Pure system or the NucliSENS easyMAG effectively removes impurities from the specimen as it isolates and washes the nucleic acids during the extraction process. Internal controls alert the end user to potential PCR inhibition; if the target and the internal control are undetected the assay is invalid.

REPRODUCIBILITY

The Reproducibility Study was performed by two operators on two integrated cycler instruments (one operator per instrument) doing two runs per day for five days. The reproducibility panel consisted of controls (NTC, HPC and LPC), quantitation standards (QS-1 to QS-5), negative pools (unspiked urine and plasma matrices) and positive BKV virus pools in urine and plasma matrices at three different concentrations, namely a low pool (spiked at a concentration of approximately 2 to 4 times Limit of Detection (LoD)), a mid pool (approximately 8 to 10 times LoD) and a high pool (upper range of the assay). Four replicates of each panel sample were run for a total of 80 determinations for each panel member for the study. Reproducibility results for each panel sample are presented in the table below.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Expected Concentration (Copies/mL)</th>
<th>Geometric Mean (Copies/mL)</th>
<th>Mean Log (Copies/mL)</th>
<th>No. of Measurable Results*</th>
<th>Between Instrument</th>
<th>Between Day</th>
<th>Between Run</th>
<th>Within Run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTC</td>
<td>0</td>
<td>Not Detected</td>
<td>Not Detected</td>
<td>0</td>
<td>Not applicable (N/A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPC</td>
<td>5.08 x 10⁷</td>
<td>4.81 x 10⁷</td>
<td>7.682</td>
<td>74</td>
<td>0.026</td>
<td>0.012</td>
<td>0.025</td>
<td>0.024</td>
<td>0.045</td>
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<tr>
<td>LPC</td>
<td>6.32 x 10⁵</td>
<td>6.44 x 10⁵</td>
<td>3.809</td>
<td>80</td>
<td>0.014</td>
<td>0.034</td>
<td>0.015</td>
<td>0.045</td>
<td>0.060</td>
</tr>
<tr>
<td>QS-1</td>
<td>4.70 x 10⁶</td>
<td>&gt;1 x 10⁸</td>
<td>N/A</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QS-2</td>
<td>5.29 x 10⁶</td>
<td>5.35 x 10⁶</td>
<td>6.728</td>
<td>79</td>
<td>0.016</td>
<td>0.021</td>
<td>0.024</td>
<td>0.024</td>
<td>0.043</td>
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<tr>
<td>QS-3</td>
<td>2.74 x 10⁶</td>
<td>5.56 x 10⁶</td>
<td>4.745</td>
<td>80</td>
<td>0.000</td>
<td>0.021</td>
<td>0.014</td>
<td>0.021</td>
<td>0.032</td>
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<tr>
<td>QS-4</td>
<td>5.83 x 10⁵</td>
<td>5.89 x 10⁵</td>
<td>3.770</td>
<td>80</td>
<td>0.005</td>
<td>0.025</td>
<td>0.017</td>
<td>0.047</td>
<td>0.056</td>
</tr>
<tr>
<td>QS-5</td>
<td>1.05 x 10⁵</td>
<td>1.02 x 10⁵</td>
<td>3.007</td>
<td>80</td>
<td>0.008</td>
<td>0.031</td>
<td>0.000</td>
<td>0.083</td>
<td>0.089</td>
</tr>
</tbody>
</table>
### Plasma

<table>
<thead>
<tr>
<th>Pool</th>
<th>Copies/mL</th>
<th>copies/mL Range</th>
<th>MagNA Pure</th>
<th>easyMag</th>
<th>MagNA Pure</th>
<th>easyMag</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Pool</td>
<td>1.00 x 10^7</td>
<td>9.11 x 10^6</td>
<td>6.960</td>
<td>0.016</td>
<td>0.009</td>
<td>0.026</td>
</tr>
<tr>
<td>Mid Pool</td>
<td>5.00 x 10^3</td>
<td>4.71 x 10^3</td>
<td>3.673</td>
<td>0.022</td>
<td>0.050</td>
<td>0.025</td>
</tr>
<tr>
<td>Low Pool</td>
<td>2.00 x 10^3</td>
<td>1.44 x 10^3</td>
<td>3.158</td>
<td>0.063</td>
<td>0.050</td>
<td>0.000</td>
</tr>
<tr>
<td>Neg Pool</td>
<td>0</td>
<td>Not Detected</td>
<td>Not Detected</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

### Urine

<table>
<thead>
<tr>
<th>Pool</th>
<th>Copies/mL</th>
<th>copies/mL Range</th>
<th>MagNA Pure</th>
<th>easyMag</th>
<th>MagNA Pure</th>
<th>easyMag</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Pool</td>
<td>1.00 x 10^7</td>
<td>5.75 x 10^6</td>
<td>6.760</td>
<td>0.000</td>
<td>0.104</td>
<td>0.014</td>
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<tr>
<td>Mid Pool</td>
<td>5.00 x 10^3</td>
<td>2.87 x 10^3</td>
<td>3.458</td>
<td>0.000</td>
<td>0.061</td>
<td>0.022</td>
</tr>
<tr>
<td>Low Pool</td>
<td>2.00 x 10^3</td>
<td>1.12 x 10^3</td>
<td>3.048</td>
<td>0.088</td>
<td>0.049</td>
<td>0.000</td>
</tr>
<tr>
<td>Neg Pool</td>
<td>0</td>
<td>Not Detected</td>
<td>Not Detected</td>
<td>0</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* Number of results within the linearity range of the Simplex BKV assay.

## LINEARITY

The linearity was determined using samples contrived from a strain of quantified BKV stock spiked into clinical negative urine and plasma matrices. The panel consisted of 10 pools of known copies, across the expected linear range. Of the pools, at least 3 concentrations were near the Lower Limit of Quantitation (LLOQ), 2 near the Upper Limit of Quantitation (ULOQ) and the remaining pools were distributed approximately evenly between the LLOQ and ULOQ. Each sample was assayed randomly, in at least 3 replicates. The Linearity protocol was run for each sample type on each of the two extraction methods. The individual reportable range values are shown in the table below.

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure</td>
<td>easyMag</td>
<td>MagNA Pure</td>
</tr>
<tr>
<td>copies/mL</td>
<td>250 to 1.00 x 10^8</td>
<td>250 to 1.00 x 10^8</td>
</tr>
</tbody>
</table>

Simplex BKV Linearity for Plasma - MagNA Pure

Regression Equation:

\[ \text{Log Simplex BKV} = 0.885935 + 0.867903 + \text{Log Expected BKV} \]
Simplex BKV Linearity for Urine - MagNA Pure

Regression Equation:
\[
\log_{10}\text{Simplex BKV} = 8.89686 + 1.08433 \cdot \log_{10}\text{Expected BKV}
\]

Simplex BKV Linearity for Plasma - easyMAG

Regression Equation:
\[
\log_{10}\text{Simplex BKV} = 0.21313 + 0.88520 \cdot \log_{10}\text{Expected BKV}
\]
REPORTABLE RANGE

All extraction methods and sample types were linear up to $1 \times 10^8$ copies/mL. The reportable range of the assay was based on the LoD since it was higher than the linearity. This was determined as $>510$ copies/mL to $<1 \times 10^8$ copies/mL. Samples greater than the linear range will be reported as $>1 \times 10^8$ copies/mL and samples below 510 will be reported as <510.

CARRY-OVER CONTAMINATION

The amplification carry-over has been evaluated for the instrument and Universal Disc using other assays. The studies searched for the presence of contamination in high negative samples. Each study was designed by alternately placing a high positive and a high negative sample on each disc. The carryover effect was evaluated by comparing the observed negative rate for the high negative sample with the expected rate under normal reproducibility conditions. No carry-over contamination effect was seen in previous testing.

REFERENCES

7. AU Dei R; Marro F; Corte D; Sampietro MG; Franceschini E; Urbano P
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