Rx Only

cobas® BKV

Quantitative nucleic acid test
for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® BKV	P/N: 09040960190
For use on the cobas® 5800 System:	
cobas® EBV/BKV Control Kit	P/N: 09040951190
cobas® Buffer Negative Control Kit	P/N: 09051953190
For use on the cobas® 6800/8800 Systems:	
cobas® EBV/BKV Control Kit	P/N: 08688214190
	P/N: 09040951190
cobas® Buffer Negative Control Kit	P/N: 07002238190
	P/N: 09051953190

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Intended use

cobas® BKV is an in vitro nucleic acid amplification test for the quantitation of BK virus (BKV) DNA in human EDTA plasma and urine stabilized in **cobas®** PCR Media on the **cobas®** 5800/6800/8800 Systems.

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.

In urine stabilized in **cobas®** PCR Media, **cobas®** BKV is intended for use as an aid in the management of BKV in transplant patients.

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 decisions.

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 (HCT/Ps).

Summary and explanation of the test

Background

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.[1,2]

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%).[3] BKV seroprevalence is > 80% in the general healthy adult population.[4] In immunocompetent persons, BKV is not associated with significant pathology. However, BKV infection may cause severe clinical disease in immunocompromised persons, including transplant recipients.[5]

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.[5] 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.[1]

Guidelines recommend regular monitoring for BKV in kidney transplant patients for up to 5 years post transplant.[6] 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.[6] 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, a BKV DNA level greater than 10,000 IU/mL is associated with a higher risk for HC in transplant patients.[7]

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 level is at an undetectable level on two consecutive measurements.

Many laboratory tests for BKV quantitation are not standardized, leading to high inter-laboratory and inter-assay variability.[6,7] In addition, urine constituents may cause BKV aggregation, which may also impact quantitative variability.[8,9] Formal assessment of the reproducibility and validity of BKV DNA levels is critical to ensuring consistent results (regardless of which lab the assay was performed in) for the clinical management of patients with BKV related diseases.

While the exact medically relevant viral threshold is still a subject of debate due to inter-assay variability, the critical threshold concept appears valid and has been reported in natural history studies showing that higher BKV DNA levels are associated with increased risks for the development of PVN and HC.[6,7]

Rationale for NAT testing

Polyoma virus serology is not routinely used in clinical settings; it is only of value for determining whether a patient has been previously infected with BKV and is at risk of reactivation. Virus culture methods have a long turnaround time, and because they are semiquantitative, have limited use in immunocompromised patients where low levels of virus are common. Direct detection of BKV DNA by real-time PCR methods potentially offers a wide dynamic range, precision, and optimal sensitivity and specificity for use in transplant patients.

Explanation of the test

cobas® BKV is a quantitative test that is run on the **cobas**® 5800/6800/8800 Systems. **cobas**® BKV enables the detection and quantitation of BKV DNA in EDTA plasma and urine stabilized in **cobas**® PCR Media of infected patients. The BKV DNA level is quantified against a non-BKV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample processing. The DNA-QS also functions to monitor for the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

cobas® BKV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 5800 System is designed as one integrated instrument. 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**® 5800 or **cobas**® 6800/8800 System 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.

Nucleic acid from patient samples and added lambda DNA-QS molecules is 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.

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).[10-12] 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.

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.[13,14] 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.

Reagents and materials

cobas® BKV reagents and controls

The materials provided for **cobas**® BKV can be found in Table 1. Materials required, but not provided can be found in Table 2 through Table 4, and Table 8 through Table 10.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

Table 1 cobas® BKV

(BKV)

Store at 2-8°C
192 test cassette (P/N 09040960190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheets available on request. EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 mL
DNA Quantitation Standard (**DNA QS**)	Tris buffer, < 0.05% EDTA, < 0.001% non-BKV DNA construct containing non-BKV primer binding and a unique probe region (non-infectious DNA), 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
BKV Master Mix Reagent 2 (BKV MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP,dUTPs, < 0.01% upstream and downstream BKV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for BKV and the BKV Quantitation Standard, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 **cobas**® EBV/BKV Control Kit

(EBV/BKV CTL)

Store at 2–8°C

For use on the **cobas**® 5800 System (P/N 09040951190)

For use on the **cobas**® 6800/8800 Systems (P/N 08688214190 or P/N 09040951190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
EBV/BKV Low Positive Control (EBV/BKV L(+)C)	< 0.001% synthetic (plasmid) BKV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, BKV DNA not detectable by PCR methods. The mean concentration of BKV DNA is approximately 1600 fold lower than the mean concentration of BKV DNA in the EBV/BKV H(+)C. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5 mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
EBV/BKV High Positive Control (EBV/BKV H(+)C)	< 0.001% synthetic (plasmid) BKV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, BKV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5 mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance or mixture

Table 3 cobas® Buffer Negative Control Kit

(BUF (-) C)

Store at 2-8°C

For use on the **cobas**® 5800 System (P/N 09051953190)

For use on the **cobas**® 6800/8800 Systems (P/N 07002238190 or P/N 09051953190)

Kit components	Reagent ingredients	Quantity per kit
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas® omni reagents for sample preparation

Table 4 cobas® omni reagents for sample preparation

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning*
cobas® omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas® omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas® omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate**, 5% (w/v) polydocanol**, 2% (w/v) dithiothreitol**, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance or mixture

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5, Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 5800 or **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® BKV	2–8°C
cobas® EBV/BKV Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas® **omni** Lysis Reagent	2–8°C
cobas® **omni** MGP Reagent	2–8°C
cobas® **omni** Specimen Diluent	2–8°C
cobas® **omni** Wash Reagent	15–30°C

Reagent handling requirements for the cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The **s**ystem allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® BKV	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® EBV/BKV Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® Buffer Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

* Single use reagents.

Time is measured from the first time that reagent is loaded onto the **cobas® 5800 System.

Reagent handling requirements for the cobas® 6800/8800 Systems

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 7 Reagent expiry conditions enforced by the **cobas®** 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® BKV	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® EBV/BKV Control Kit	Date not passed	Not applicable*	Not applicable	Max 8 hours
cobas® Buffer Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 10 hours
cobas® omni Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® omni MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® omni Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® omni Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

* Single use reagents

Time is measured from the first time that reagent is loaded onto the **cobas® 6800/8800 Systems.

Additional materials required for the cobas® 5800 System

Table 8 Materials and consumables for use on the **cobas®** 5800 System

Material	P/N
cobas® omni Processing Plate 24	08413975001
cobas® omni Amplification Plate 24	08499853001
cobas® omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1 mL	04639642001
Tip CORE TIPS with Filter, 300 µL	07345607001
cobas® omni Liquid Waste Container	07094388001
cobas® omni Lysis Reagent	06997538190
cobas® omni MGP Reagent	06997546190
cobas® omni Specimen Diluent	06997511190
cobas® omni Wash Reagent	06997503190
Solid Waste Bag or Solid Waste Bag With Insert	07435967001 or 08030073001
cobas® omni Secondary Tubes 13x75 (optional)	06438776001
cobas® PCR Media Secondary Tube Kit	07958048190
cobas® PCR Media Tube Replacement Cap Kit	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 2001-2050*,**	03143449001
RD5 RACK – RD Standard rack 0001-0050 LR*,**	11902997001
16-position tube carrier*	09224319001
5-position rack carrier*	09224475001

* Contact your local Roche representative for a detailed order list for sample racks.

MPA 16mm rack or 16-position tube carrier are the preferred racks for use with samples collected in **cobas® PCR Media tubes.

Additional materials required for the cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **cobas®** 6800/8800 Systems

Material	P/N
cobas® omni Processing Plate	05534917001
cobas® omni Amplification Plate	05534941001
cobas® omni Pipette Tips	05534925001
cobas® omni Liquid Waste Container	07094388001
cobas® omni Lysis Reagent	06997538190
cobas® omni MGP Reagent	06997546190
cobas® omni Specimen Diluent	06997511190
cobas® omni Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer Solid Waste Update	07435967001 and 07094361001 or 08030073001 and 08387281001
cobas® omni Secondary Tubes 13x75 (optional)	06438776001
cobas® PCR Media Secondary Tube Kit	07958048190
cobas® PCR Media Tube Replacement Cap Kit (Optional)	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 7001-7050*,**	03143449001

* Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments

MPA 16mm rack are the preferred racks for use with samples collected in **cobas® PCR Media tubes.

Table 10 Urine specimen collection kits used with **cobas**® BKV

Collection Kit	P/N
cobas® PCR Urine Sample Kit	05170486190

Note: The **cobas**® PCR Urine Sample Kit is used to collect and transport urine specimens. Each **cobas**® PCR Urine Sample Kit contains 100 **cobas**® PCR Urine Sample Packets. Each Packet contains 1 disposable pipette and 1 **cobas**® PCR Media tube, containing 4.3 mL of **cobas**® PCR Media. **cobas**® PCR Media serves as a nucleic acid stabilizing transport and storage medium for urine specimens.
For urine samples directly sent to the laboratory without the use of **cobas**® PCR Urine Sample Kit at collection, the **cobas**® PCR Media kit containing 100 **cobas**® PCR Media tubes (without disposable pipettes) can be used as an alternative, given that urine must be transferred within 24 hours from collection.

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® BKV analysis package for the **cobas**® 5800 System must be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

The **cobas**® 6800/8800 System software and **cobas**® BKV analysis package must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 11 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for additional information.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments. **cobas**® BKV accepts the primary tube used for urine specimen types collected in **cobas**® PCR Media.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- **cobas**® BKV has not been evaluated 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 (HCT/Ps).
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[15,16] Only personnel proficient in handling infectious materials and the use of **cobas**® BKV and **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water or follow appropriate site procedures.
- **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.
- **Do not freeze whole blood or urine samples stored in primary tubes.**
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- **cobas**® PCR Media (from primary specimen tube) contains guanidine hydrochloride. **Do not allow direct contact between guanidine hydrochloride and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas.** If liquid containing guanidine hydrochloride is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, **FIRST** clean the affected area with laboratory detergent and water, and then with 0.6% sodium or potassium hypochlorite.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- 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.
- **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.

- **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.
- 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.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® BKV kits, EBV/BKV Low Positive Control (EBV/BKV L(+)C), EBV/BKV High Positive Control (EBV/BKV H(+)C), **cobas**® Buffer Negative Control Kits (BUF (-) C) and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 or **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® Systems – User Assistance and/or User Guides to properly clean and decontaminate the surface of instrument(s).

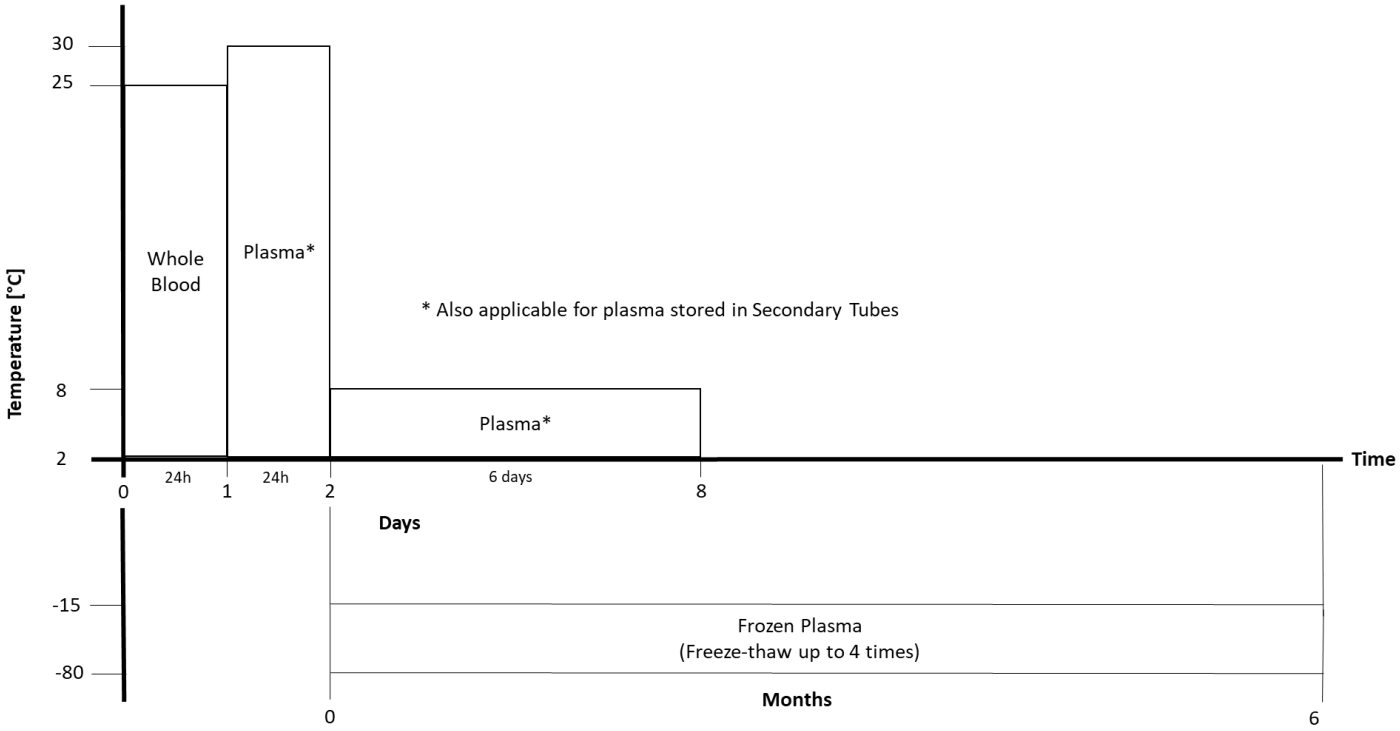
Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

EDTA plasma samples

- Store all samples at specified temperatures. Sample stability is affected by elevated temperatures.
- If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.
- 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. Refer to Figure 1.
- 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 2-25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation plasma samples may be stored for 24 hours at 2-30°C in primary or secondary tubes, followed by:
    - Storage in primary or secondary tubes for up to 6 days at 2-8°C.
    - Storage in secondary tubes for up to 6 months at -15°C to -80°C.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at -15°C to -80°C.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

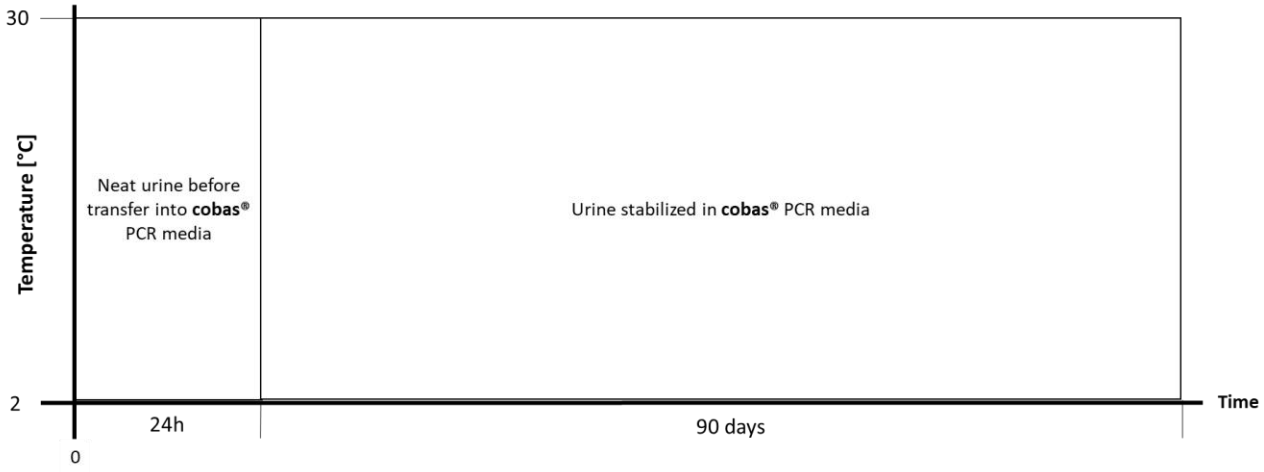
Figure 1 Sample storage conditions for EDTA plasma



Urine samples

- Use only the **cobas**® PCR Urine Sample Kit to collect and stabilize urine specimens for **cobas**® BKV. **cobas**® BKV has not been validated for use with other urine collection devices or media types. Using **cobas**® BKV with other urine collection devices or other media types may lead to false negative, false positive, and/or invalid results.
- Urine specimens must be transferred into the **cobas**® PCR Media tube (stabilized) immediately. If specimens cannot be transferred immediately, they can be stored at 2°C to 30°C for up to 24 hours.
  Once the urine samples are stabilized in **cobas**® PCR Media, samples may be stored for up to 90 days at 2-30°C. Refer to Figure 2.
- Untested urine specimens must show the top of the liquid level between the two black lines on the **cobas**® PCR Media tube label window. If the liquid level is above or below these lines, the specimen has not been collected properly and cannot be used for testing.
- If not enough volume of urine (4.3 mL) is available for diluting in the **cobas**® PCR Urine Sample tube, urine may be diluted manually with **cobas**® PCR Media. Before testing with **cobas**® BKV, at least 0.5 mL of neat urine must be manually diluted in **cobas**® PCR Media (1:1 ratio).
- To avoid cross contamination of processed specimens, additional caps for **cobas**® PCR Media tubes in an alternate color (neutral; see **Additional materials required**) should be used to recap specimens after testing.
- If additional testing is required, ensure that there is at least 1.2 mL of specimen remaining in the **cobas**® PCR Media tube.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Figure 2 Sample storage conditions for urine



Instructions for use

Procedural notes

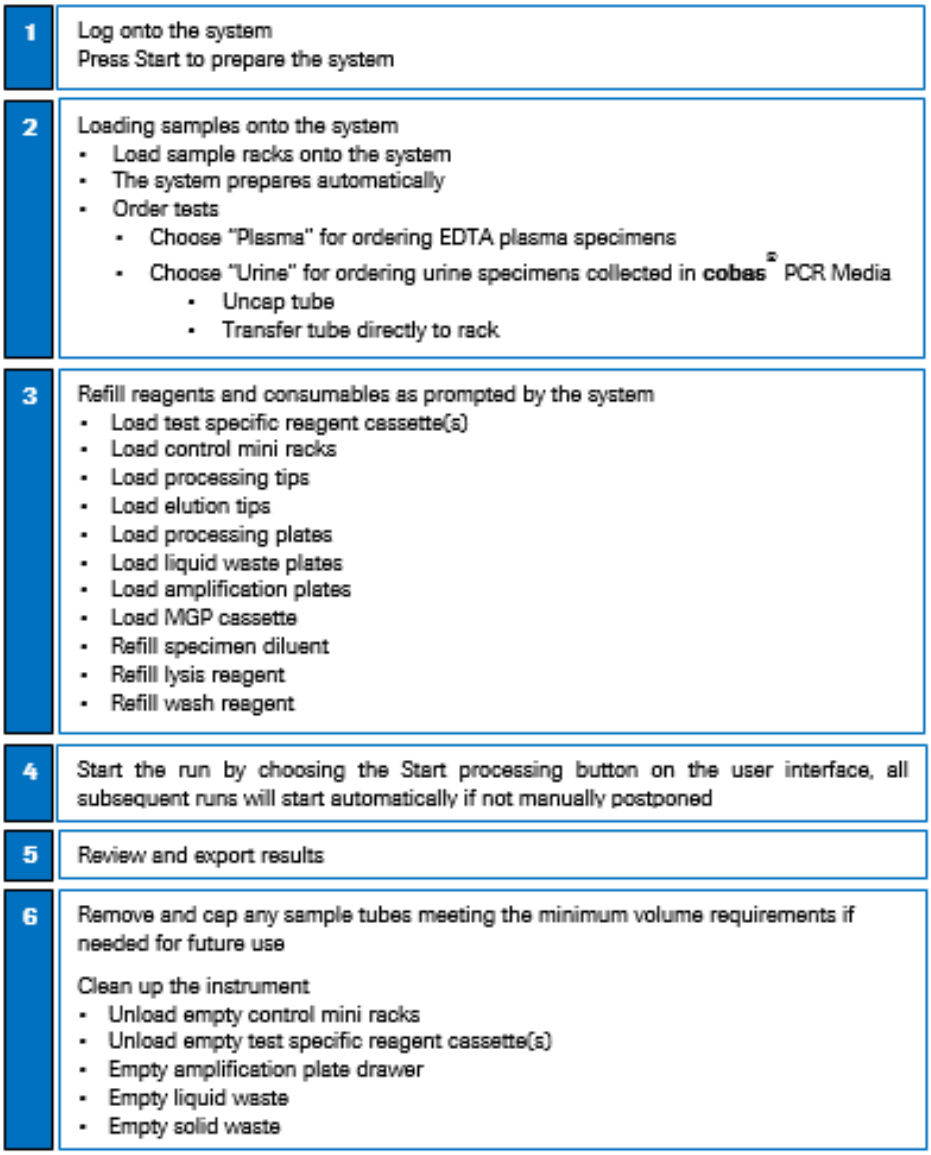
- Do not use **cobas**® BKV reagents, **cobas**® EBV/BKV Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas**® **omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of RD5 or MPA sample racks. Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems User Guides for proper barcode specifications and additional information on loading sample tubes.
- Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for proper maintenance of instruments.

Running cobas® BKV on the cobas® 5800 System

cobas® BKV can be run with a minimum required sample volume of 350 µL for EDTA plasma of which 200 µL sample is processed when using **cobas**® **omni** Secondary Tubes and 550 µL for stabilized urine specimens of which 400 µL sample is processed when using **cobas**® **omni** Secondary Tubes. The test procedure is described in detail in the **cobas**® 5800 Systems User Assistance and/or User Guide. Figure 3 below summarizes the procedure.

- Specimens must be uncapped and loaded directly onto racks for processing on the **cobas**® 5800 System.
- A single run can have a combination of specimens (plasma, stabilized urine).
- EDTA plasma and stabilized urine specimens should be processed using the sample type selection in the user interface (UI) of **cobas**® BKV as described in Figure 3, step 1.

Figure 3 **cobas**® BKV test procedure on the **cobas**® 5800 System

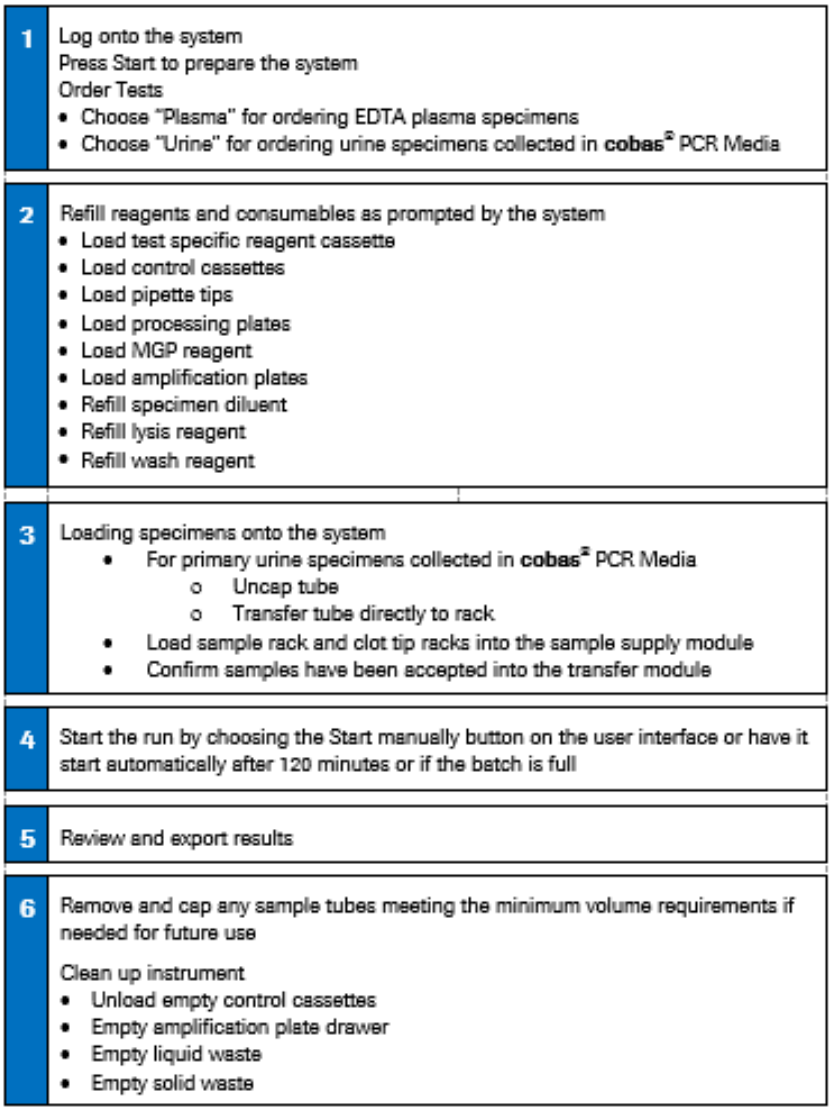


Running cobas® BKV on the cobas® 6800/8800 Systems

cobas® BKV can be run with a minimum required sample volume of 350 µL for EDTA plasma when using **cobas**® **omni** Secondary Tubes if 200 µL sample is processed and 550 µL for stabilized urine specimens when using **cobas**® **omni** Secondary Tubes if 400 µL sample is processed. The test procedure of the instrument is described in detail in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide. Figure 4 below summarizes the procedure.

- Specimens must be uncapped and loaded directly onto racks for processing on the **cobas**® 6800/8800 Systems.
- A single run can have a combination of specimens (plasma, stabilized urine).
- EDTA plasma and stabilized urine specimens should be processed using the sample type selection in the user interface (UI) of **cobas**® BKV as described in Figure 4, step 1.

Figure 4 **cobas**® BKV procedure on the **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800/6800/8800 Systems automatically determine the BKV DNA concentration for the samples and controls. The BKV DNA concentration is expressed in International Units per milliliter (IU/mL).

Quality control and validity of results on the cobas® 5800 System

- One negative control [(-) Ctrl] and two positive controls, a low positive control [EBV/BKV L (+) C] and a high positive control [EBV/BKV H (+) C] are processed at least every 72 hours and with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 software and/or report, check for flags and their associated results to ensure the result validity.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Control results on the cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with "Invalid" in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with "Invalid" show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view.
- If one of the controls is invalid, repeat testing of all controls and all associated samples is required.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One negative control [(–) Ctrl] and two positive controls, a low positive control [EBV/BKV L (+) C] and a high positive control [EBV/BKV H (+) C] is processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: EBV/BKV L (+) C, EBV/BKV H (+) C. The negative control result is displayed as (–) Ctrl and the low and high positive controls are displayed as EBV/BKV L (+) C and EBV/BKV H (+) C.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

Control flags on the cobas® 6800/8800 Systems

Table 12 Control flags for negative and positive controls

Negative Control	**Flag**	**Result**	**Interpretation**
(–) Ctrl	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	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
EBV/BKV H (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

If the control batch is invalid, repeat testing of all samples of affected batch.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas®** 5800 and **cobas®** 6800/8800 Systems software and/or reports. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.

Table 13 Target results for individual target result interpretation

Results	**Interpretation**
Target Not Detected	BKV DNA not detected. Report results as “BKV not detected”.
< Titer Min[a]	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “BKV detected, less than (Titer Min)”. EDTA plasma Titer Min = 21.5 IU/mL Urine Titer Min = 200 IU/mL
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as “(Titer) of BKV detected”.
> Titer Max[b]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “BKV detected, greater than (Titer Max)”. EDTA plasma and urine Titer Max = 1.0E+08 IU/mL

[a] Sample results < Titer min (Target Detected < LLoQ) should be interpreted with the context of other clinical data and should not be the sole basis for treatment decisions.

[b] Sample result > Titer Max refers to BKV positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired for EDTA plasma specimens, the original sample should be diluted with BKV-negative human EDTA plasma and the test should be repeated. Multiply the reported result by the dilution factor.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.

For a valid control batch, check each individual sample for flags in the **cobas**® 5800 software and/or report. The result interpretation should be as follows:

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
    - Q05D : Result validation failure because of an invalid positive control
    - Q06D :Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 13 above.

If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Interpretation of results on the cobas® 6800/8800 Systems

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as shown in Table 13 above.

Procedural limitations

- Recommendations regarding monitoring BKV viral load post-transplant and medically relevant BKV DNA thresholds vary among transplant type and transplant institutions.
- **cobas**® BKV test results should be interpreted in the context of other clinical data and should not be the sole basis for treatment decisions.
- **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**® 5800/6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma and stabilized urine. Testing of other sample types with **cobas**® BKV may result in inaccurate results. Plasma and stabilized urine DNA level measurements are not directly comparable to each other and to those of other sample types.
- Quantitation of BKV DNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Degradation of BKV DNA in neat urine can affect quantification.[17] Transfer of urine into **cobas**® PCR Media is required to achieve specimen stability.

- Quantitative variability of BKV DNA inherent to urine has been observed in specimen stability experiments at different sampling timepoints (neat urine) or in different aliquots of the same sample (neat urine and urine stabilized in **cobas**® PCR Media).
- Given these limitations, urine BKV DNA results should be interpreted with caution in context with clinical and other laboratory findings and should not be the sole basis for treatment decisions.
- Urine may contain high levels of BKV DNA, with the risk of carry over contamination.[18]
- 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.
- 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. Users should follow their own specific policies/procedures.
- 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.
- **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 (HCT/Ps).

Non-clinical performance evaluation

Key performance characteristics for EDTA plasma sample type performed on the cobas® 6800/8800 Systems

Limit of Detection (LoD)

The limit of detection (LoD) of **cobas**® BKV was determined by analysis of serial dilutions of the WHO International Standard (subgroup Ib) and verified for subgroups Ia, Ic and subtypes II, III and IV. The overall concentration for which 95% hit rate is expected by PROBIT is 21.5 IU/mL for EDTA plasma.

WHO International Standard

The limit of detection of **cobas**® BKV for the WHO International Standard was determined by analysis of serial dilutions of the 1st WHO BKV International Standard obtained from NIBSC (NIBSC 14/212), in BKV-negative human EDTA plasma. Panels of six concentration levels plus a blank were tested over three lots of **cobas**® BKV reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma are shown in Table 14 through Table 16. The study demonstrates that with the least sensitive lot, the concentration for which 95% hit rate is expected by PROBIT is 21.5 IU/mL with a 95% confidence range of 16.3 – 32.4 IU/mL in EDTA plasma. The lowest concentration with a hit rate ≥ 95% is 19.0 IU/mL in EDTA plasma.

Table 14 BKV DNA 1st WHO International Standard Limit of Detection in EDTA plasma, Lot 1

Input titer concentration (BKV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
80.0	63	63	100.0
38.0	63	63	100.0
19.0	63	60	95.2
9.5	63	46	73.0
4.75	63	36	57.1
2.38	63	23	36.5
0	62	0	0.0

LoD by PROBIT at 95% hit rate: 21.5 IU/mL. 95% confidence range: 16.3 – 32.4 IU/mL

Table 15 BKV DNA 1st WHO International Standard Limit of Detection in EDTA plasma, Lot 2

Input titer concentration (BKV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
80.0	62	62	100.0
38.0	63	63	100.0
19.0	63	61	96.8
9.5	63	48	76.2
4.75	63	34	54.0
2.38	62	23	37.1
0	62	0	0.0

LoD by PROBIT at 95% hit rate: 19.7 IU/mL, 95% confidence range: 15.0 – 29.2 IU/mL

Table 16 BKV DNA 1st WHO International Standard Limit of Detection in EDTA plasma, Lot 3

Input titer concentration (BKV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
80.0	63	63	100.0
38.0	63	63	100.0
19.0	63	60	95.2
9.5	63	50	79.4
4.75	63	35	55.6
2.38	63	22	35.0
0	63	0	0.0

LoD by PROBIT at 95% hit rate: 19.3 IU/mL, 95% confidence range: 14.8 – 28.5 IU/mL

Limit of Detection for subgroups Ia, Ic and Subtypes II, III and IV

BKV armored DNA for subgroup Ic and subtype III, and clinical specimens for subgroup Ia and subtypes II and IV were diluted to three different concentration levels in BKV-negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® BKV reagents.

The combined results from three lots shown in Table 17 verify that – consistent with an LoD of 21.5 IU/mL – **cobas**® BKV detected BKV DNA for subgroups Ia and Ic, and subtypes II, III and IV at a concentration of 21.5 IU/mL with a ≥ 95% hit rate.

Table 17 BKV DNA subgroups Ia, Ic and subtypes II, III and IV verification of limit of detection in EDTA plasma

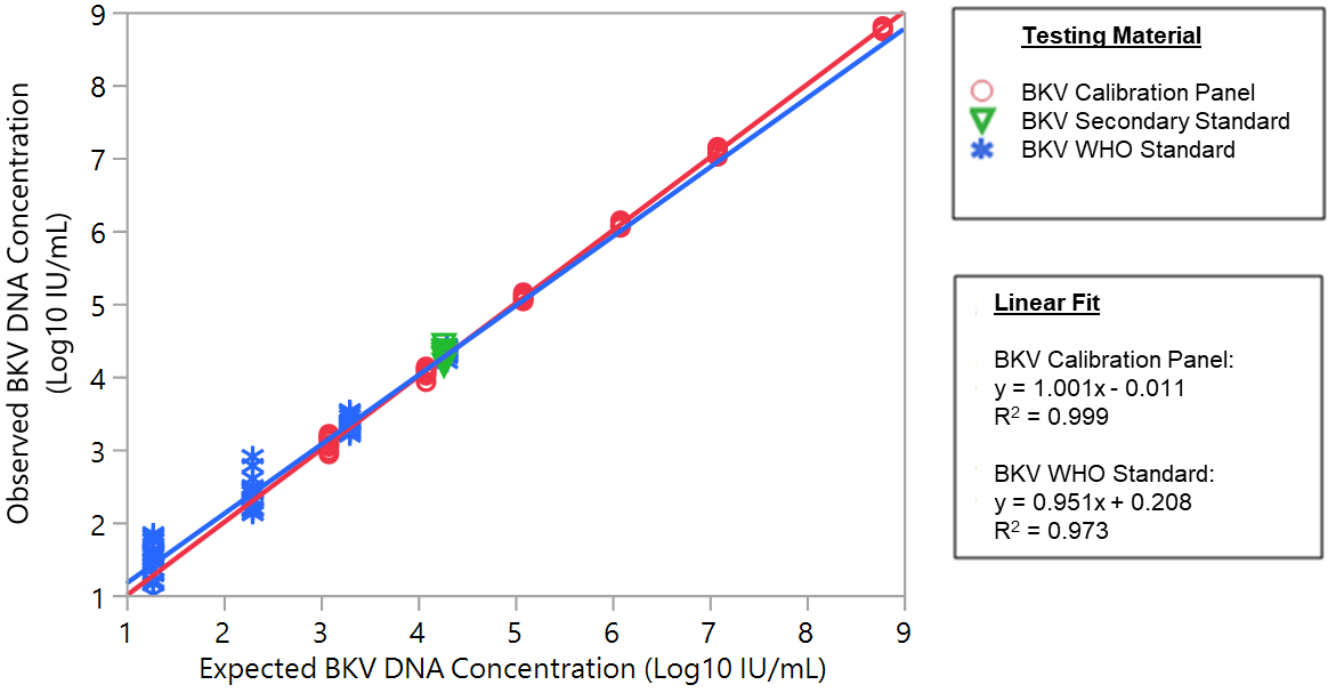
Genotype	Test concentration	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100
Subgroup Ia	5.4 IU/mL	Not Tested	Not Tested	Not Tested
Subgroup Ia	10.8 IU/mL	63	54	85.7%
Subgroup Ia	21.5 IU/mL	63	63	100.0%
Subgroup Ic	5.4 IU/mL	62	57	91.9%
Subgroup Ic	10.8 IU/mL	63	61	96.8%
Subgroup Ic	21.5 IU/mL	62	62	100.0%
Subtype II	5.4 IU/mL	63	54	85.7%
Subtype II	10.8 IU/mL	63	63	100.0%
Subtype II	21.5 IU/mL	63	63	100.0%
Subtype III	5.4 IU/mL	63	49	77.8%
Subtype III	10.8 IU/mL	63	63	100.0%
Subtype III	21.5 IU/mL	63	63	100.0%
Subtype IV	5.4 IU/mL	63	57	90.5%
Subtype IV	10.8 IU/mL	63	63	100.0%
Subtype IV	21.5 IU/mL	63	63	100.0%

Traceability to the 1st WHO International Standard for BK Virus for Nucleic Acid Amplification Techniques (NAT)-based assays

Several standards and controls have been used during development of this test to provide traceability to the WHO standard [the 1st WHO International Standard for BK Virus DNA (NIBSC 14/212)[19]]. The standards used during development of the test include the BKV WHO Standard, the RMS BKV Secondary Standard, and the RMS BKV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the BKV WHO Standard was from 1.90E+01 IU/mL to 2.00E+04 IU/mL (1.28-4.30 $\log_{10}$ IU/mL), the RMS BKV Secondary Standard was tested at 1.86E+04 IU/mL (4.27 $\log_{10}$ IU/mL), and the RMS BKV Calibration Panel was tested from 1.00E+03 IU/mL to 5.00E+08 IU/mL (3.00-8.70 $\log_{10}$ IU/mL).

The calibration and standardization process of **cobas**® BKV provides quantitation values for the calibration panel, the RMS BKV Secondary Standard, and the BKV WHO Standard that are similar to the expected values with deviation of not more than 0.19 $\log_{10}$ IU/mL (Figure 5**).** The maximum deviation was obtained at 19.0 IU/mL (approximately LLoQ).

Figure 5 Traceability to WHO International Standard [bivariate fit of observed BKV DNA concentration ($\log_{10}$ IU/mL) by expected BKV DNA concentration ($\log_{10}$ IU/mL)] using **cobas**® BKV



Linear range

Linearity of **cobas**® BKV in the EDTA plasma was evaluated using a dilution series consisting of 18 panel members with BKV subgroup Ib DNA spanning the assay linear range. A high titer lambda DNA stock was used to prepare 11 panel members spanning the entire linear range. A clinical specimen was used to prepare seven panel members covering the intermediate - and lower levels of the linear range.

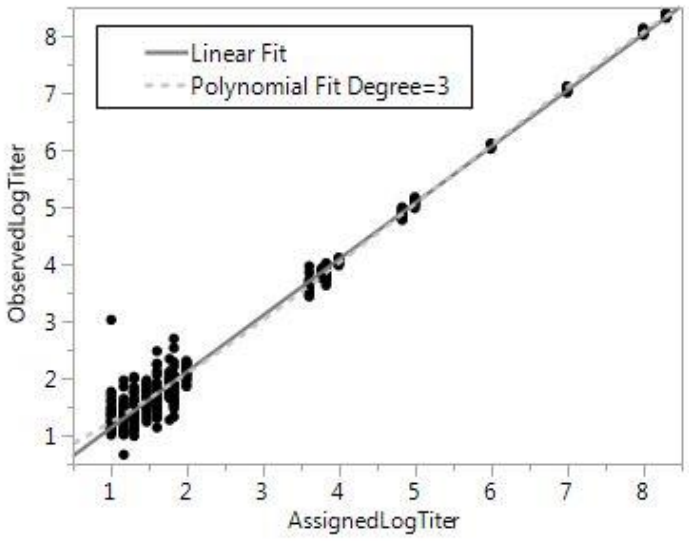
Each panel member was tested in 36 replicates across three lots of **cobas**® BKV reagents and the results of the study are presented in Figure 6.

cobas® BKV was demonstrated to be linear from 1.01E+01 to 1.97E+08 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less or equal than ± 0.1 $\log_{10}$ in human EDTA plasma (see Figure 6). Across the linear range, the accuracy of the test was within ± 0.2 $\log_{10}$.

The LLoQ is 21.5 IU/mL, calculated based on a goal for acceptable total analytical error (TAE) of ≤ 1.0 $\log_{10}$, where TAE = |bias| + 2 standard deviations in alignment with the CLSI EP-17A guideline, and
TAE = SQUARE ROOT(2) x 2 standard deviations based on the "difference between 2 measurements" approach.

Based on the LLoQ and the determined linear range, as well as the medical value the linear measurement range of the test was set to 21.5-1.0E+08 IU/mL. The results of calculation and claimed LLoQ are shown in Table 19.

Figure 6 Linear range determination in EDTA plasma



Linearity for subgroups Ia, Ic and subtypes II, III and IV

The dilution series used in the verification of subgroup/subtype linearity study of **cobas**® BKV consisted of eight panel members spanning the linear range of the assay. Testing was conducted with three lots of **cobas**® BKV reagent, 12 replicates per level were tested in EDTA plasma. The results of the study are presented in Table 18.

The linearity within the linear range of **cobas**® BKV was verified for subgroups Ia, Ic and subtypes II, III and IV. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.2 $\log_{10}$.

Table 18 Linearity verification on subgroups Ia, Ic and subtypes II, III and IV in EDTA plasma

Genotype	Linear regression	Better fitting higher order model regression	Maximum difference between linear regression and the better fitting higher order model (log_{10} IU/mL)
Subgroup Ia	y = 0.9794912x + 0.2792632	$y = -0.0120248x^3 + 0.1792256x^2 + 0.2014722x + 1.1614041$	0.21
Subgroup Ic	y = 0.9820273x + 0.1365877	$y = -0.0024169x^3 + 0.0403425x^2 + 0.7853471x + 0.3881234$	0.06
Subtype II	y = 0.9856895x + 0.1313346	$y = -0.0063337x^3 + 0.0966686x^2 + 0.5547977x + 0.6352469$	0.12
Subtype III	y = 0.9742446x + 0.1747927	$y = -0.0039425x^3 + 0.0693297x^2 + 0.6211286x + 0.6415592$	0.12
Subtype IV	y = 0.9802729x + 0.1452696	$y = -0.0054353x^3 + 0.0880830x^2 + 0.5657132x + 0.6484089$	0.14

Lower Limit of Quantitation

The analysis for LLoQ of **cobas®** BKV in EDTA plasma was performed with data obtained from the LoD study at concentration levels of 19.0 IU/mL, 38.0 IU/mL and 80.0 IU/mL. The LLoQ is the lowest titer within the linear range that is not lower than the LoD and meets the acceptance criterion for the Total Analytical Error (|Bias| + 2x SD) (TAE). The TAE criterion is ≤ 1 log_{10}.

The results of calculation and claimed LLoQ are shown in Table 19. The LLoQ is 21.5 IU/mL.

Table 19 LLoQ of **cobas®** BKV using the 1st WHO International Standard for BK Virus (BKV) (NIBSC 14/212) (plasma)

Lot	Nominal concentration (IU/mL)	log_{10} titer nominal	Mean log_{10} titer observed	SD (log_{10})	Absolute Bias	TAE (|Bias| + 2x SD)	Difference between Measurements in SD (= SQRT(2) x 2x SD)
1	19.0	1.28	1.39	0.25	0.11	0.61	0.71
1	38.0	1.58	1.62	0.25	0.04	0.53	0.69
1	80.0	1.90	1.89	0.26	0.01	0.52	0.73
2	19.0	1.28	1.50	0.26	0.22	0.74	0.74
2	38.0	1.58	1.76	0.21	0.18	0.60	0.59
2	80.0	1.90	2.02	0.27	0.11	0.65	0.76
3	19.0	1.28	1.47	0.27	0.19	0.72	0.75
3	38.0	1.58	1.66	0.26	0.08	0.59	0.72
3	80.0	1.90	1.91	0.19	0.00	0.38	0.53
3 Lots combined	19.0	1.28	1.45	0.26	0.18	0.69	0.73
3 Lots combined	38.0	1.58	1.68	0.24	0.10	0.57	0.67
3 Lots combined	80.0	1.90	1.94	0.24	0.04	0.52	0.68

Precision – within laboratory

Precision of **cobas®** BKV was determined by analysis of serial dilutions of high titer BKV DNA (subgroup Ib) in BKV-negative EDTA plasma. Five dilution levels were tested in 72 replicates for each level across three lots of **cobas®** BKV reagents using four instruments and two operators over 12 days. Each sample was carried through the entire **cobas®** BKV procedure on fully automated **cobas®** 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 22. The results of the variance component estimation are shown in Table 21.

cobas® BKV showed high precision for three lots of reagents tested across a concentration range of 5.90E+01 IU/mL to 9.83E+05 IU/mL.

Table 20 Within-laboratory precision of **cobas®** BKV in EDTA plasma*

Nominal Concentration [IU/mL]	Assigned Concentration [IU/mL]	EDTA plasma Lot 1 SD	EDTA plasma Lot 2 SD	EDTA plasma Lot 3 SD	EDTA plasma All lots Pooled SD
1.00E+06	9.83E+05	0.02	0.02	0.04	0.03
1.00E+05	9.83E+04	0.03	0.04	0.04	0.04
1.00E+04	9.83E+03	0.04	0.05	0.03	0.04
6.00E+03	5.90E+03	0.03	0.05	0.03	0.04
1.00E+02	9.83E+01	0.09	0.11	0.11	0.11
6.00E+01	5.90E+01	0.14	0.11	0.13	0.13

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 21 Lognormal Percent Coefficient of Variation (%CV) of **cobas®** BKV by positive panel and contributing components of variance in EDTA plasma*

Nominal concentration Titer (IU/mL)	Nominal concentration $\log_{10}$ titer (IU/mL)	Assigned concentration Titer (IU/mL)	Assigned concentration $\log_{10}$ titer (IU/mL)	N	Instrument / Operator %CV	Lot %CV	Day %CV	Run %CV	Within Run %CV	Total %CV
1.00E+06	6.00	9.83E+05	5.99	72	2%	5%	3%	2%	5%	8%
1.00E+05	5.00	9.83E+04	4.99	71	3%	6%	3%	0%	8%	11%
1.00E+04	4.00	9.83E+03	3.99	70	3%	7%	5%	3%	9%	13%
6.00E+03	3.78	5.90E+03	3.77	72	2%	8%	2%	1%	8%	12%
1.00E+02	2.00	9.83E+01	1.99	72	5%	8%	6%	4%	24%	26%
6.00E+01	1.78	5.90E+01	1.77	71	4%	14%	7%	15%	29%	36%

* Titer data are considered to be log-normally distributed and the %CV values are analyzed as Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100%

cobas® BKV showed high precision for three lots of reagents tested across a concentration range of 9.83E+01 IU/mL to 9.83E+05 IU/mL.

Table 22 Within-laboratory precision of **cobas®** BKV*

Nominal Concentration [IU/mL]	Assigned Concentration [IU/mL]	EDTA plasma Lot 1 SD	EDTA plasma Lot 2 SD	EDTA plasma Lot 3 SD	EDTA plasma All lots Pooled SD
1.00E+06	9.83E+05	0.02	0.02	0.04	0.03
1.00E+05	9.83E+04	0.03	0.04	0.04	0.04
1.00E+04	9.83E+03	0.04	0.05	0.03	0.04
6.00E+03	5.90E+03	0.03	0.05	0.03	0.04
1.00E+02	9.83E+01	0.09	0.11	0.11	0.11

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Analytical specificity

The analytical specificity of **cobas**® BKV was evaluated by testing a panel of microorganisms at a concentration of 1.00E+06 units/mL (CFU/mL, cells/mL, CCU/mL, IFU/mL) for bacteria and yeast and between 1.00E+05 units/mL and 1.00E+06 units/mL (copies/mL, $TCID_{50}$/mL, IU/mL, cells/mL) for viruses. Microorganisms were diluted into BKV DNA negative human EDTA plasma as well as human EDTA plasma containing (100 IU/mL) BKV DNA. The specific organisms tested are listed in Table 23. Each sample was tested in replicates of three. None of the non-BKV pathogens interfered with test performance at the concentrations tested. Negative results were obtained with **cobas**® BKV for all microorganism samples without BKV target and positive results were obtained for all of the microorganism samples with BKV target. Furthermore, the mean log_{10} titer of each of the positive BKV samples containing potentially cross-reacting organisms was within ± 0.5 log_{10} of the mean log_{10} titer of the respective positive spike control.

Table 23 Microorganisms tested for cross-reactivity

Viruses	**Bacteria**	**Yeast**
Adenovirus Type 5	*Propionibacterium acnes*	*Aspergillus niger*
Cytomegalovirus	*Staphylococcus aureus*	*Candida albicans*
Epstein-Barr Virus	*Chlamydia trachomatis*	*Cryptococcus neoformans*
Hepatitis B Virus	*Clostridium perfringens*	-
Hepatitis C Virus	*Enterococcus faecalis*	-
Herpes Simplex Virus Type1	*Escherichia coli*	-
Herpes Simplex Virus Type 2	*Klebsiella pneumoniae*	-
Human Herpes Virus Type 6	*Listeria monocytogenes*	-
Human Herpes Virus Type 7	*Mycobacterium avium*	-
Human Herpes Virus Type 8	*Neisseria gonorrhoeae*	-
Human Immunodeficiency Virus-1	*Staphylococcus epidermidis*	-
Human Immunodeficiency Virus-2	*Streptococcus pyogenes*	-
Human Papillomavirus*	*Mycoplasma pneumoniae*	-
JC virus	*Salmonella enterica*	-
Parvovirus B19	*Streptococcus pneumoniae*	-
Simian Virus 40	-	-
Varicella-Zoster Virus	-	-

* Highest concentration with no interference observed: 1.00E+05 cells/mL

Analytical specificity – interfering substances

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 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.

In addition, drug compounds listed in Table 24 were tested at three times the C_{max} in presence (100 IU/mL) and absence of BKV DNA.

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 $\log_{10}$ titer of each of the positive BKV samples containing potentially interfering substances was within ± 0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 24 Drug compounds tested for interference with the quantitation of BKV DNA by **cobas**® BKV in EDTA plasma

Class of drug	Generic drug name	Test concentration
Antimicrobial	Cefotetan	711 µg/mL
Antimicrobial	Clavulanate potassium	3 µg/mL
Antimicrobial	Fluconazole	20.1 µg/mL*
Antimicrobial	Piperacillin	894 µg/mL*
Antimicrobial	Tazobactam sodium	112 µg/mL
Antimicrobial	Sulfamethoxazole	204 µg/mL
Antimicrobial	Ticarcillin disodium	972 µg/mL
Antimicrobial	Trimethoprim	41 µg/mL
Antimicrobial	Vancomycin	189 µg/mL
Antimicrobial	Micafungin	49.2 µg/mL*
Compounds for Treatment of Herpes Viruses	Ganciclovir	27 µg/mL
Compounds for Treatment of Herpes Viruses	Valganciclovir	16.8 µg/mL
Compounds for Treatment of Herpes Viruses	Acyclovir	16.8 µg/mL*
Compounds for Treatment of Herpes Viruses	Cidofovir	60 µg/mL
Compounds for Treatment of Herpes Viruses	Foscarnet	1869 µmol/L
Compounds for Treatment of Herpes Viruses	Letermovir	39 µg/mL
Immune suppressant	Azathioprine	3 µg/mL
Immune suppressant	Cyclosporine	5.4 µg/mL
Immune suppressant	Everolimus	12 µg/mL
Immune suppressant	Mycophenolate mofetil	75 µg/mL
Immune suppressant	Prednisone	36 µg/mL
Immune suppressant	Sirolimus	0.045 µg/mL
Immune suppressant	Tacrolimus	0.21 µg/mL
Immune suppressant	Mycophenolic acid	111 µg/mL

* Drug compounds were tested at concentrations lower than three times the C_{max}

Cross contamination

The cross-contamination rate for **cobas**® BKV was determined by testing 240 replicates of a BKV-negative matrix sample and 225 replicates of a high titer BKV DNA EDTA plasma sample at approximately 2.00E+07 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0% (upper one-sided 95% confidence interval 1.24%).

Key performance characteristics for urine sample type performed on the cobas® 6800/8800 Systems

Limit of Detection (LoD)

The limit of detection (LoD) of **cobas**® BKV was determined by analysis of serial dilutions of the WHO International Standard (subgroup Ib) and verified for subgroups Ia, Ic and subtypes II, III and IV. The overall concentration for which 95% hit rate is expected by PROBIT is 12.2 IU/mL for neat urine.

WHO International Standard

The limit of detection of **cobas**® BKV for the WHO International Standard was determined by analysis of serial dilutions of the 1st WHO BKV International Standard obtained from NIBSC (NIBSC 14/212), in BKV-negative pooled urine stabilized in **cobas**® PCR Media. Panels of six concentration levels plus a blank were tested over three lots of **cobas**® BKV reagents, multiple runs, days, operators, and instruments.

The results for pooled urine stabilized in **cobas**® PCR Media are shown in Table 25 through Table 27. The study demonstrates that with the least sensitive lot, the concentration for which 95% hit rate is expected by PROBIT is 12.2 IU/mL with a 95% confidence range of 9.2 – 18.3 IU/mL in neat urine. The lowest concentration with a hit rate ≥ 95% is 10.0 IU/mL in neat urine.

Table 25 Limit of detection in urine, Lot 1

Input titer concentration (BKV DNA IU/mL)*	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
40.0	63	63	100.0
20.0	63	63	100.0
10.0	63	60	95.2
5.0	63	47	74.6
2.5	63	25	39.7
1.25	63	26	41.3
0	63	0	0.0

LoD by PROBIT at 95% hit rate: 12.2 IU/mL. 95% confidence range: 9.2 – 18.3 IU/mL

* Urine samples tested stabilized in **cobas**® PCR Media. Input titer concentration used for calculation based on neat urine.

Table 26 Limit of detection in urine, Lot 2

Input titer concentration (BKV DNA IU/mL)*	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
40.0	63	63	100.0
20.0	63	63	100.0
10.0	63	60	95.2
5.0	63	42	66.7
2.5	63	32	50.8
1.25	63	17	27.0
0	63	0	0.0

LoD by PROBIT at 95% hit rate: 11.9 IU/mL. 95% confidence range: 9.2 – 17.3 IU/mL

* Urine samples tested stabilized in **cobas**® PCR Media. Input titer concentration used for calculation based on neat urine.

Table 27 Limit of detection in urine, Lot 3

Input titer concentration (BKV DNA IU/mL)*	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
40.0	63	63	100.0
20.0	63	63	100.0
10.0	63	61	96.8
5.0	63	46	73.0
2.5	63	39	61.9
1.25	63	19	30.2
0	63	0	0.0

LoD by PROBIT at 95% hit rate: 10.1 IU/mL. 95% confidence range: 7.8 – 14.7 IU/mL

* Urine samples tested stabilized in **cobas**® PCR Media. Input titer concentration used for calculation based on neat urine.

Limit of Detection for subgroups Ia, Ic and Subtypes II, III and IV

BKV armored DNA for subgroup Ic and clinical specimens for subgroup Ia and subtypes II, III and IV were diluted to three different concentration levels in BKV-negative urine stabilized in **cobas**® PCR Media. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® BKV reagents.

The combined results from three lots shown in Table 28 verify that – consistent with an LoD of 12.2 IU/mL – **cobas**® BKV detected BKV DNA for subgroups Ia and Ic, and subtypes II, III and IV at a concentration of 12.2 IU/mL with a ≥ 95% hit rate.

Table 28 BKV DNA subgroups Ia, Ic and subtypes II, III and IV verification of limit of detection in urine

Genotype	Test concentration	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100
Subgroup Ia	6.1 IU/mL	63	53	84.1%
Subgroup Ia	12.2 IU/mL	63	61	96.8%
Subgroup Ia	18.3 IU/mL	63	62	98.4%
Subgroup Ic	6.1 IU/mL	63	50	79.4%
Subgroup Ic	12.2 IU/mL	63	62	98.4%
Subgroup Ic	18.3 IU/mL	63	63	100.0%
Subtype II	6.1 IU/mL	63	56	88.9%
Subtype II	12.2 IU/mL	63	61	96.8%
Subtype II	18.3 IU/mL	63	63	100.0%
Subtype III	6.1 IU/mL	63	60	95.2%
Subtype III	12.2 IU/mL	63	62	98.4%
Subtype III	18.3 IU/mL	63	63	100.0%
Subtype IV	6.1 IU/mL	63	54	85.7%
Subtype IV	12.2 IU/mL	63	63	100.0%
Subtype IV	18.3 IU/mL	63	63	100.0%

Linear range

Linearity of **cobas®** BKV was evaluated using a dilution series consisting of 10 panel members using a clinical specimen (BKV subgroup Ib) spanning the assay linear range. A high titer lambda DNA stock was used to prepare 12 panel members spanning the entire linear range.

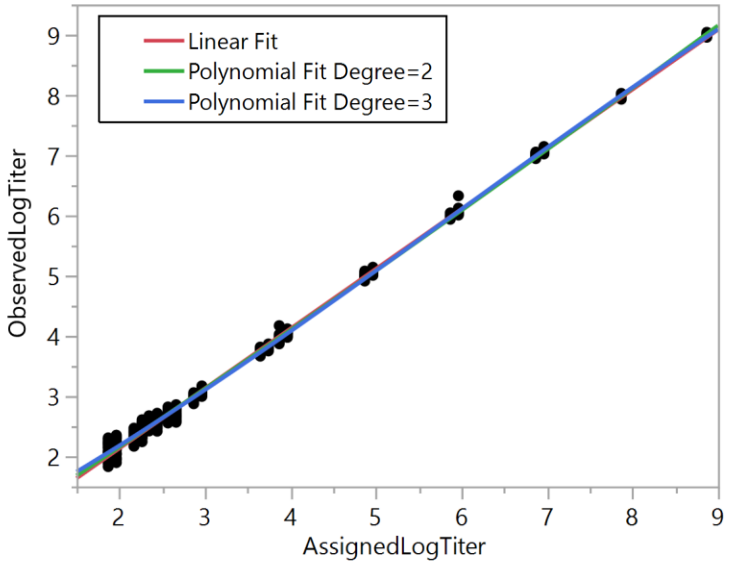
Each panel member was tested in 36 replicates across three lots of **cobas®** BKV reagents and the results of the study are presented in Figure 7.

cobas® BKV was demonstrated to be linear from 7.41E+01 IU/mL to 7.41E+08 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less or equal than ± 0.1 $\log_{10}$ in pooled urine stabilized in **cobas®** PCR Media (see Figure 7). Across the linear range, the accuracy of the test was within ± 0.2 $\log_{10}$.

The lower limit of quantitation (LLoQ) was set to 200 IU/mL, to include the mean deviation between the observed vs. the assigned $\log_{10}$ titer (Accuracy) being equal or less than ±0.3 $\log_{10}$, based on the upper 95% confidence interval of the worst performing lot using clinical specimen and calculated based on a goal for acceptable total analytical error (TAE) of ≤ 1.0 $\log_{10}$, where TAE = |bias| + 2 standard deviations in alignment with the CLSI EP-17A guideline, and TAE = SQUARE ROOT(2) x 2 standard deviations based on the "difference between 2 measurements" approach.

Based on the LLoQ and the determined linear range, as well as the medical value the linear measurement range of the test was set to 2.0E+02 IU/mL to-1.0E+08 IU/mL. The results of calculation and claimed LLoQ are shown in Table 30.

Figure 7 Linear range determination in urine



Linearity for subgroups Ia, Ic and subtypes II, III and IV

The dilution series used in the verification of subtype/subgroup linearity study of **cobas**® BKV consisted of eight panel members spanning the linear range of the assay. Testing was conducted with three lots of **cobas**® BKV reagent, 12 replicates per level were tested in urine stabilized in **cobas**® PCR Media. The results of the study are presented in Table 29.

The linearity within the linear range of **cobas**® BKV was verified for subgroups Ia, Ic and subtypes II, III and IV. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.2 $\log_{10}$.

Table 29 Linearity verification on subgroups Ia, Ic and subtypes II, III and IV in urine

Genotype	Linear regression	Better fitting higher order model regression	Maximum difference between linear regression and the better fitting higher order model ($\log_{10}$ IU/mL)
Subgroup Ia	$y = 0.9756016x + 0.2367214$	$y = -0.0081339x^3 + 0.1373308x^2 + 0.2602376x + 1.3507964$	0.12
Subgroup Ic	$y = 0.9773177x + 0.2051674$	$y = -0.005087x^3 + 0.0902742x^2 + 0.4865276x + 0.9928655$	0.10
Subtype II	$y = 0.9762885x + 0.2271355$	$y = -0.0072558x^3 + 0.1259492x^2 + 0.3032666x + 1.2971372$	0.12
Subtype III	$y = 0.9762129x + 0.2270439$	$y = -0.0081255x^3 + 0.1370235x^2 + 0.2645895x + 1.327922$	0.12
Subtype IV	$y = 0.9758502x + 0.2028958$	$y = -0.0086141x^3 + 0.1427957x^2 + 0.2499516x + 1.2930774$	0.13

Lower Limit of Quantitation

The analysis for LLoQ of **cobas**® BKV in urine was performed with data obtained from the Linearity study at concentration levels of 100 IU/mL, 200 IU/mL and 300 IU/mL. The LLoQ was set at the concentration level of 200 IU/mL to include the mean deviation between the observed vs. the assigned $\log_{10}$ titer (Accuracy) being equal or less than ± 0.3 $\log_{10}$, based on the upper 95% confidence interval of the worst performing lot using clinical specimen. The LLoQ within the linear range meets the acceptance criterion for the Total Analytical Error (|Bias| + 2x SD) (TAE). The TAE criterion is ≤ 1 $\log_{10}$.

The results of calculation and claimed LLoQ are shown in Table 30. The LLoQ is 200 IU/mL.

Table 30 LLoQ of **cobas**® BKV using clinical sample (urine)

Lot	Nominal concentration (IU/mL)	$\log_{10}$ titer assigned	Mean $\log_{10}$ titer observed	SD ($\log_{10}$)	Absolute Bias	TAE (|Bias| + 2x SD)	Difference between Measurements in SD (= SQRT(2) x 2x SD)
1	3.00E+02	2.44	2.50	0.05	0.06	0.15	0.13
1	2.00E+02	2.27	2.37	0.07	0.11	0.25	0.21
1	1.00E+02	1.96	2.06	0.11	0.10	0.32	0.32
2	3.00E+02	2.44	2.63	0.05	0.19	0.29	0.14
2	2.00E+02	2.27	2.49	0.06	0.22	0.34	0.17
2	1.00E+02	1.96	2.22	0.09	0.26	0.44	0.25
3	3.00E+02	2.44	2.58	0.07	0.13	0.27	0.20
3	2.00E+02	2.27	2.41	0.07	0.15	0.28	0.19
3	1.00E+02	1.96	2.14	0.09	0.17	0.36	0.26
3 Lots combined	3.00E+02	2.44	2.57	0.08	0.13	0.28	0.22
3 Lots combined	2.00E+02	2.27	2.42	0.08	0.16	0.32	0.23
3 Lots combined	1.00E+02	1.96	2.14	0.12	0.18	0.41	0.33

Precision – within laboratory

Precision of **cobas**® BKV was determined by analysis of serial dilutions of high titer BKV DNA (subgroup Ib) in BKV-negative pooled urine stabilized in **cobas**® PCR Media. Five dilution levels were tested in 72 replicates for each level across three lots of **cobas**® BKV reagents using two instruments and two operators over 12 days. Each sample was carried through the entire **cobas**® BKV procedure on fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 31 . The results of the variance component estimation are shown in Table 32.

cobas® BKV showed high precision for three lots of reagents tested across a concentration range of 7.41E+02 IU/mL to 7.41E+07 IU/mL.

Table 31 Within-laboratory precision of **cobas®** BKV* in stabilized urine

Nominal Concentration [IU/mL]	Assigned Concentration [IU/mL]	Urine stabilized in cobas® PCR Media Lot 1 SD	Urine stabilized in cobas® PCR Media Lot 2 SD	Urine stabilized in cobas® PCR Media Lot 3 SD	Urine stabilized in cobas® PCR Media All lots Pooled SD
1.00E+08	7.41E+07	0.02	0.01	0.02	0.02
1.00E+06	7.41E+05	0.02	0.02	0.02	0.02
1.00E+05	7.41E+04	0.02	0.03	0.02	0.03
1.00E+04	7.41E+03	0.03	0.03	0.03	0.03
6.00E+03	4.44E+03	0.04	0.03	0.04	0.03
1.00E+03	7.41E+02	0.05	0.05	0.04	0.05
3.00E+02	2.22E+02	0.08	0.07	0.05	0.07

* Titer data are considered to be log-normally distributed and are analyzed following log_{10} transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 32 Lognormal Percent Coefficient of Variation (% CV) of **cobas®** BKV by positive panel and contributing components of variance in urine*

Nominal concentration Titer (IU/mL)	Nominal concentration Log_{10} titer (IU/mL)	Assigned concentration Titer (IU/mL)	Assigned concentration Log_{10} titer (IU/mL)	N	Instrument / Operator %CV	Lot %CV	Day %CV	Run %CV	Within Run %CV	Total %CV
1.00E+08	8.00	7.41E+07	7.87	57**	3%	5%	1%	1%	4%	7%
1.00E+06	6.00	7.41E+05	5.87	72	2%	6%	1%	2%	4%	8%
1.00E+05	5.00	7.41E+04	4.87	72	4%	7%	1%	2%	5%	10%
1.00E+04	4.00	7.41E+03	3.87	71	6%	9%	2%	1%	6%	12%
6.00E+03	3.78	4.44E+03	3.65	72	6%	7%	0%	1%	7%	11%
1.00E+03	3.00	7.41E+02	2.87	72	3%	11%	2%	2%	11%	16%
3.00E+02	2.48	2.22E+02	2.35	70	5%	15%	5%	6%	15%	23%

* Titer data are considered to be log-normally distributed and the % CV values are analyzed as Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100%.

**15/72 replicates had results above the Upper Limit of Quantification and were excluded from the analysis.

Analytical specificity

The analytical specificity of **cobas®** BKV was evaluated by testing a panel of microorganisms to a concentration between 1.00E+06 units/mL and 2.00E+06 units/mL (CFU/mL, cells/mL, CCU/mL, IFU/mL) for bacteria and yeast and at 1.00E+05 units/mL (copies/mL, $TCID_{50}$/mL, IU/mL, cells/mL) for viruses. Microorganisms were diluted into BKV DNA negative urine as well as urine containing (600 IU/mL) BKV DNA. The specific organisms tested are listed in Table 33. Each sample was tested in replicates of three. None of the non-BKV pathogens interfered with test performance at the concentrations tested. Negative results were obtained with **cobas®** BKV for all microorganism samples without BKV target and positive results were obtained for all of the microorganism samples with BKV target. Furthermore, the mean log_{10} titer of each of the positive BKV samples containing potentially cross-reacting organisms was within ± 0.5 log_{10} of the mean log_{10} titer of the respective positive spike control.

Table 33 Microorganisms tested for cross-reactivity in urine

Bacteria	**Yeast**	**Viruses**
Bacillus cereus	*Candida albicans*	*Herpes Simplex Virus-2*
Bacillus subtilis	*Candida glabrata*	*Human Papillomavirus 16*
Chlamydia trachomatis	*Candida parapsilosis*	-
Corynebacterium diphteriae	*Candida tropicalis*	-
Enterobacter cloacae	-	-
Enterococcus faecalis	-	-
Enterococcus faecium	-	-
Escherichia coli	-	-
Klebsiella pneumoniae	-	-
Lactobacillus acidophilus	-	-
Lactobacillus crispatus	-	-
Lactobacillus jensenii	-	-
Lactobacillus vaginalis	-	-
Morganella morganii	-	-
Mycoplasma genitalium	-	-
Neisseria gonorrhoeae	-	-
Proteus mirabilis	-	-
Pseudomonas aeruginosa	-	-
Staphylococcus aureus	-	-
Staphylococcus epidermidis	-	-
Staphylococcus saphrophyticus	-	-
Streptococcus agalactiae	-	-
Streptococcus bovis	-	-
Streptococcus oralis/viridans	-	-
Streptococcus pneumoniae	-	-
Treponema pallidum	-	-
Trichomonas vaginalis	-	-
Ureaplasma urealyticum	-	-

Analytical specificity – interfering substances

Elevated levels of albumin (0.5% w/v), conjugated bilirubin (1% w/v) , glucose (1% w/v), peripheral blood mononuclear cells (1.00E+06 cells/mL), mucus (in presence of 1 mucus swab per 4.3mL of specimen), acidic pH (pH 4), alkaline pH (pH 9), semen (1 swab dipped into semen per 4.3mL of specimen) , sodium (300 mEq/L) and whole blood (10% v/v) in samples were tested in the presence (600 IU/mL) and absence of BKV DNA. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® BKV.

In addition, drug compounds listed in Table 34 were tested in presence and absence of BKV DNA.

All potentially interfering substances, with the exception of talcum powder, have been shown to not interfere with the test performance. Talcum powder at ≤ 0.05% showed no interference with **cobas**® BKV.

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 $\log_{10}$ titer of each of the positive BKV samples containing potentially interfering substances was within ± 0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 34 Drug compounds tested for interference with the quantitation of BKV DNA by **cobas**® BKV in urine

Class of drug	Active Ingredient	Concentration	Generic drug name
Antimicrobial	Clotrimazole	100 µg/mL	Gyne-Lotrimin 7
Antimicrobial	Metronidazole	701 µmol/L	Arilin rapid, Vaginal suppositories, Vagi Metro Cream, Nidazea Gel
Estrogen steroid hormone	Estradiol	4.41 nmol/L	Estrace
Analgesics	Phenazopyridine Hydrochloride	200 µg/mL	Azo Standard
Analgesics	Acetaminophen	1324 µmol/L	Acetaminophen
Lubricant	Propylene Glycol	1000 µg/mL	K-Y UltraGel
Nonsteroidal anti-inflammatory drug	Acetylsalicylic Acid	3.62 mmol/L	Acetylsalicylic Acid
Nonsteroidal anti-inflammatory drug	Naproxen	2170 µmol/L	Naproxen
Nonsteroidal anti-inflammatory drug	Ibuprofen	2425 µmol/L	Ibuprofen
Not applicable	Talc	0.05% (w/v)	Talcum powder

Cross contamination

The cross-contamination rate for **cobas**® BKV was determined by testing 240 replicates of a BKV-negative matrix sample and 225 replicates of a high titer BKV DNA urine sample stabilized in **cobas**® PCR Media at approximately 1.00E+09 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0.0% (upper one-sided 95% confidence interval 1.24%).

Clinical performance evaluation performed on the cobas® 6800/8800 Systems

Reproducibility of cobas® BKV for EDTA plasma sample type

The reproducibility of **cobas®** BKV was evaluated across factors (reagent lot, test site, batch and testing days) that could affect reported results in routine clinical testing. The evaluation was conducted at 3 testing sites, using 3 reagent lots, of a positive and a negative sample panel with a total number 270 tests per concentration (not including controls). The panels were made from EDTA plasma that was BKV VCA IgG negative and were tested for BKV with a plasma NAT release protocol, and spiked with a BKV WHO international standard, or BKV genotype Ib (most common genotype) cultured virus DNA. Two operators at each site tested each reagent lot for 5 days. Two runs (1 run = 1 batch; 1 batch = 1 panel + 3 controls) were performed each day and 3 replicates of each panel member were performed for each run. The evaluation results are summarized in Table 35.

Table 35 Attributable percentage of total variance (%TV), total precision Standard Deviation (SD), and lognormal CV(%) of BKV DNA concentration (log_{10} IU/mL) by positive panel member (EDTA plasma)

Expected BKV DNA Concentration	Observed Mean[a] BKV DNA Concentration	Number of Tests[b]	Lot %TV[c] (CV%)[d]	Site %TV[c] (CV%)[d]	Day/ Operator %TV[c] (CV%)[d]	Batch %TV[c] (CV%)[d]	Within -Batch %TV[c] (CV%)[d]	Total Precision SD[e]	Total Precision CV(%)[d]
1.81	1.74	270	9% (20.63)	6% (17.69)	0% (0.00)	7% (19.15)	78% (68.05)	0.304	79.43
3.70	3.52	270	10% (9.79)	10% (9.57)	14% (11.44)	25% (15.16)	40% (19.38)	0.131	30.91
4.70	4.51	270	3% (4.42)	24% (13.46)	0% (0.00)	56% (20.58)	17% (11.27)	0.118	27.71
5.70	5.54	270	7% (5.66)	28% (11.50)	0% (0.00)	40% (13.85)	25% (10.84)	0.094	21.94
7.70	7.62	269	4% (3.27)	49% (11.00)	0% (0.00)	13% (5.60)	34% (9.10)	0.068	15.74

Note: The table only includes results with detectable DNA level. SD = standard deviation; CV = percent coefficient of variation; BKV = BK Virus.

[a] Calculated using SAS MIXED procedure.

[b] Number of valid tests with detectable DNA level.

[c] %TV = Percent contribution to Total Variance.

[d] CV% = Lognormal percent coefficient of variation = sqrt(10^[SD^2 * ln(10)] - 1) * 100.

[e] Calculated using the total variability from the SAS MIXED procedure.

cobas® BKV showed acceptable clinical reproducibility at concentrations throughout the linear range. In addition, the system detected 100% of the 3 x LLoQ samples. The **cobas®** 6800 and **cobas®** 8800 Systems share a modular design and they showed equivalency when using **cobas®** BKV. All of the estimated 95% confidence limits (CLs) for the difference between 2 measurements from the same subject were within ± 0.84 log_{10} IU/mL, indicating that the assay can assess changes in BKV DNA levels that are thought to be clinically significant.

Of the 270 valid tests for the negative panel members performed on the **cobas®** 6800/8800 Systems, all samples showed a "Target Not Detected" result, therefore the negative percent agreement (NPA) was 100% with the 95% Exact CI of 98.6% to 100%.

Performance of cobas® BKV for EDTA plasma sample type

The clinical performance of **cobas®** BKV was further evaluated at three testing sites by measuring BKV DNA levels in clinical samples (neat and diluted) of BKV infected and non-infected patients and contrived EDTA plasma samples spiked with cultured BK virus, compared with a well-established laboratory developed nucleic acid test (LDT) (comparator BKV LDT).

From all samples tested with **cobas®** BKV and the comparator BKV test, there were a total of 550 samples (217 neat and 303 diluted clinical samples from 129 transplant subjects and 30 contrived samples) that were valid on both assays and evaluable for the clinical concordance analysis (Table 36).

Table 36 Concordance analysis between **cobas®** BKV and the comparator LDT on BKV DNA level (log_{10} IU/mL) results for all samples (EDTA plasma)

cobas® BKV (log_{10} IU/mL)	**Comparator BKV LDT Target Not Detected**	**Comparator BKV LDT < LLoQ (< 2.3)**	**Comparator BKV LDT 2.3 to < 3.0**	**Comparator BKV LDT 3.0 to < 3.7**	**Comparator BKV LDT 3.7 to 4.4**	**Comparator BKV LDT > 4.4**	**Total**
Target Not Detected	107	7	5	0	0	0	119
< LLoQ (< 2.3)	23	51	39	0	0	0	113
2.3 to < 3.0	0	3	40	62	1	0	106
3.0 to < 3.7	0	0	1	71	42	0	114
3.7 to 4.4	0	0	0	0	26	26	52
> 4.4	0	0	0	0	1	45	46
Total	130	61	85	133	70	71	550
Column Agreement (%)	(130/130) 100.0%	(61/61) 100.0%	(80/85) 94.1%	(133/133) 100.0%	(69/70) 98.6%	(71/71) 100.0%	-
(95% Score CI)[a]	(97.1%, 100.0%)	(94.1%, 100.0%)	(87.0%, 97.5%)	(97.2%, 100.0%)	(92.3%, 99.7%)	(94.9%, 100.0%)	-

Note: CI = Confidence Interval; LLoQ = lower limit of quantitation of Comparator BKV LDT (200 IU/mL = 2.3 log_{10} IU/mL); LDT = laboratory developed test; BKV = BK virus;
Standard Deviation of Comparator BKV LDT estimated at 0.37 log_{10} IU/mL (from Indiana University BKV LDT analytical precision study).
Analyte concentration of 3.0 log_{10} IU/mL represented LLoQ + 2σ, 3.7 log_{10} IU/mL represented LLoQ + 4σ and 4.4 log_{10} IU/mL represented LLoQ + 6σ with a range interval of 2σ.
Paired samples evaluable for clinical concordance analysis were included in this table.
[a] Assumed independence between all samples.

Discordant results were defined as those that are more than one box away from the diagonal (indicated by shading). For Target Not Detected (TND) by LDT Column Agreement the **cobas®** BKV Target Not Detected and < LLoQ (< 2.3) cells were combined. The rationale for adding the adjacent <LLoQ and TND cells for the TND column is that the difference between a TND and <LLoQ is not clinically meaningful and that these are analytically at the lower end of the measuring range, which may be impacted by random error.

Of the 43 BKV DNA-negative samples collected for the estimation of the NPA with the **cobas®** BKV, all 43 samples were negative by **cobas®** BKV, therefore the NPA was 100% with the 95% Exact CI of 91.8% to 100%.

Concordance between **cobas®** BKV and the comparator BKV LDT was also evaluated using different clinical thresholds (Table 37).

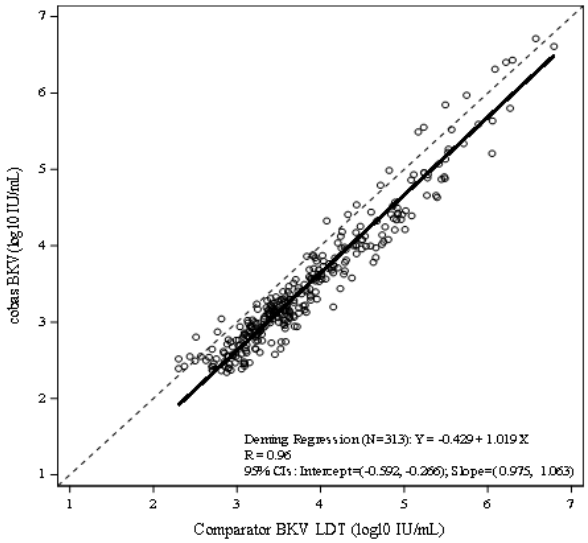
Table 37 Summary of concordance of **cobas**® BKV and comparator BKV LDT using different thresholds for all samples (EDTA plasma)

Thresholds*	Percent Agreement < Threshold 95% CI (n/N)	Percent Agreement ≥ Threshold 95% CI (n/N)
Target Not Detected	82.3% (107/130) (74.8%, 87.9%)	97.1% (408/420) (95.1%, 98.4%)
LLoQ (2.3 $\log_{10}$ IU/mL)	98.4% (188/191) (95.5%, 99.5%)	87.7% (315/359) (83.9%, 90.7%)
3.0 $\log_{10}$ IU/mL	99.6% (275/276) (98.0%, 99.9%)	77.0% (211/274) (71.7%, 81.6%)
4.0 $\log_{10}$ IU/mL	100.0% (447/447) (99.1%, 100.0%)	67.0% (69/103) (57.4%, 75.3%)

Note: Samples with Target Not Detected results were categorised as < threshold value in IU/mL.
LLoQ = lower limit of quantitation of Comparator BKV LDT (200 IU/mL = 2.3 $\log_{10}$ IU/mL).
95% confidence interval (CI) calculated by Score method assuming independence between all samples.
* Thresholds of 1000 IU/mL = 3.0 $\log_{10}$ IU/mL and 10,000 IU/mL = 4.0 $\log_{10}$ IU/mL.

From all samples tested with **cobas**® BKV that were BKV positive with the comparator BKV test, there were a total of 313 (133 neat and 159 diluted clinical samples from 68 transplant subjects and 21 contrived samples), which were evaluable for the correlation analysis at the three testing sites (Figure 8).

Figure 8 Correlation between **cobas**® BKV and comparator BKV LDT for all samples: Deming linear regression plot of DNA levels ($\log_{10}$ IU/mL) (EDTA plasma)



Additional bias plot analysis of DNA level differences indicated a systematic difference between both assays that is constant across the overlapping linear range. The 95% CI of the intercept of the fitted line in the bias plots was (-0.404 to -0.168), which is within ±0.74 $\log_{10}$ IU/mL (± 2 times analytical precision standard deviation of comparator BKV LDT). Furthermore, the mean bias was estimated at -0.357 $\log_{10}$ IU/mL and using the equation of the fitted line in the bias plots, the systematic difference between both assays was -0.343 $\log_{10}$ IU/mL and -0.362 $\log_{10}$ IU/mL for samples with DNA levels at 3 and 4 $\log_{10}$ IU/mL, respectively.

Reproducibility of cobas® BKV for stabilized urine sample type

The reproducibility of **cobas®** BKV was evaluated across factors (reagent lot, test site, batch and testing days) that could affect reported results in routine clinical testing. The evaluation was conducted at 3 testing sites, using 3 reagent lots, of a positive and a negative sample panel with a total number 270 tests per concentration (not including controls). The panels were made from urine stabilized with **cobas®** PCR Media that was confirmed negative for BKV DNA using a urine nucleic acid test (NAT) release protocol and spiked with a BKV WHO international standard, or BKV genotype Ia cultured virus DNA. Two operators at each site tested each reagent lot for 5 days. Two runs (1 run = 1 batch; 1 batch = 1 panel + 3 controls) were performed each day and 3 replicates of each panel member were performed for each run. The evaluation results are summarized in Table 38.

Table 38 Attributable percentage of total variance (%TV), total precision Standard Deviation (SD), and lognormal CV (%) of BKV DNA concentration (log_{10} IU/mL) by positive panel member (stabilized urine)

Expected BKV DNA Concentration	Observed Mean[a] BKV DNA Concentration	Number of Tests[b]	Lot %TV[c] (CV%)[d]	Site %TV[c] (CV%)[d]	Day/ Operator %TV[c] (CV%)[d]	Batch %TV[c] (CV%)[d]	Within -Batch %TV[c] (CV%)[d]	Total Precision SD[e]	Total Precision CV(%)[d]
2.78	2.92	270	59% (12.64)	0% (1.15)	0% (0.00)	0% (0.00)	40% (10.41)	0.071	16.47
3.70	3.78	270	47% (8.14)	2% (1.62)	8% (3.31)	0% (0.00)	43% (7.72)	0.051	11.83
4.70	4.80	270	38% (5.02)	2% (1.28)	6% (2.07)	0% (0.00)	53% (5.96)	0.035	8.17
5.70	5.70	270	21% (3.12)	0% (0.00)	0% (0.00)	0% (0.00)	79% (6.12)	0.030	6.87
7.70	7.69	270	2% (1.51)	19% (4.84)	6% (2.79)	0% (0.00)	73% (9.53)	0.048	11.17

Note: The table only includes results with detectable DNA level. SD = standard deviation; CV = percent coefficient of variation; BKV = BK Virus.

[a] Calculated using SAS MIXED procedure.

[b] Number of valid tests with detectable DNA level.

[c] %TV = Percent contribution to Total Variance.

[d] CV% = Lognormal percent coefficient of variation = sqrt(10^[SD^2 * ln(10)] - 1) * 100.

[e] Calculated using the total variability from the SAS MIXED procedure.

cobas® BKV showed acceptable clinical reproducibility at concentrations throughout the linear range. In addition, the system detected 100% of the 3 x LLoQ samples. The **cobas®** 6800 and **cobas®** 8800 Systems share a modular design and they showed equivalency when using **cobas®** BKV. All of the estimated 95% confidence limits (CLs) for the difference between 2 measurements from the same subject were within ± 0.20 log_{10} IU/mL, indicating that the assay can assess changes in BKV DNA levels that are thought to be clinically significant. The system showed a 99.26% negative percent agreement with a CI of 97.3% to 99.9%. Of the 270 valid tests for the negative panel members, 2 samples (0.74%) showed a DNA level of < LLoQ positivity. Further investigation of these results showed that they were not associated with a particular instrument/site or reagent lot. Additional DNA sequencing confirmed the presence of BKV. The identified BKV sequences were different from those of the positive control and the BKV strain used for panel preparation, excluding contamination during panel preparation and suggesting trace viruria in one of the 25 urine specimens of the pooled urine sample that was used for the negative panel preparation.

Performance of cobas® BKV for stabilized urine sample type

The clinical performance of **cobas®** BKV was further evaluated at three testing sites by measuring BKV DNA levels in clinical urine samples of BKV infected and non-infected patients that were stabilized in **cobas®** PCR Media, compared with a well-established LDT (comparator BKV LDT).

From all samples tested with **cobas®** BKV and the comparator BKV test, there were a total of 308 neat urine samples stabilized in **cobas®** PCR Media from 84 transplant subjects that were valid on both assays and evaluable for the clinical concordance analysis (Table 39).

Table 39 Concordance analysis between **cobas®** BKV and the comparator LDT on BKV DNA level (log_{10} IU/mL) results for all samples (stabilized urine)

cobas® BKV (log_{10} IU/mL)	**Comparator BKV LDT Target Not Detected**	**Comparator BKV LDT < LLoQ (<3.0)**	**Comparator BKV LDT 3.0 to < 3.3**	**Comparator BKV LDT 3.3 to < 3.6**	**Comparator BKV LDT 3.6 to 3.9**	**Comparator BKV LDT > 3.9**	**Total**
Target Not Detected	62	6	0	0	0	0	68
< LLoQ (<3.0)	4	22	0	0	0	1	27
3.0 to < 3.3	0	2	0	0	0	0	2
3.3 to < 3.6	0	0	6	3	0	0	9
3.6 to 3.9	0	0	2	11	10	0	23
> 3.9	0	0	0	2	8	169	179
Total	66	30	8	16	18	170	308
Column Agreement (%)	(66/66) 100.0%	(30/30) 100.0%	(6/8) 75.0%	(14/16) 87.5%	(18/18) 100.0%	(169/170) 99.4%	-
(95% Score CI)[a]	(94.5%, 100.0%)	(88.6%, 100.0%)	(40.9%, 92.9%)	(64.0%, 96.5%)	(82.4%, 100.0%)	(96.7%, 99.9%)	-

Note: CI = Confidence Interval; LLoQ = lower limit of quantitation of Comparator BKV LDT (1000 IU/mL = 3.0 log_{10} IU/mL); LDT = laboratory developed test; BKV = BK virus.
Standard Deviation of Comparator BKV LDT estimated at 0.15 log_{10} IU/mL (comparator BKV LDT validation study).
Analyte concentration of 3.3 log_{10} IU/mL represented LLoQ + 2σ, 3.6 log_{10} IU/mL represented LLoQ + 4σ and 3.9 log_{10} IU/mL represented LLoQ + 6σ with a range interval of 2σ.
Paired samples evaluable for clinical concordance analysis were included in this table.
[a] Assumed independence between all samples.

DNA sequencing on representative samples from subjects with results consistently offset by more than 1 log_{10} IU/mL DNA level did not reveal any sequence mismatches for any primer or probe targets for **cobas®** BKV. Discordant results were defined as those that are more than one box away from the diagonal (indicated by shading). For Target Not Detected (TND) by LDT Column Agreement the **cobas®** BKV Target Not Detected and < LLoQ (< 3.0) cells were combined. The rationale for adding the adjacent < LLoQ and TND cells for the TND column is that the difference between a TND and < LLoQ is not clinically meaningful and that these are analytically at the lower end of the measuring range, which may be impacted by random error. Of the 66 BKV DNA-negative samples collected for the estimation of the NPA with the **cobas®** BKV 61 provided valid results, all 61 samples were negative by **cobas®** BKV, therefore the NPA was 100% with the 95% Exact CI of 94.1% to 100%.

Concordance between **cobas®** BKV and the comparator BKV LDT was also evaluated using different clinical thresholds (Table 40).

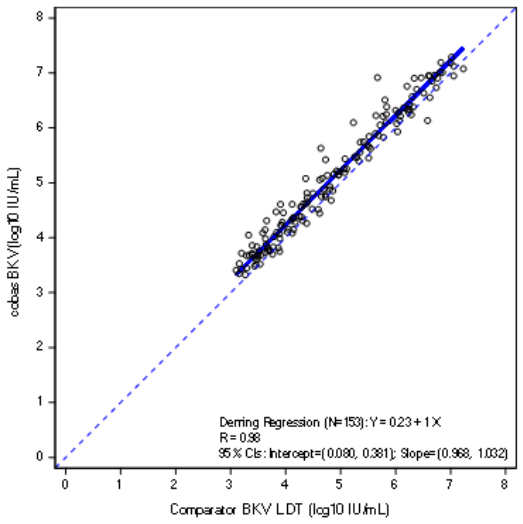
Table 40 Summary of concordance of **cobas**® BKV and comparator BKV LDT using different thresholds for all samples (stabilized urine)

Threshold*	Percent Agreement < Threshold 95% CI (n/N)	Percent Agreement ≥ Threshold 95% CI (n/N)
Target Not Detected	93.9% (62/66) (85.4%, 97.6%)	97.5% (236/242) (94.7%, 98.9%)
LLoQ (3.0 $\log_{10}$ IU/mL)	97.9% (94/96) (92.7%, 99.4%)	99.5% (211/212) (97.4%, 99.9%)
4.0 $\log_{10}$ IU/mL	90.9% (130/143) (85.1%, 94.6%)	99.4% (164/165) (96.6%, 99.9%)
7.0 $\log_{10}$ IU/mL	97.2% (242/249) (94.3%, 98.6%)	94.9% (56/59) (86.1%, 98.3%)

Note: Samples with Target Not Detected results were categorized as < threshold value in IU/mL.
LLoQ = lower limit of quantitation of Comparator BKV LDT (1000 IU/mL = 3.0 $\log_{10}$ IU/mL).
95% confidence interval (CI) calculated by Score method assuming independence between all samples.
* Thresholds of 10,000 IU/mL = 4.0 $\log_{10}$ IU/mL and 10,000,000 IU/mL = 7.0 $\log_{10}$ IU/mL.

From all samples tested with **cobas**® BKV that were BKV positive with the comparator BKV test, there were a total of 153 neat urine samples stabilized in **cobas**® PCR Media from 55 transplant subjects evaluable for the correlation analysis at the three testing sites (Figure 9).

Figure 9 Correlation between **cobas**® BKV and comparator BKV LDT for all samples: Deming linear regression plot of DNA levels ($\log_{10}$ IU/mL) (stabilized urine)



Additional bias plot analysis of DNA level differences indicated a systematic difference between both assays that is constant across the overlapping linear range. The 95% CI of the intercept of the fitted line in the bias plots was (0.168 to 0.488), which is within ± 0.5 $\log_{10}$ IU/mL. Furthermore, the mean bias was estimated at 0.231 $\log_{10}$ IU/mL and using the equation of the fitted line in the bias plots, the systematic difference between both assays was 0.248 $\log_{10}$ IU/mL and 0.188 $\log_{10}$ IU/mL for samples with DNA levels at 4 $\log_{10}$ IU/mL and 7 $\log_{10}$ IU/mL, respectively.

System equivalency / system comparison

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 Systems was demonstrated via performance studies. The results presented in the Instructions for Use support equivalent performance for all systems.

Additional information

Key test features

Sample type	EDTA plasma	Urine stabilized in **cobas**® PCR Media
Minimum amount of sample required	350 µL*	550 µL*
Sample processing volume	200 µL	400 µL
Analytical sensitivity	21.5 IU/mL (two-sided 95% confidence interval: 16.3 IU/mL – 32.4 IU/mL)	12.2 IU/mL (two-sided 95% confidence interval: 9.2 IU/mL – 18.3 IU/mL)
Linear range	21.5 IU/mL to 1E+08 IU/mL	200 IU/mL to 1E+08 IU/mL
Subtypes detected	BKV Subtypes I (with Subgroups Ia, Ib and Ic), II, III and IV	BKV Subtypes I (with Subgroups Ia, Ib and Ic), II, III and IV

*Dead volume of 150 µL identified for the **cobas**® **omni** Secondary tubes. Other tubes used for testing may have different dead volume and require more or less minimum volume. Contact your local Roche service representative for further information.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 41 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	# Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and distributor

Table 42 Manufacturer and distributor

Roche Molecular Systems, Inc.
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Made in USA

Distributed by

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(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

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Document revision

Document Revision Information	
Doc Rev. 1.0 11/2022	First Publishing.
Doc Rev. 2.0 05/2023	Added footnote to **Table 23**, added column and footnote to **Table 24** specifying tested drug concentration. Return minimum sample volume to original level in **Instructions for use** and **Key test features** section. Updated **cobas**® branding. Minor wording corrections. Please contact your local Roche Representative if you have any questions.