BKV Quant Assay (Panther Fusion™)

For *in vitro* diagnostic use

For US export only

CONTENTS

General Information

Intended Use

The Panther Fusion™ BKV Quant assay is a fully automated real-time PCR (RT-PCR) *in vitro* nucleic acid amplification test for the quantitation of human BK virus (BKV) DNA in human plasma and urine samples.

The Panther Fusion BKV Quant assay is intended for use to aid in the diagnosis and to aid in the management of solid-organ transplant patients and in hematopoietic stem cell transplant patients.

The Panther Fusion BKV Quant assay is not intended for use as a screening assay for the presence of BKV in plasma or urine. This assay is designed for use on the Panther Fusion system.

Summary and Explanation of the Test

BKV is a highly prevalent small non-enveloped virus with a closed circular double stranded DNA genome. BKV is a human polyomavirus which belongs to the papovaviridae family.

Primary exposure to BKV occurs in childhood resulting in 80 to 90% of adults having developed antibodies against BKV. The majority of primary BKV infections are asymptomatic or minimally symptomatic. After primary infection the virus is thought to remain latent in the urinary tract without disease manifestation in immunocompetent individuals.[1]

Viral reactivation occurs in immunocompromised individuals and occurs frequently in renal transplant patients and hematopoietic stem cell transplant (HSCT) patients. In renal transplant patients, BKV reactivation is associated with nephropathy (BKVN) and ureteral stenosis, BKVN occurs in approximately 5% of renal transplant patients within one year of transplantation. BKV reactivation is important for HSCT recipients with late onset haemorrhagic cystitis occurring in 6% to 29% of patients within 2 months of transplantation.[2]

Quantitative nucleic acid amplification testing from plasma or urine specimens is an important laboratory marker for the diagnosis and monitoring of BKV infection in transplant recipients. Recent guidelines recommend that kidney transplant patients should be regularly screened for BKV DNA levels in plasma post-transplant to identify those patients considered for preemptive treatment for nephropathy. The risk of developing BKVN is increased when high levels of BKV DNA are observed in plasma or urine but can occur in patients with lower BKV levels.[3,4]

Principles of the Procedure

The Panther Fusion system fully automates specimen processing, including cell lysis, nucleic acid capture, amplification, and detection for the Panther Fusion BKV Quant assay. The Panther Fusion BKV Quant assay targets the highly conserved VP2 gene to ensure an accurate quantification of BKV DNA. The assay is standardized to the 1st WHO international standard (NIBSC code: 14/212) for BKV.[5]

Sample processing and nucleic acid capture: An internal control (IC-B) is added automatically to each specimen via the working Fusion Capture Reagent-B (wFCR-B) to monitor for interference during specimen processing, amplification, and detection caused by reagent failure or inhibitory substances. Specimens are first added to the Fusion Capture Reagent-B (FCR-B)

and Fusion Enhancer Reagent-B (FER-B) to release nucleic acid for hybridization to magnetic particles. The capture particles are then separated from residual specimen matrix in a magnetic field by a series of wash steps with a mild detergent. The captured nucleic acid is then eluted from the magnetic particles with a reagent of low ionic strength (Panther Fusion Elution Buffer).

Note: *The Panther Fusion system adds the IC-B to the FCR-B. After the IC-B is added to the FCR-B, it is referred to as wFCR-B.*

PCR amplification and fluorescence detection: Lyophilized single unit dose PCR master mix is reconstituted with the Panther Fusion Reconstitution Buffer I and then combined with the eluted nucleic acid into a reaction tube. Panther Fusion Oil reagent is added to prevent evaporation during the PCR reaction. PCR-based target amplification subsequently occurs with target-specific forward and reverse primers generating a fluorescence signal.

The Panther Fusion system provides a Ct value proportional to the BKV concentration in the test samples. The sample concentration is determined by the Panther Fusion system software using the BKV Ct values for each reaction and comparing them to the calibration curve. BKV results are reported in IU/mL and $\log_{10}$ IU/mL for both plasma and urine specimens. When the urine conversion factor is selected on the Panther Fusion software, a dilution factor of 2 is automatically applied to BKV viral load results to account for the dilution step during urine specimen processing.

The targets and the channels used for their detection on the Panther Fusion system are summarized in the table below:

Target	Gene Targeted	Instrument Channel
BKV	VP2	ROX
Internal Control	Not Applicable	Quasar 705

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. Carefully read the entire package insert and the *Panther/Panther Fusion System Operator's Manual* prior to performing this assay.

D. The Panther Fusion Enhancer Reagent-B (FER-B) is corrosive, harmful if swallowed, and causes severe skin burns and eye damage.

E. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

F. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[6]

G. Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

H. Use only supplied or specified disposable laboratory ware.

I. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

J. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

M. Do not use the reagents, calibrators, or controls after the expiration date. Do not use the Aptima™ urine specimen transport tube after its expiration date.

N. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* and *Panther Fusion System Test Procedure* for more information.

O. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

P. Avoid microbial and nuclease contamination of reagents.

Q. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

R. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic Technical Support if either occurs.

S. Do not use the fluid packs if the foil seal is not intact. Contact Hologic Technical Support if this occurs.

T. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

U. Some reagents of this kit are labeled with risk and safety symbols.

Note: *Hazard communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.*

EU Hazard Information	
	Panther Fusion BKV Quant Assay Cartridge ***ALPHA-CYCLODEXTRIN 20-25%*** — H412 - Harmful to aquatic life with long lasting effects P273 - Avoid release to the environment P280 - Wear eye protection/ face protection
	**Panther Fusion Oil** ***POLYDIMETHYLSILOXANE 100%*** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
 	**Panther Fusion Enhancer Reagent-B (FER-B)** ***LITHIUM HYDROXIDE MONOHYDRATE 5-10%*** **DANGER** H302 - Harmful if swallowed H314 - Causes severe skin burns and eye damage P260 - Do not breathe dust/fume/gas/mist/vapours/spray P280 - Wear protective gloves/protective clothing/eye protection/face protection P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P310 - Immediately call a POISON CENTER or doctor/physician

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[1]	Opened Storage
Panther Fusion BKV Quant Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C[2]
Panther Fusion Capture Reagent-B (FCR-B)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-B (FER-B)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-B (IC-B)	2°C to 8°C	(In wFCR-B)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion BKV Quant Calibrators (1-5)	-15°C to -35°C	Single use vial	Not applicable-single use
Panther Fusion EBV–BKV Quant High Positive Control	-15°C to -35°C	Single use vial	Not applicable-single use
Panther Fusion EBV–BKV Quant Low Positive Control	-15°C to -35°C	Single use vial	Not applicable-single use
Panther Fusion Transplant Negative Control (III)	-15°C to -35°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[1] On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion BKV Quant assay cartridge, FCR-B, FER-B, and IC-B. On board stability for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer, and Panther Fusion Oil Reagent starts when the reagent pack is first used.

[2] If removed from the Panther Fusion system, store the assay cartridge in an air-tight container with desiccant at the recommended storage temperature.

B. Working Panther Fusion Capture Reagent-B (wFCR-B) and Panther Fusion Enhancer Reagent-B (FER-B) are stable for 60 days when capped and stored at 15ºC to 30ºC. Do not refrigerate.

C. Discard any unused reagents that have surpassed their stability.

D. Avoid cross-contamination during reagent handling and storage.

E. **Do not freeze reagents.**

F. **Do not re-freeze controls or calibrators.**

Specimen Collection, Processing, and Storage

Specimens – Clinical material collected from patient and placed in an appropriate transport system. For the Panther Fusion BKV Quant assay this includes urine specimens collected in primary container, the plasma specimens in tubes containing EDTA anticoagulants or plasma preparation tubes (PPTs).

Samples – Represents a more generic term to describe any material for testing on the Panther Fusion system including specimens, processed specimens transferred into an Aptima urine specimen transport tube, calibrators, and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

Note: *Only plastic secondary tubes are recommended for sample storage.*

A. Specimen Collection

1. Whole blood specimens collected in the following glass or plastic tubes may be used to prepare plasma:
   - Tubes containing EDTA anticoagulants
   - Plasma preparation tubes (PPTs)
2. Urine specimens should be collected in a cup.
   a. After collection, urine specimens in the primary collection container must be transferred within an hour at 30°C into the Aptima urine specimen transport tube.
   b. Before urine specimens can be tested, urine in the primary cup should be mixed thoroughly by inversion prior transfer into the Aptima urine specimen transport tube containing urine transport medium.
   c. Exactly 2000 µL of urine must be transferred to an Aptima urine specimen transport tube.
   d. Replace the cap and gently mix the sample for at least 5 seconds.

B. Specimen Processing

1. Plasma: Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Plasma can be prepared from either EDTA or PPT primary tubes. Separate the plasma from the pelleted red blood cells following the manufacturer's instructions for the tube used. Plasma can be tested on the Panther Fusion system in a primary tube or transferred to a secondary tube such as an Aptima Specimen Aliquot Tube (SAT).

   To ensure adequate sample volume, refer to the following table:

Table 1: Minimum Sample Volume

Tube (Size and Type)	Minimum Volume for 1 Replicate
Aptima Sample Aliquot Tube (SAT)	0.6 mL
12x75 mm	0.9 mL
13x100 mm	0.9 mL
13x100 mm with Gel	0.7 mL
16x100 mm with Gel	1.1 mL

If not tested immediately, plasma can be stored in accordance with the *Specimen Storage Conditions*. If transferred to a secondary tube, plasma may be frozen at -20°C or -70°C. Do not freeze plasma specimens in EDTA primary collection tubes.

2. Urine must be transferred within an hour at 30°C into pre-filled Aptima urine specimen transport tubes before being tested on the Panther Fusion system (see *Urine Specimen Handling* for specimen handling).

C. Specimen Storage Conditions

Specimens can be stored under one of the following conditions:

1. Plasma Stability
   - Unprocessed specimens are stable for 24 hours at 2°C to 30°C after centrifugation.
   - Unprocessed specimens are stable for 5 days at 2°C to 8°C after centrifugation.
   - Unprocessed and processed specimens are stable for 60 days at -20°C or -70°C after centrifugation.
2. Urine Specimen Stability
   - Processed specimens are stable for 24 hours at 2°C to 30°C.
   - Processed specimens are stable for 5 days at 2°C to 8°C.
   - Processed specimens are stable for 60 days at -20°C or -70°C.

Samples Onboard the Panther Fusion System

Plasma and processed urine samples may be left on the Panther Fusion system uncapped for up to 8 hours. Samples may be removed from the Panther Fusion system and tested as long as the total time onboard does not exceed 8 hours prior to the pipetting of the sample by the Panther Fusion system.

Specimen Transport

Maintain specimen storage conditions during transport as described under *Specimen Collection, Processing, and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther Fusion System

The Panther Fusion system is an integrated nucleic acid testing system that fully automates all steps necessary to perform various Panther Fusion assays from sample processing through amplification, detection, and data reduction.

Reagents and Materials Provided

Assay Packaging

Components	Part No.	Storage
Panther Fusion BKV Quant Assay Calibrators PCAL 1 qBKV, 3 per box PCAL 2 qBKV, 3 per box PCAL 3 qBKV, 3 per box PCAL 4 qBKV, 3 per box PCAL 5 qBKV, 3 per box	PRD-07234	-15°C to -35°C
Panther Fusion EBV–BKV Quant Assay Controls HPC High Positive Control tube, 5 per box LPC Low Positive Control tube, 5 per box NC III Transplant Negative Control tube, 5 per box	PRD-07158	-15°C to -35°C
Panther Fusion BKV Quant Assay Cartridge 96 Tests Panther Fusion qBKV assay cartridge, 12 tests, 8 per box	PRD-07232	2°C to 8°C
Panther Fusion Internal Control-B 960 Tests Panther Fusion Internal Control-B tube, 4 per box	PRD-06234	2°C to 8°C
Panther Fusion Extraction Reagent-B 960 Tests Panther Fusion Capture Reagent-B bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-B bottle, 240 tests, 4 per box	PRD-06232	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I, 960 Tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil Reagent 1920 Tests Panther Fusion Oil Reagent, 960 tests, 2 per box	PRD-04335	15°C to 30°C

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	303095
Panther Fusion Module	PRD-04173
Panther Fusion System	PRD-04172
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects**	303096 (5000 tests)
Tips, 1000 µL, filtered, liquid-sensing, conductive, and disposable: *Not all products are available in all regions. Contact your representative for region-specific information.*	901121 (10612513 Tecan) 903031(10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Panther Fusion Tube Trays, 1008 Tests, 18 trays per box	PRD-04000
Aptima Urine Specimen Transport Tubes *for processing urine specimens only*	105575 (100 pre-filled tubes per bag)
Replacement Hologic Solid Caps (single-use tube cap)	PRD-06720 (100 caps per bag)
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable powderless gloves	—
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tubes (EDTA and PPT) options: 13 mm x 100 mm 12 mm x 75 mm 16 mm x 100 mm	—
Centrifuge	—
Vortex mixer	—

*Needed only for Panther Aptima TMA assays.

Optional Materials

Material	Cat. No.
Secondary tube options: 12 mm x 75 mm 13 mm x 100 mm 16 mm x 100 mm	 — — —
Aptima Specimen Aliquot Tubes (SATs) (100 pack)	503762
Transport tube cap (100 pack) *cap for SAT*	504415
Aptima Specimen Diluent	PRD-03003
Aptima Specimen Diluent Kit *contains Aptima Specimen Diluent, 100 SATs and 100 caps*	PRD-03503
Transfer pipets	—
Tube rocker	—

Panther Fusion System Test Procedure

Note: *Refer to the Panther/Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (Step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (Step A.1).

B. Calibrators and Controls Preparation

Allow the calibrators and controls to reach 15°C to 30°C prior to processing as follows:

1. Remove the calibrators and controls from storage (-15°C to -35°C) and place at 15°C to 30°C. Throughout the thawing process, gently invert each tube to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Option. Calibrator and control tubes may be placed on a tube rocker to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Note: *Avoid creating excessive foam when inverting the calibrators and controls. Foam compromises the level-sensing by the Panther Fusion system.*
2. When the tube contents have thawed, dry the outside of the tube with a clean, dry disposable wipe.
3. To prevent contamination, do not open the tubes at this time.

C. Reagent Preparation

1. Remove the bottles of IC-B, FCR-B, and FER-B from storage.
2. Mix FCR-B until the beads are fully suspended. Avoid creating foam during this step.
3. Open the bottles of IC-B, FCR-B, and FER-B, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
4. Place the IC-B, FCR-B, and FER-B bottles in the appropriate positions on the TCR carousel.
5. Close the TCR door.

Note: *The Panther Fusion system adds the IC-B to the FCR-B. After the IC-B is added to the FCR-B, it is referred to as wFCR-B (working FCR-B). If the wFCR-B and FER-B are removed from the system, use new caps and immediately store according to the proper storage conditions.*

D. Specimen Handling

Note: *Prepare specimens per the instructions in the Specimen Collection, Processing, and Storage section before loading specimens onto the Panther Fusion system.*

Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

E. Plasma Specimen Handling

1. Ensure that processed specimens in primary tubes or undiluted specimens in secondary tubes are stored properly per *Specimen Collection, Processing, and Storage*.
2. Ensure frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
3. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther Fusion System* for additional onboard information.
4. Ensure each primary or secondary tube contains adequate specimen. Refer to Table 1 for minimum sample volume for 1 replicate.
5. Just prior to loading specimens into a Sample Rack, centrifuge each specimen at 1000 to 3000*g* for 10 minutes. Do not remove caps at this step.

   See Step G.2 below, for information about loading the rack and removing the caps.

F. Urine Specimen Handling

1. Ensure that specimens in primary tubes or processed specimens in Aptima urine specimen transport tubes are stored properly per *Specimen Collection, Processing, and Storage*.
2. Ensure frozen specimens in Aptima urine specimen transport tubes are thoroughly thawed. Allow the specimens to reach 15°C to 30°C prior to testing on the Panther Fusion system. See *Samples Onboard the Panther Fusion System* for additional onboard information.

   Note: *Avoid loading specimens that contain precipitates onto the Panther Fusion system.*
3. Gently invert the Aptima urine specimen transport tubes at least 3 times, or mix gently on a rocker, until urine is homogeneous.

Note: *Avoid creating excessive foam when inverting or mixing the tubes. Foam may compromise the level-sensing by the Panther Fusion system.*

See Step G.2 below, for information about loading the rack and removing the caps.

G. System Preparation

1. For instructions on setting up the Panther Fusion system including loading samples, reagents, assay cartridges and universal fluids, refer to the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes.*
2. Load samples into the Sample Rack. Perform the following steps for each sample tube (specimen, and, when necessary, calibrators, and controls):
   a. Loosen one sample tube cap, but do not remove it yet.

      Note: *Be especially careful to avoid contamination by the spread of aerosols. Gently loosen caps on samples.*

   b. Load the sample tube into the Sample Rack.
   c. Repeat Steps 2.a and 2.b for each remaining sample.
   d. After the samples have been loaded into the Sample Rack, remove and discard each sample tube cap in one Sample Rack. To avoid contamination, do not pass a cap over any other Sample Racks or sample tubes.
   e. If necessary, use a new, disposable transfer pipet to remove any bubbles or foam. Bubbles in the tube compromise the level-sensing by the Panther Fusion system.
   f. When the last cap has been removed, load the Sample Rack into the Sample Bay.

      Note: *If running other assays and sample types at the same time, secure the Sample Retainer prior to loading the Sample Rack into the Sample Bay.*

   g. Repeat Steps 2.a to 2.f for the next Sample Rack.

H. System Preparation: Applying the Urine Specimen Conversion Factor

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual.*
2. Load specimen rack.
3. Apply Urine Conversion Factor to assay test orders for urine specimens.

   Note: The Urine *Conversion Factor may be applied to an entire rack or a single test order.*

   To apply the Urine Conversion Factor to an entire rack of urine specimens:

   a. From the *Sample Rack Bay* screen, double-click the loaded rack of interest. The *Sample Rack Loading* screen appears for the selected rack.
   b. Select **Dilute All**.

The *Dilution Factor* window appears (Figure 1).

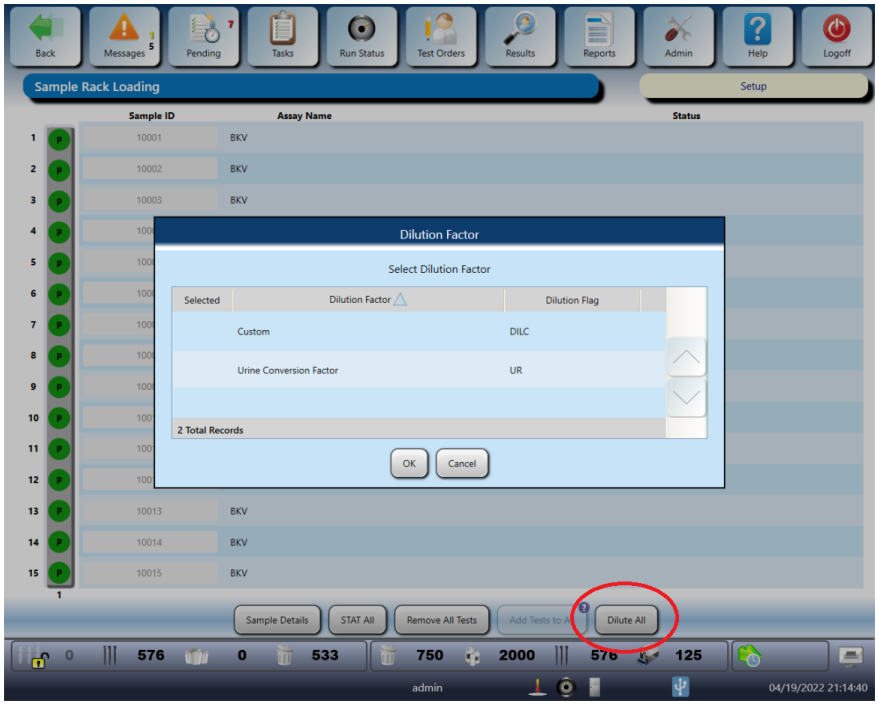

Figure 1. The Dilution Factor Window in the Sample Rack Loading Screen (Example)

c. Select **Urine Conversion Factor**.

d. Select **OK.**

   A *Set Dilution Factor for Rack* window appears.

e. Select **Yes** to apply the Urine Conversion Factor flag to the entire rack of urine specimens.

To apply the Urine Conversion Factor to a single test order (Figure 2):

a. From the *Sample Rack Bay* screen, double-click the loaded rack with the specimen(s) of interest.

   The *Sample Rack Loading* screen appears for the selected sample rack.

b. From the *Sample Rack Loading* screen, double-click the specimen of interest.

   The *Sample Details* screen appears with the current test orders for the selected specimen.

c. Select the test order of interest from the *Test Orders* panel.

d. Select **Apply Dilution**.

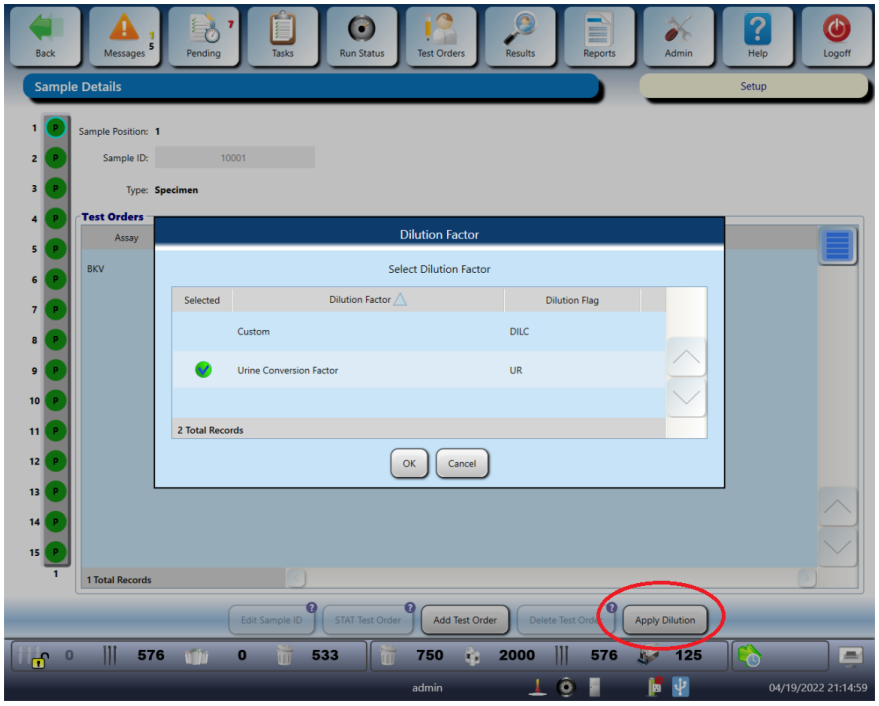

Figure 2. The Dilution Factor Window in the Sample Details Screen (Example)

e. Select **Urine Conversion Factor**.

f. Select **OK** to apply the Urine Conversion Factor flag to all selected test orders.

4. If necessary, the Urine Factor can be removed from test orders prior to the start of processing.

   To delete the Urine Conversion Factor from an entire rack:

   a. From the *Sample Rack Bay* screen, double-click the loaded rack of interest.

      The *Sample Rack Loading* screen appears for the selected rack.

   b. Select **Dilute All**.

   c. From the *Dilution Factor* window, de-select **Urine Conversion Factor**.

   d. Select **OK.**

      A *Set Dilution Factor for Rack* window appears.

   e. Select **Yes** to delete the Urine Conversion Factor from an entire rack.

   To delete the Urine Conversion Factor assay test orders:

   a. From the *Sample Rack Bay* screen, double-click the loaded rack with the specimen(s) of interest.

      The *Sample Rack Loading* screen appears for the selected sample rack.

   b. From the *Sample Rack Loading* screen, double-click the specimen of interest.

      The *Sample Details* screen appears with the current test orders for the selected specimen.

c. Select the test order of interest from the *Test Orders* panel.

d. Select **Apply Dilution**.

e. From the *Dilution Factor* window, de-select **Urine Conversion Factor**.

f. Select **OK** to delete the Urine Conversion Factor from the test order.

Procedural Notes

A. Calibrators and Controls

1. The qBKV calibrators (5 tubes), the EBV–BKV low positive control (LPC), the EBV–BKV high positive control (HPC), and the Transplant negative control (NC III) tubes can be loaded in any position in the Sample Rack and in any Sample Bay lane on the Panther Fusion system. Calibrator and control pipetting will begin when BKV specimens have been loaded onto the system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. The calibrators and controls are currently being processed by the system.
   b. Valid results for the calibrators and controls are registered on the system.
2. After the calibrator and control tubes have been pipetted and are being processed for the Panther Fusion BKV Quant assay, specimens can be tested. Calibration results are valid for 60 days and control results are valid for up to 30 days (frequency configured by an administrator) **unless:**
   a. The calibrator results are invalid.
   b. The control results are invalid.
   c. The operator requests to run new controls/calibrators in Panther Fusion system software.
3. A calibration is required for each new assay cartridge lot that is loaded onto the Panther Fusion system prior to using it for specimen processing.
4. Each calibrator and each control tube can be used once.

Quality Control

Assay Calibration

To generate valid results, assay calibration must be completed. The five positive calibrators are run in triplicate each time a new assay cartridge lot is loaded on the Panther Fusion system. Once established, the assay calibration is valid for up to 60 days. Software on the Panther Fusion system alerts the operator when calibration is required.

During processing, the Panther Fusion software automatically verifies the validity of the calibration curve. If the calibration fails the validity checks, the Panther Fusion system automatically invalidates any affected samples, and will require a new set of assay calibrators to be run prior to pipetting any additional samples.

By default, the assay will process samples as undiluted plasma. To process urine samples, the Urine Conversion Factor dilution must be selected from the instrument user interface.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the NC III (transplant negative control), the LPC (low positive control), and the HPC (high positive control) must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active cartridge lot has expired.

The Panther Fusion system is configured to require assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion system and a new set of assay controls will be required prior to pipetting any additional samples.

If any of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected samples, and a new set of assay controls will be required prior to pipetting any additional samples.

Internal Control

An internal control is added to each sample during the extraction process. During processing, the internal control acceptance criteria are automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for BKV. The internal control must be detected in all samples that are negative for BKV; samples that fail to meet that criterion will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther Fusion system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual*.

Interpretation of Results

The Panther Fusion system automatically determines the concentration of BKV DNA for specimens and controls by comparing the results to a calibration curve. BKV DNA concentrations are reported in IU/mL and $\log_{10}$ IU/mL. The interpretation of results is provided in Table 2 and Table 3.

Table 2: Plasma Result Interpretation

Reported BKV Quant Assay Results		
IU/mL	**$\log_{10}$ Value**	**Interpretation**
Not Detected	Not Detected	BKV DNA not detected.
< 79 detected	< 1.90	BKV DNA is detected but at a level below the lower limit of quantification (LLoQ).
79 to 1.0E09	1.90 to 9.00	BKV DNA concentration is within the quantitative range between LLoQ to ULoQ IU/mL.
> 1.0E09	> 9.00	BKV DNA concentration is above the upper limit of quantification (ULoQ).
Invalid[a]	Invalid[a]	There was an error in the generation of the result. Specimen should be retested.

[a] Invalid results are displayed in blue-colored font.

Table 3: Urine Result Interpretation

Reported BKV Quant Assay Results		
IU/mL	**$\log_{10}$ Value**	**Interpretation**
Not Detected	Not Detected	BKV DNA not detected.
< 162 detected	< 2.21	BKV DNA is detected but at a level below the lower limit of quantification (LLoQ).
162 to 2.0E09	2.21 to 9.30	BKV DNA concentration is within the quantitative range between LLoQ to ULoQ IU/mL.
> 2.0E09	> 9.30	BKV DNA concentration is above the upper limit of quantification (ULoQ).
Invalid[a]	Invalid[a]	There was an error in the generation of the result. Specimen should be retested.

[a] Invalid results are displayed in blue-colored font.

Limitations

A. Use of this assay is limited to personnel who are trained in this procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Panther Fusion BKV Quant assay may result in under quantification of or failure to detect the virus.

E. Negative results do not preclude BKV infections and should not be used as sole basis for treatment or other management decisions.

F. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

Performance

Limit of Detection Using the 1st WHO International Standard

The limit of detection (LoD) of the assay is defined as the concentration of BKV DNA that is detected at 95% or greater probability according to CLSI EP17-A2.[7]

Limit of Detection Using WHO Standards in Plasma

The LoD was determined by testing panels of the 1st WHO International Standard (NIBSC code 14/212) for BKV diluted in BKV-negative human plasma. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values shown in Table 4 are the results from the reagent lot with the highest predicted detection limit. The LoD for the Panther Fusion BKV Quant assay using the 1st WHO International Standard is 43.1 IU/mL for plasma.

Table 4: Limit of Detection for Plasma Using the 1st WHO International Standard for BKV

Predicted Detection Limit	Concentration (IU/mL)
10%	1.6
20%	2.1
30%	2.7
40%	3.5
50%	4.5
60%	6.1
70%	8.6
80%	13.3
90%	25.3
95%	43.1

Limit of Detection Using WHO Standards in Urine

The LoD was determined by testing panels of the 1st WHO International Standard for BKV diluted in BKV-negative human urine. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values shown in Table 5 are the results from the reagent lot with the highest predicted detection limit. The LoD for the Panther Fusion BKV Quant assay using the 1st WHO International Standard is 143.6 IU/mL for urine.

Table 5: Limit of Detection for Urine Using the 1st WHO International Standard for BKV

Predicted Detection Limit	Concentration (IU/mL)
10%	3.7
20%	6.0
30%	9.1
40%	13.0
50%	18.5
60%	26.2
70%	38.1
80%	58.1
90%	99.5
95%	143.6

Linear Range

Linear Range in Plasma

The linear range was established by testing panels of BKV diluted in BKV-negative human plasma according to CLSI EP06-A.[8] Panels ranged in concentration from 1.80 log IU/mL to 9.08 log IU/mL. The Panther Fusion BKV Quant assay demonstrated linearity across the range tested. The upper limit of quantitation (ULoQ) of the assay is 9.00 log IU/mL as shown in Figure 3.

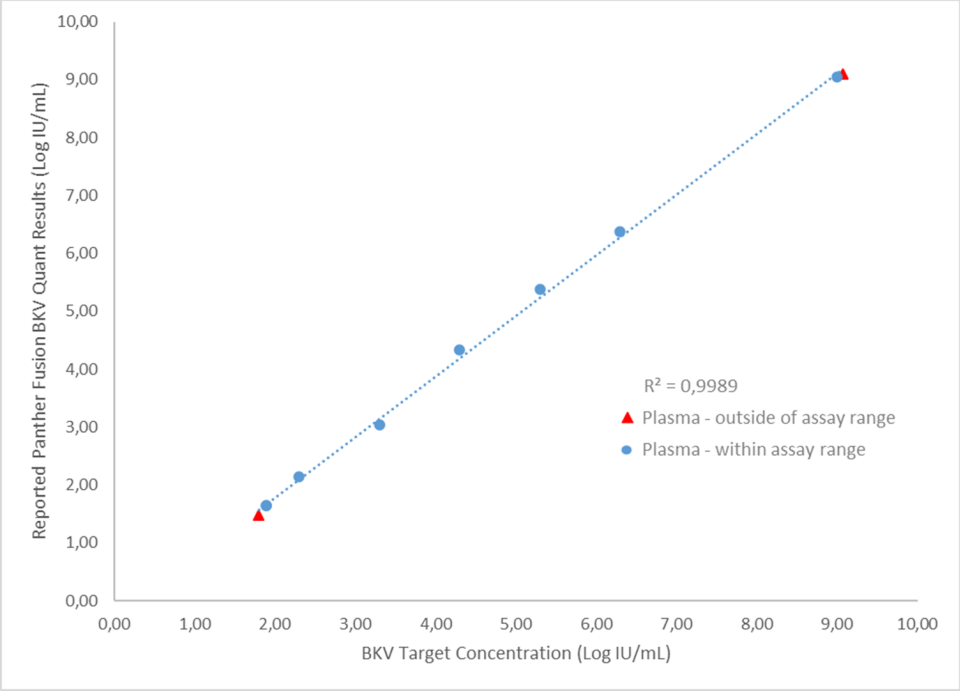

Figure 3. Linearity in Plasma

Linear Range in Urine

The linear range was established by testing panels of BKV diluted in BKV-negative human urine according to CLSI EP06-A.[8] Panels ranged in concentration from 2.11 log IU/mL to 9.38 log IU/mL. The Panther Fusion BKV Quant assay demonstrated linearity across the range tested. The upper limit of quantitation (ULoQ) of the assay is 9.30 log IU/mL as shown in Figure 4.

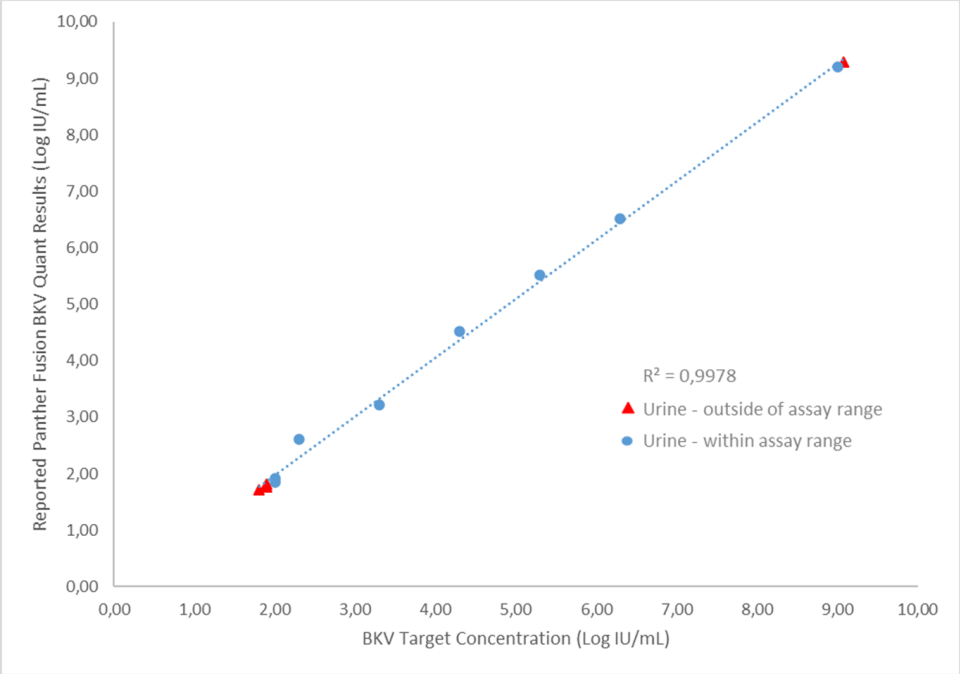

Figure 4. Linearity in Urine

Lower Limit of Quantitation Using the 1st WHO International Standard

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which BKV is reliably quantitated, according to CLSI EP17-A2.[7] Total error was estimated using the Westgard Model: Total Error (TE) = |bias| + 2 SD. To ensure precision and accuracy of the measurements, the Total Error of the Panther Fusion BKV Quant assay was set at 1.2 log IU/mL, with a bias to the truth and a SD that must be ≤ 0.5 log IU/mL and ≤ 0.35 log IU/mL, respectively.

Lower Limit of Quantitation Using the WHO Standard in Plasma

The LLoQ was determined by testing panels of the 1st WHO International Standard (NIBSC code 14/212) for BKV diluted in BKV-negative human plasma. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. The LLoQ results for the three reagent lots are shown in Table 6. The LLoQ generated with the 1st WHO International Standard for BKV in plasma is 79 IU/mL (1.90 log IU/mL).

Table 6: Determination of LLoQ Using the 1st WHO International Standard for BKV Diluted in Plasma

Reagent Lot	N	N Detected	Target Concentration (log IU/mL)	BKV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)	Calculated TE (log IU/mL
1	20	20	1.90	1.95	0.19	0.2	0.5
	20	20	2.06	2.09	0.14	0.1	0.4
	20	20	2.18	2.26	0.12	0.1	0.4
	20	20	2.26	2.35	0.15	0.2	0.5
2	20	20	1.90	1.96	0.14	0.1	0.4
	20	20	2.06	2.13	0.16	0.2	0.5
	20	20	2.18	2.24	0.16	0.1	0.4
	20	20	2.26	2.35	0.14	0.1	0.4
3	20	20	1.90	1.98	0.20	0.2	0.6
	20	20	2.06	2.06	0.15	0.1	0.4
	20	20	2.18	2.27	0.09	0.1	0.3
	20	20	2.26	2.35	0.11	0.1	0.4

SD=standard deviation ≤ 0.35 (log IU/mL).
|Bias|=bias to the truth ≤ 0.5 (log IU/mL).
The dilution corresponding to the LLoQ concentration and tested on each reagent lot is highlighted in gray.

Lower Limit of Quantitation Using the WHO Standard in Urine

The LLoQ was determined by testing panels of the 1st WHO International Standard (NIBSC code 14/212) for BKV diluted in BKV-negative human urine. Twenty (20) replicates of each dilution were tested with each of three reagent lots for a total of 60 replicates per dilution. The LLoQ results for the three reagent lots are shown in Table 7. The LLoQ generated with the 1st WHO International Standard for BKV in urine is 162 IU/mL(2.21 log IU/mL).

Table 7: Determination of LLoQ Using the 1st WHO International Standard for BKV Diluted in Urine

Reagent Lot	N	N Detected	Target Concentration (log IU/mL)	BKV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)	Calculated TE (log IU/mL
1	20	20	2.21	2.09	0.24	0.2	0.7
	20	20	2.26	2.15	0.20	0.2	0.5
	20	20	2.30	2.15	0.23	0.2	0.7
	20	20	2.38	2.27	0.20	0.2	0.6
2	20	20	2.21	1.98	0.22	0.2	0.7
	20	20	2.26	2.14	0.27	0.2	0.7
	20	20	2.30	2.23	0.20	0.2	0.6
	20	20	2.38	2.27	0.25	0.2	0.7
3	20	20	2.21	1.97	0.24	0.3	0.7
	20	20	2.26	2.03	0.22	0.3	0.7
	20	20	2.30	2.08	0.18	0.2	0.6
	20	20	2.38	2.13	0.23	0.3	0.7

SD=standard deviation ≤ 0.35 (log IU/mL).
|Bias|=bias to the truth ≤ 0.5 (log IU/mL).
The dilution corresponding to the LLoQ concentration and tested on each reagent lot is highlighted in gray.

Confirmation of the Lower Limit of Quantitation Across BKV Genotypes

Lower Limit of Quantitation Across Genotypes in Plasma

The LLoQ established using the WHO standard was assessed by testing BKV genotypes I (1b-2) and IV spiked at 3X the LLoQ in BKV-negative human plasma. Three replicates of each panel member were tested with one reagent lot. The results are shown in Table 8.

Table 8: Confirmation of LLoQ Across Genotypes in Plasma

Isolate (Genotype)	N	N Detected	Target Concentration (log IU/mL)	BKV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)
Genotype I (1b-2)	3	3	2.37	2.59	0.08	0.2
Genotype IV	3	3	2.37	2.25	0.05	0.1

SD=standard deviation.

Lower Limit of Quantitation Across Genotypes in Urine

The LLoQ established using the WHO standard was assessed by testing dilutions of BKV genotypes I (1b-2) and IV in BKV-negative human urine. Three replicates of each panel member were tested with one reagent lot. The results are shown in Table 9.

Table 9: Confirmation of LLoQ Across Genotypes in Urine

Isolate (Genotype)	N	N Detected	Target Concentration (log IU/mL)	BKV Quant Assay (log IU/mL)	SD (log IU/mL)	|Bias| (log IU/mL)
Genotype I (1b-2)	3	3	2.69	2.43	0.27	0.0
Genotype IV	3	3	2.69	2.57	0.16	0.2

SD=standard deviation.

Traceability to the 1st WHO International Standard

A series of secondary standards with known concentrations were used throughout product development and product manufacturing to establish traceability to the WHO standard. The BKV 1st WHO standard was diluted and tested along with the secondary standards, as well as assay controls, and calibrators used in the Panther Fusion BKV Quant assay to evaluate traceability according to CLSI EP32-R.[9] The secondary standards ranged in concentration from 2.30 to 6.30 $\log_{10}$ IU/mL.

Traceability to the WHO Standard Using Plasma

The concentrations tested for the BKV 1st WHO standard were between 2.07 and 4.70 log IU/mL. The WHO plasma panels, secondary standards, assay controls, and assay calibrators recovered as expected across the linear range of the assay, as can be seen from Figure 5.

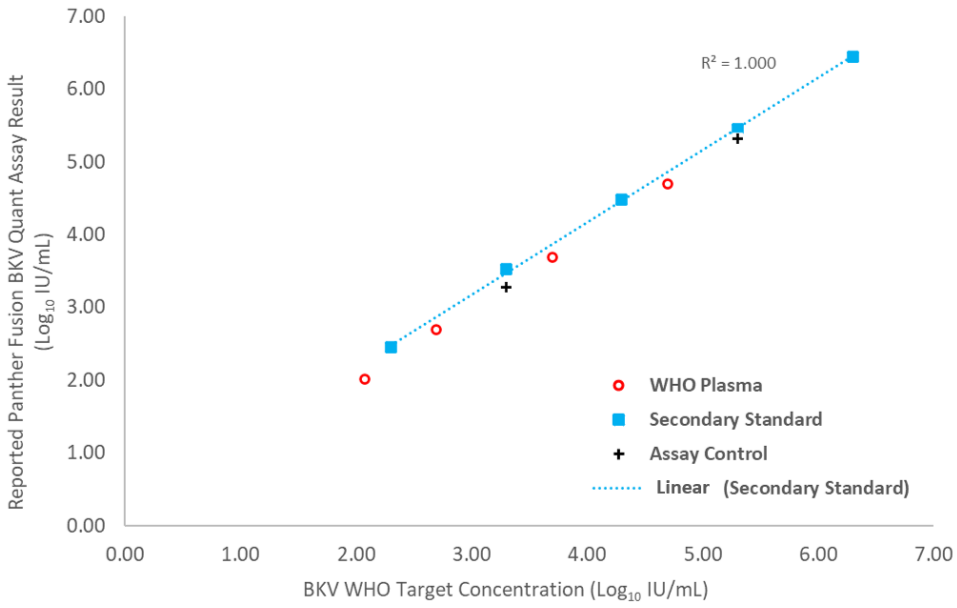

Figure 5. Traceability Between the BKV 1st WHO Standard Target Concentrations and Reported Concentrations in the Panther Fusion BKV Quant Assay (WHO Standard Diluted in Plasma)

Traceability to the WHO Standard Using Urine

The concentrations tested for the BKV 1st WHO standard in urine were between 2.38 and 5.00 log_{10} IU/mL. The WHO urine panels, secondary standards, assay controls, and assay calibrators recovered as expected across the linear range of the assay, as can be seen from Figure 6.

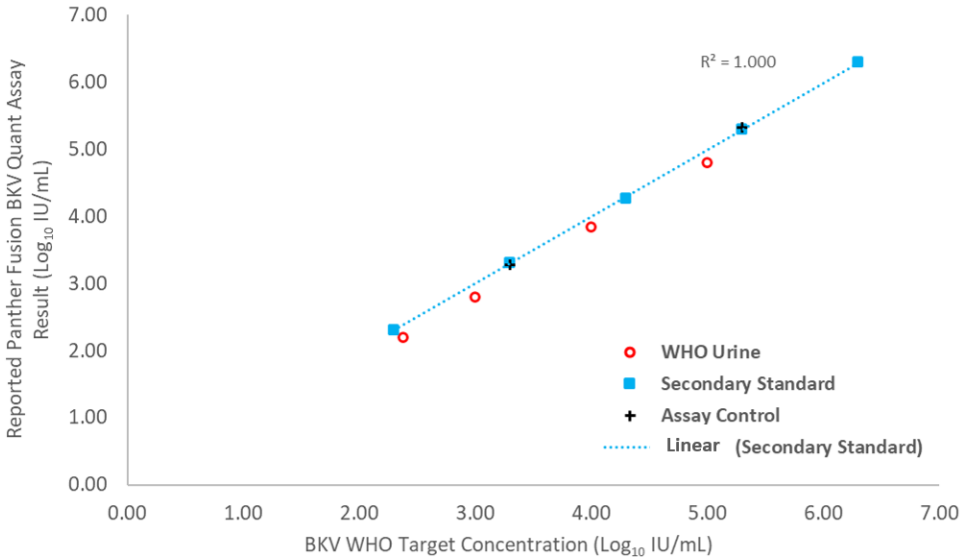

Figure 6. Traceability Between the BKV 1st WHO Standard Target Concentrations and Reported Concentrations in the Panther Fusion BKV Quant Assay (WHO Standard Diluted in Urine)

Within Lab Precision

Urine

To assess within lab precision, a negative panel and a 3-member panel were made by diluting BKV DNA into BKV-negative urine. The positive and negative panels were tested by 2 operators using 3 reagent lots on 3 Panther Fusion systems over 6 non-consecutive test days. Each operator performed 2 runs per day and each panel member was tested in triplicate in each run. The study was designed and analyzed following the recommendations of CLSI EP-05-A3.[10]

Table 10 shows the reproducibility of assay results (in log IU/mL) for the positive panel between instruments, operators, cartridge lots, runs, days, within runs, and overall. Total variability was primarily due to within-run variability (i.e., random error). All replicates of the negative panel were negative.

Table 10: Reproducibility of the Panther Fusion BKV Quant Assay in Urine

N	Mean Concentration (log IU/mL)	Inter-Lot	Inter-Instrument	Inter-Operator	Inter-Day	Inter-Run	Intra-Run	Total
		SD	SD	SD	SD	SD	SD	SD
54	2.59	0.06	0.08	0.02	0.08	0.14	0.13	0.16
54	3.55	0.08	0.04	0.01	0.02	0.05	0.25	0.10
54	4.58	0.07	0.01	0.01	0.01	0.06	0.28	0.07

SD=standard deviation.

Potentially Interfering Substances

The susceptibility of the Panther Fusion BKV Quant assay to interference by elevated levels of endogenous substances, anticoagulants, and drugs commonly prescribed to transplant patients was evaluated in BKV-negative matrices in the presence or absence of 2.37 log IU/mL and 2.69 log IU/mL of BKV in plasma and urine, respectively. The test concentrations for each of the interfering substances were selected based on available literature references and guidance provided by CLSI EP07[11] and EP37.[12]

No interference in the accuracy of quantification was observed in plasma or in urine samples in the presence of potential interfering substances listed in Table 11 and Table 12.

Table 11: Plasma Endogenous Substances

Potential Interfering Substance	Number of Replicates	Tested Concentration
Albumin	3	6000 mg/dL
Conjugated bilirubin	3	40 mg/dL
Hemoglobin	3	10 mg/dL
Human Genomic DNA	3	0.2 mg/dL
Triglycerides	3	3.45 mg/dL
Unconjugated bilirubin	3	40 mg/dL

Table 12: Urine Endogenous Substances

Potential Interfering Substance	Number of Replicates	Tested Concentration
Albumin	3	6000 mg/dL
Conjugated bilirubin	3	40 mg/dL
Estradiol	3	8E-05 mg/dL
Glucose	3	200 mg/dL
Mucin	3	6 mg/dL
Peripheral blood mononuclear cells	3	1E+06 cells/mL
pH, acidic (HCl)	3	2 mM
pH, alkaline (NaOH)	3	0.2 mM
Semen	3	5%
Whole blood	3	2%

No interference in the accuracy of quantification was observed in the presence of the exogenous substances listed in Table 13 and Table 14.

Table 13: Exogenous Substances for Plasma

Potential Interfering Substance	Number of Replicates	Tested Concentration
Acyclovir	3	6.6 mg/dL
Azathioprine	3	0.258 mg/dL
Cefotetan	3	71.1 mg/dL
Cidofovir	3	12.4 mg/dL
Clavulanate potassium	3	1.47 mg/mL
Cyclosporine	3	0.180 mg/dL
Everolimus	3	0.0183 mg/dL
Fluconazole	3	2.55 mg/dL
Foscarnet	3	108 mg/dL
Ganciclovir	3	3.96 mg/dL
Letermovir	3	3.9 mg/dL
Micafungin	3	6.6 mg/dL
Mycophenolate mofetil	3	18.1 mg/dL
Mycophenolate mofetil related compound B	3	18.1 mg/dL
Naproxen	3	36 mg/dL
Piperacillin	3	110 mg/dL
Prednisone	3	0.0099 mg/dL
Sirolimus	3	0.0213 mg/dL
Sulfamethoxazole	3	35.7 mg/dL
Tacrolimus	3	0.0144 mg/dL
Tazobactam sodium	3	10.2 mg/dL
Ticarcillin disodium	3	151 mg/dL
Trimethoprim	3	4.2 mg/dL
Valganciclovir	3	4.83 mg/dL
Vancomycin	3	12 mg/dL

Table 14: Exogenous Substances for Urine

Potential Interfering Substance	Number of Replicates	Tested Concentration
Acetaminophen	3	3 mg/dL
Acetylsalicylic acid	3	3 mg/dL
Clotrimazole	3	0.5 mg/dL
Ibuprofen	3	21.9 mg/dL
Metronidazole	3	12.3 mg/dL
Naproxen	3	36 mg/dL
Phenazopyridine hydrochloride	3	79.5 mg/dL
Propylene glycol	3	130 mg/dL
Talc	3	5 mg/dL

Analytical Specificity

Potential cross-reactivity to the pathogens listed in Table 15 was evaluated in BKV-negative matrices in the presence or absence of 2.37 log IU/mL and 2.69 log IU/mL of BKV in plasma and urine, respectively. Pathogens were tested at the highest concentration available. No cross-reactivity or interference in the accuracy of quantification was observed.

Table 15: Pathogens Tested for Analytical Specificity

Microorganism/Pathogen	Concentration	Microorganism/Pathogen	Concentration
ADV-5	1.00E+05 $TCID_{50}$/mL	Human Herpes Virus 7	1.00E+03 $TCID_{50}$/mL
Aspergillus niger	1.00E+06 CFU/mL	Human Herpes Virus 8	1.00E+05 $TCID_{50}$/mL
Bacillus cereus	1.00E+06 CFU/mL	*Klebsiella pneumoniae*	1.00E+06 cp/mL
Bacillus subtilis	1.00E+06 CFU/mL	*Lactobacillus acidophilus*	1.00E+06 CFU/mL
Candida albicans	1.00E+06 CFU/mL	*Lactobacillus crispatus*	1.00E+06 CFU/mL
Candida glabrata	1.00E+06 CFU/mL	*Listeria monocytogenes*	1.00E+06 CFU/mL
Candida parapsilosis	1.00E+06 CFU/mL	*Mycobacterium avium*	1.00E+06 CFU/mL
Candida tropicalis	1.00E+06 CFU/mL	*Mycoplasma pneumoniae*	1.00E+06 CCU/mL
Chlamydia trachomatis	1.00E+06 IFU/mL	*Neisseria gonorrhoeae*	1.00E+06 CFU/mL
Clostridium perfringens	1.00E+06 CFU/mL	Human Parvovirus B19	1.00E+05 IU/mL
CMV	1.00E+05 cp/mL	*Propionibacterium acnes*	1.00E+06 CFU/mL
Corynebacterium diphtheriae	1.00E+06 CFU/mL	*Proteus miribalis*	1.00E+06 CFU/mL
Cryptococcus neoformans	1.00E+06 CFU/mL	*Pseudomonas aeruginosa*	1.00E+06 CFU/mL
EBV	1.00E+05 cp/mL	*Salmonella enterica*	1.00E+06 CFU/mL
Enterobacter cloacae	1.00E+06 CFU/mL	*Staphylococcus aureus*	1.00E+06 CFU/mL
Enterococcus faecalis	1.00E+06 CFU/mL	*Staphylococcus epidermidis*	1.00E+06 CFU/mL
Enterococcus faecium	1.00E+06 CFU/mL	*Staphylococcus saprophyticus*	1.00E+06 CFU/mL
Escherichia coli	1.00E+06 CFU/mL	*Streptococcus agalactiae*	1.00E+06 CFU/mL

Table 15: Pathogens Tested for Analytical Specificity (continued)

Microorganism/Pathogen	Concentration	Microorganism/Pathogen	Concentration
HBV	1.00E+05 IU/mL	*Streptococcus bovis*	1.00E+06 CFU/mL
HCV	1.00E+04 IU/mL	*Streptococcus oralis*	1.00E+06 CFU/mL
HIV-1	1.00E+05 IU/mL	*Streptococcus pneumoniae*	1.00E+06 CFU/mL
HIV-2	1.00E+04 IU/mL	*Streptococcus pyogenes*	1.00E+06 CFU/mL
HSV-1	1.00E+06 $TCID_{50}$/mL	*Trichomonas vaginalis*	1.00E+05 Trophozoites/mL
HSV-2	1.00E+04 $TCID_{50}$/mL	*Ureaplasma urealyticum*	1.00E+06 cp/mL
HPV-16 (SiHa cells infected)	1.00E+05 cells/mL	Varicella Zoster Virus	1.00E+05 cp/mL
Human Herpes Virus 6	1.00E+05 cp/mL	—	—

CCU/mL=colony changing units/mL
CFU/mL=colony forming units per mL.
cp/mL=viral copies per mL.
IFU/mL=inclusion forming units per mL.
IU/mL=International units per mL.
$TCID_{50}$/mL=tissue culture infectious dose units per mL.

Method Correlation

This study was designed in accordance with CLSI EP09c.[13]

Plasma Method Correlation

The performance of the Panther Fusion BKV Quant assay was assessed against a comparator assay by testing retrospectively collected specimens and contrived specimens covering the entire linear range. A total of 108 specimens within the linear range common to both assays were used for the Deming regression as shown in Figure 7.

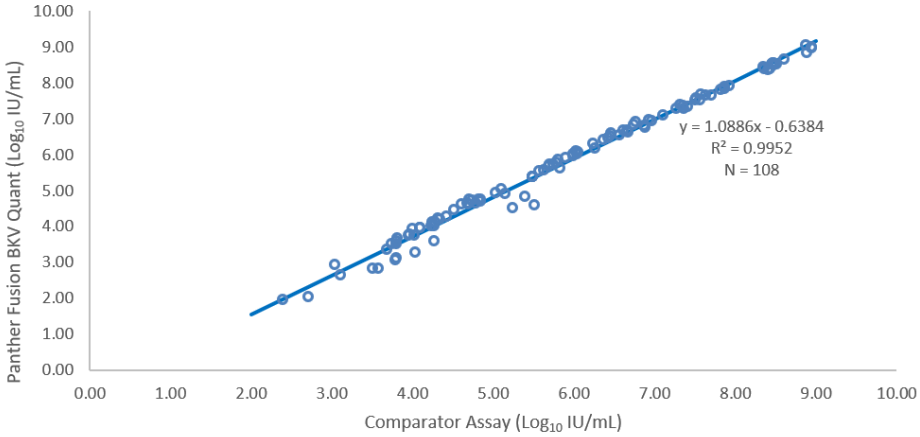

Figure 7. Correlation Between BKV Viral Load in the Panther Fusion BKV Quant Assay and the Comparator Assay on Testing Plasma Samples

Urine Method Correlation

The performance of the Panther Fusion BKV Quant assay was assessed against a comparator assay by testing retrospectively collected specimens and contrived specimens covering the entire linear range. A total of 153 specimens within the linear range common to both assays were used for the Deming regression as shown in Figure 8.

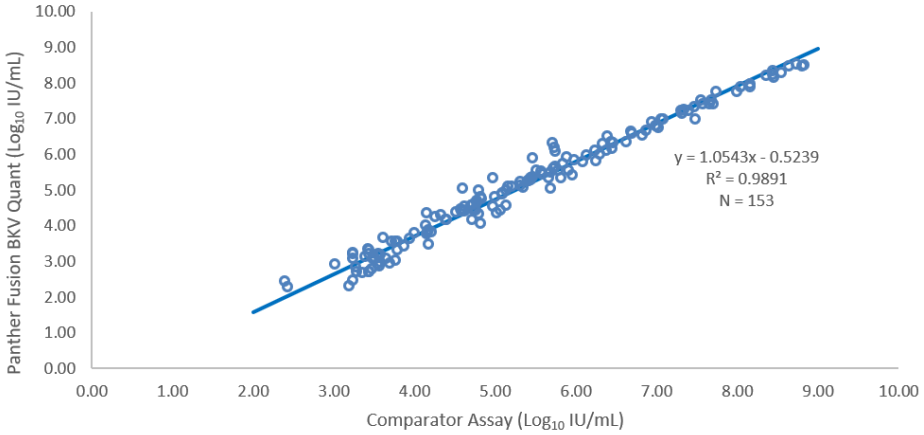

Figure 8. Correlation Between BKV Viral Load in the Panther Fusion BKV Quant Assay and the Comparator Assay on Testing Urine Samples

Carryover/Cross-contamination

Carryover was assessed using high-titer BKV-spiked STM samples (1.00E+09 IU/mL) interspersed between BKV-negative samples in a checkerboard pattern. Testing was carried out over 5 runs. The overall carryover rate was 0.00% (0/150).

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