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 or P/N: 09040951190
cobas® Buffer Negative Control Kit	P/N: 07002238190 or P/N: 09051953190

Table of contents

Additional information 46

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.

In EDTA plasma, **cobas**® BKV is intended for use as an aid in the diagnosis and 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 diagnosis and management of BKV in transplant patients.

The results from **cobas**® BKV must be interpreted within the context of all relevant clinical and laboratory findings.

cobas® BKV is not intended for use as a screening test for blood or blood products.

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 copies/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, commonly defined as a measurement above 10,000 copies/mL, 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

Polyomavirus 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. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to BKV 1st WHO International Standard (NIBSC code: 14/212). Each Amplification/Detection kit lot is calibrated traceable to 1st BKV WHO International Standard (NIBSC code: 14/212).

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 systems 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.

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® BKV

cobas® 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

cobas® EBV/BKV Control Kit
Store at 2–8°C

For use on the **cobas**® 5800 system, and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09040951190)
For use on the **cobas**® 6800/8800 systems with software version 1.4 (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. <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 dust/fumes/gas/ mist/vapours/spray. 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-4-isothiazolin-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 dust/fumes/gas/ mist/vapours/spray. 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-4-isothiazolin-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

cobas® Buffer Negative Control Kit
Store at 2-8°C

For use on the **cobas®** 5800 system, and **cobas®** 6800/8800 systems with software version 2.0 or higher (P/N 09051953190)
For use on the **cobas®** 6800/8800 systems with software version 1.4 (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 EUH032: Contact with acids liberates very toxic gas.	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe burns and eye damage. H411: Toxic to aquatic life with long lasting effects. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face 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 system 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 or cobas® 6800/8800 systems

Reagents loaded onto the **cobas**® 5800 system or **cobas**® 6800/8800 systems are stored at appropriate temperatures their expiration is monitored and enforced by the system. The system allows reagents to be used only if all of the reagent handling conditions shown in Table 6, Table 7 and Table 8 are met. The system automatically prevents use of expired reagents. Remaining open-kit stability and number of kit uses information for assay specific reagents is accessible through the system user interface.

Table 6 Reagent expiry conditions monitored and enforced by the **cobas**® 5800 system

Reagent	Open-kit stability	Number of kit uses	On-board stability
cobas® BKV	90 days from first usage	40	36 days from loading
cobas® EBV/BKV Control Kit	single use vial	8	36 days from loading
cobas® Buffer Negative Control Kit	single use vial	16	36 days from loading

Table 7 Reagent expiry conditions monitored and enforced by the **cobas**® 6800/8800 systems

Reagent	Open-kit stability	Number of kit uses	On-board stability (outside on board refrigerator)
cobas® BKV	90 days from first usage	40	40 hours from loading
cobas® EBV/BKV Control Kit	single use vial	8	8 hours from loading
cobas® Buffer Negative Control Kit	single use vial	16	10 hours from loading

Table 8 shows the open-kit stability of the **cobas**® **omni** reagents. Prior to each run, the system verifies the open-kit stability and ensures sufficient fill volume. Therefore, these reagents have no number of kit uses or on-board stability assigned.

Table 8 **cobas® omni** reagent expiry condition monitored and enforced by the **cobas®** 5800/6800/8800 systems

Reagent	Open-kit stability
cobas® omni Lysis Reagent	30 days from loading
cobas® omni MGP Reagent	30 days from first usage
cobas® omni Specimen Diluent	30 days from loading
cobas® omni Wash Reagent	30 days from loading

Additional materials required for the cobas® 5800/6800/8800 systems

Table 9 Materials for use on the **cobas®** 5800/6800/8800 systems

Material	P/N
cobas® omni Lysis Reagent	06997538190
cobas® omni MGP Reagent	06997546190
cobas® omni Specimen Diluent	06997511190
cobas® omni Wash Reagent	06997503190

Table 10 Consumables for use on **cobas®** 5800 system*

Material
cobas® omni Processing Plate 24
cobas® omni Liquid Waste Plate 24
cobas® omni Amplification Plate 24
Tip CORE TIPS with Filter, 1ml
Tip CORE TIPS with Filter, 300µL
cobas® omni Liquid Waste Container
Solid Waste Bag or Solid Waste Bag With insert
16-position tube S-carrier complete
5-position Rack Carrier

*For Part Numbers please refer to the **cobas®** 5800 system User Assistance.

Table 11 Consumables for use on the **cobas®** 6800/8800 systems*

Material
cobas® omni Processing Plate
cobas® omni Amplification Plate
cobas® omni Pipette Tips
cobas® omni Liquid Waste Container
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer
MPA RACK 16 MM LIGHT GREEN 2001-2050**,***

*For Part Numbers please refer to the **cobas®** 6800/8800 systems User Assistance

** 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 12 Urine specimen collection kits used with **cobas®** BKV

Collection Kit	P/N
cobas® PCR Urine Sample Kit	05170486190
cobas® PCR Media Kit	06466281190
cobas® PCR Media Secondary Tube Kit*	07958048190
cobas® PCR Media Tube Replacement Cap Kit*	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)*	07958064190

* Contact your local Roche representative for details

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, the **cobas**® 6800/8800 systems software and **cobas**® BKV analysis package (ASAP) for the **cobas**® 5800/6800/8800 systems must be installed.

For **cobas**® 5800 and the **cobas**® 6800/8800 systems with software 2.0 or higher, the x800 Data Manager software and PC (or server) will be provided with the system.

For the **cobas**® 6800/8800 systems software version 1.4, the Instrument Gateway (IG) server will be provided with the system.

Table 13 Instrumentation

Equipment	P/N
cobas® 5800 system	08707464001
cobas® 6800 system	05524245001 and 09575154001
cobas® 8800 system	05412722001 and 09575146001
Sample Supply Module for **cobas**® 6800/8800 systems	06301037001 and 09936882001

Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems – User Assistance for additional information

Note: Contact your local Roche representative for a detailed order list for primary and secondary sample tubes, for sample ra cks, 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.
- 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 0.5% 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 or potassium hypochlorite 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 0.5% sodium or potassium hypochlorite.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

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 test kits, **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 or potassium hypochlorite 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 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 0.5% 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**® 5800 system or **cobas**® 6800/8800 systems – User Assistance to properly clean and decontaminate the surface of instrument(s).

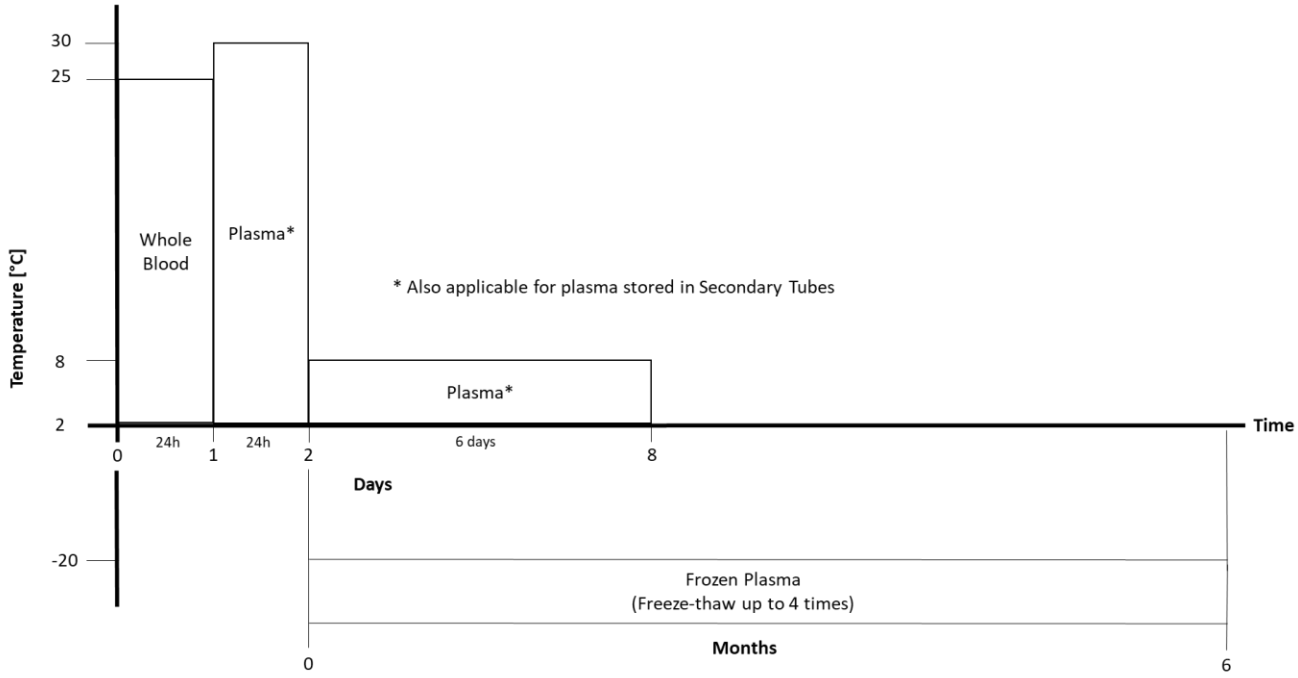
Sample collection, transport, and storage

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 ≤ -20°C.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -20°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.

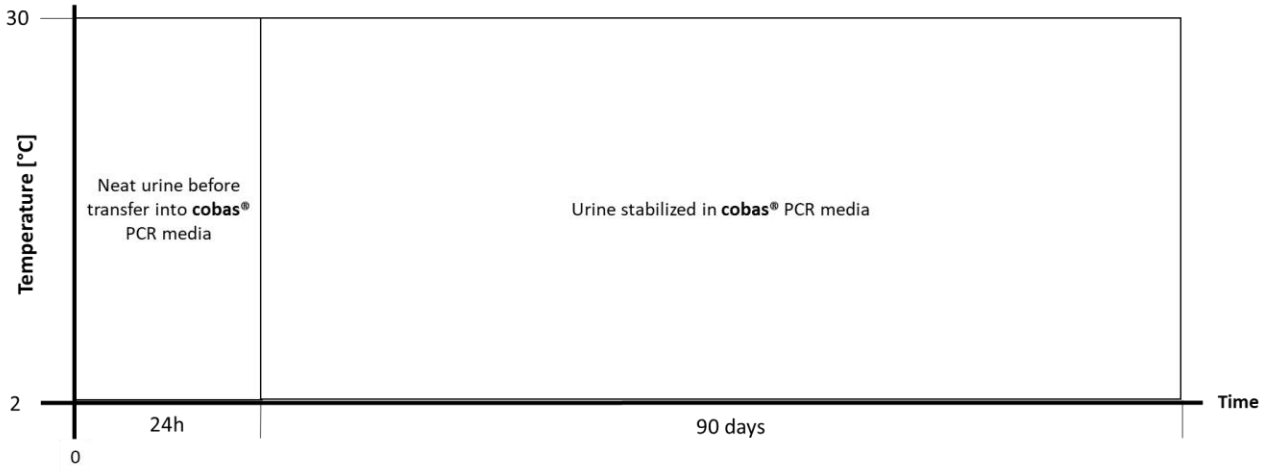
Figure 1 Sample storage conditions for EDTA plasma



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.
- **cobas**® BKV can be run with a minimum required sample volume of 350 µL for EDTA plasma (for the 200 µL sample workflow) and 550 µL for stabilized urine specimens (for the 400 µL sample workflow).
- 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).

Running cobas® BKV on the cobas® 5800/6800/8800 systems

- The operation of the instruments is described in detail in the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance.
- Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper maintenance of instruments.
- 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 2 and described in Figure 4, step 1.
- 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 Assistance for proper barcode specifications and additional information on loading sample tubes.

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

1

Log onto the system
Press Start to prepare the system

2

Loading samples onto the system
- Load sample racks onto the system
- The system prepares automatically
- Order tests
  - Choose “Plasma” for ordering EDTA plasma specimens
  - Choose “Urine” for ordering urine specimens collected in **cobas**® PCR Media
    - Uncap tube
    - Transfer tube directly to rack

3

Refill reagents and consumables as prompted by the system
- Load test specific reagent cassette(s)
- Load control mini racks
- Load processing tips
- Load elution tips
- Load processing plates
- Load liquid waste plates
- Load amplification plates
- Load MGP cassette
- Refill specimen diluent
- Refill lysis reagent
- Refill wash reagent

4

Start the run by choosing the Start processing button on the user interface, all subsequent runs will start automatically if not manually postponed

5

Review and export results

6

Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use

Clean up the instrument
- Unload empty control mini racks
- Unload empty test specific reagent cassette(s)
- Empty amplification plate drawer
- Empty liquid waste
- Empty solid waste

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

1	Log onto the system Press Start to prepare the system Order Tests • Choose “Plasma” for ordering EDTA plasma specimens • Choose “Urine” for ordering urine specimens collected in **cobas®** PCR Media
2	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette • Load control cassettes • Load pipette tips • Load processing plates • Load MGP reagent • Load amplification plates • Refill specimen diluent • Refill lysis reagent • Refill wash reagent
3	Loading specimens onto the system • For primary urine specimens collected in **cobas®** PCR Media o Uncap tube o Transfer tube directly to rack • Load sample rack and clot tip racks into the sample supply module • Confirm samples have been accepted into the transfer module
4	Start the run by choosing the Start manually button on the user interface or have it start automatically after 120 minutes or if the batch is full
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use Clean up instrument • Unload empty control cassettes • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

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 and cobas® 6800/8800 systems with software version 2.0 or higher

- One **cobas®** Buffer Negative Control [(-) Ctrl] and two **cobas®** EBV/BKV 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.
- The results of the controls are shown in the "Controls" app.
- In the software and/or report, check for flags to ensure the validity of the corresponding test results (refer to the x800 Data Manager User Assistance for a 'List of flag codes').
- Controls are marked with "Valid" in the column "Control result" if the respective target of the controls are reported valid. Controls are marked with "Invalid" in the column "Control result" if the respective target of the controls 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.

Validation of results is performed automatically by the instrument software based on control results.

NOTE: The **cobas®** 5800 system and the **cobas®** 6800/8800 systems with software version 2.0 or higher 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.

Quality control and validity of results on the cobas® 6800/8800 systems software version 1.4

- One **cobas®** Buffer Negative Control [(–) Ctrl] and two **cobas®** EBV/NKV 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 software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas®** 6800/8800 systems User Assistance.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch is required.

Validation of results is performed automatically by the instrument software based on control results.

Control flags on the cobas® 6800/8800 systems with software version 1.4

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 14 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.

Interpretation of results for cobas® 5800/6800/8800 systems

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 15 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 and cobas® 6800/8800 with software version 2.0 or higher

The results of the samples are shown in the "Results" app of the software.

For a valid control batch, check each individual sample for flags in the 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 the respective Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if the respective 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 shown in Table 15 above.
- If one or more sample targets are marked with "Invalid" the 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 with software version 1.4

For a valid batch, check each individual sample for flags in the 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 15 above.

Procedural limitations

- **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 time points (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.
- **cobas**® BKV is not intended for use as a screening test for the presence of BKV in blood or blood products.

Non-clinical performance evaluation

System equivalency

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

Key performance characteristics for EDTA plasma sample type

Limit of Detection (LoD) WHO International Standard

The limit of detection of **cobas**® BKV for the 1st 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 16 through Table 18. 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 16 Limit of detection in EDTA plasma, Lot 1

Input titer concentration (BKV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
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 17 Limit of detection in EDTA plasma, Lot 2

Input titer concentration (BKV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
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 18 Limit of detection in EDTA plasma, Lot 3

Input titer concentration (BKV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
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		

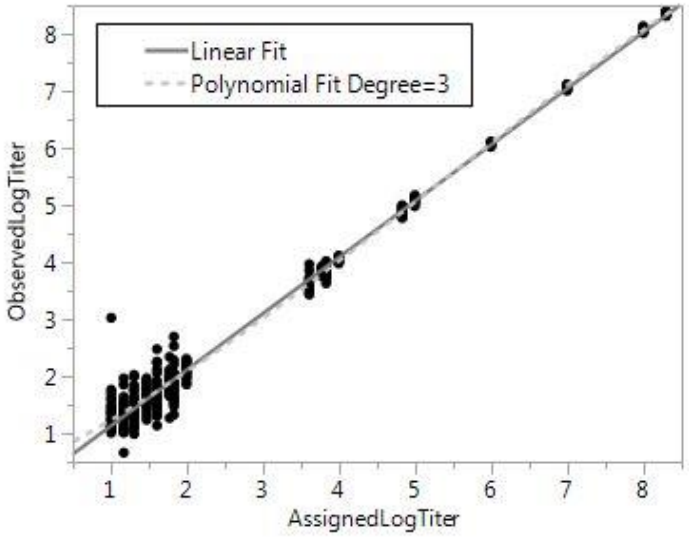
Linear range

Linearity of **cobas®** BKV 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 5.

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 5). Across the linear range, the accuracy of the test was within ± 0.2 $\log_{10}$.

Figure 5 Linear range determination in EDTA plasma



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 19.

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 19 Within-laboratory precision of **cobas**® BKV*

Nominal Concentration [IU/mL]	Assigned Concentration [IU/mL]	EDTA plasma			
		Lot 1	Lot 2	Lot 3	All lots
		SD	SD	SD	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.

Subtype verification

The performance of **cobas**® BKV on BKV subtypes I (with subgroups Ia and Ic), II, III and IV was evaluated by:

- Verification of the limit of detection
- Verification of the linear range

Verification of limit of detection for subtypes I (with subgroups Ia and Ic), II, III and IV

BKV DNA for the five different subtypes/subgroups (Ia, Ic, II, III 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, multiple runs, days, operators, and instruments. These results verify that **cobas**® BKV detected BKV DNA for five different subtypes/subgroups at concentrations of 21.5 IU/mL with a hit rate of ≥ 95%.

Verification of linear range for subtypes I (with subgroups Ia and Ic), 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 EDTA plasma.

The linear range of **cobas**® BKV was verified for all five subtypes/subgroups (Ia, Ic, II, III and IV).

Specificity

The specificity of **cobas**® BKV was determined by analyzing BKV-negative EDTA plasma samples from individual donors. 104 individual EDTA plasma samples were tested with three lots of **cobas**® BKV reagents. All samples tested negative for BKV DNA. In the test panel the specificity of **cobas**® BKV was 100% (lower one-sided 95% confidence interval: 97.16%).

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 20. 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 20 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	-	-

Analytical specificity – interfering substances

Elevated levels of triglycerides (33 g/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 21 were tested at three times the C_{max} in presence 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 21 Drug compounds tested for interference with the quantitation of BKV DNA by **cobas**® BKV

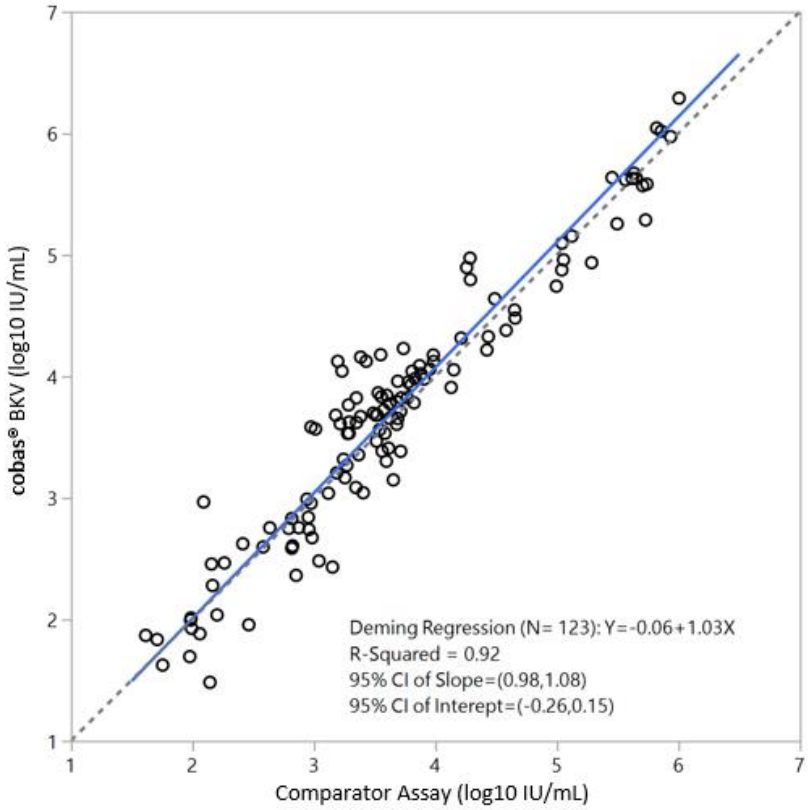
Class of drug	**Generic drug name**	
Antimicrobial	Cefotetan	Sulfamethoxazole
	Clavulanate potassium	Ticarcillin disodium
	Fluconazole	Trimethoprim
	Piperacillin	Vancomycin
	Tazobactam sodium	Micafungin
Compounds for Treatment of Herpes Viruses	Ganciclovir	Cidofovir
	Valganciclovir	Foscarnet
	Acyclovir	Letermovir
Immune suppressant	Azathioprine	Prednisone
	Cyclosporine	Sirolimus
	Everolimus	Tacrolimus
	Mycophenolate mofetil	Mycophenolic acid

Method correlation

The performance of **cobas**® BKV was assessed against a comparator assay by analyzing EDTA plasma specimens from BKV-infected patients. EDTA plasma specimens within the quantitation range of both tests, were tested as single replicates. Deming regression analysis was performed.

The Deming regression results are shown in Figure 6.

Figure 6 Regression analysis of **cobas**® BKV versus comparator assay



Whole system failure

The whole system failure rate for **cobas**® BKV was determined by testing 100 replicates of EDTA plasma spiked with a BKV positive clinical specimen. These samples were tested at a concentration of 3 x LoD.

The results of this study determined that all replicates were valid and positive for the BKV target, resulting in a whole system failure rate of 0% (upper one-sided 95% confidence interval 2.95%).

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 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

Limit of Detection (LoD) 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 22 through Table 24. 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 22 Limit of detection in urine, Lot 1

Input titer concentration (BKV DNA IU/mL)*	Number of valid replicates	Number of positives	Hit rate in %
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 23 Limit of detection in urine, Lot 2

Input titer concentration (BKV DNA IU/mL)*	Number of valid replicates	Number of positives	Hit rate in %
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 24 Limit of detection in urine, Lot 3

Input titer concentration (BKV DNA IU/mL)*	Number of valid replicates	Number of positives	Hit rate in %
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.

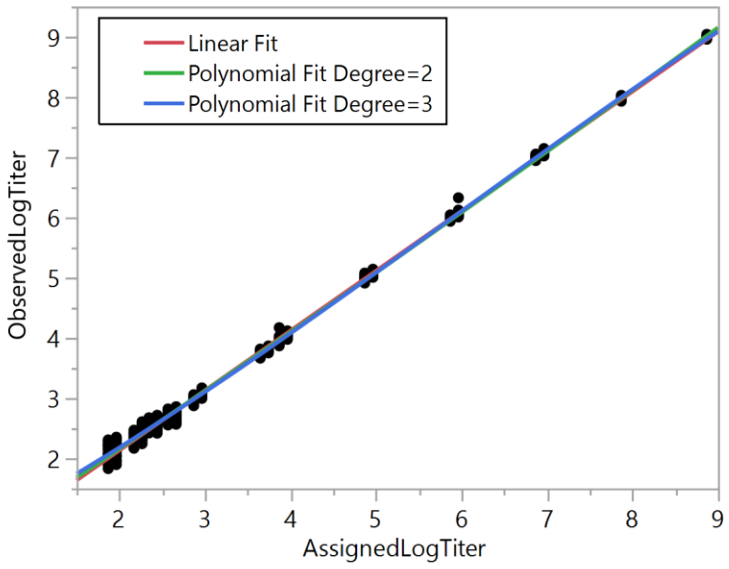
Linear range

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 $\log_{10}$.

Figure 7 Linear range determination in urine



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 25.

cobas® BKV showed high precision for three lots of reagents tested across a concentration range of 7.41E+02 IU/mL to 7.41E+05 IU/mL.

Table 25 Within-laboratory precision of **cobas**® BKV*

Nominal Concentration [IU/mL]	Assigned Concentration [IU/mL]	Urine stabilized in cobas® PCR Media			
		Lot 1	Lot 2	Lot 3	All lots
		SD	SD	SD	Pooled SD
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

* 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.

Subtype verification

The performance of **cobas**® BKV on BKV subtypes I (with subgroups Ia and Ic), II, III and IV was evaluated by:

- Verification of the limit of detection
- Verification of the linear range

Verification of limit of detection for subtypes I (with subgroups Ia and Ic), II, III and IV

BKV DNA for the five different subtypes/subgroups (Ia, Ic, II, III and IV) were diluted to three different concentration levels in BKV-negative pooled 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, multiple runs, days, operators, and instruments. These results verify that **cobas**® BKV detected BKV DNA for five different subtypes/subgroups at concentrations of 12.2 IU/mL with a hit rate of ≥ 95%.

Verification of linear range for subtypes I (with subgroups Ia and Ic), 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 linear range of **cobas**® BKV was verified for all five subtypes/subgroups (Ia, Ic, II, III and IV).

Specificity

The specificity of **cobas**® BKV was determined by analyzing BKV-negative urine samples stabilized in **cobas**® PCR Media from individual donors. One hundred individual urine samples were tested with three lots of **cobas**® BKV reagents. All samples tested negative for BKV DNA. In the test panel the specificity of **cobas**® BKV was 100% (lower one-sided 95% confidence interval: 97.05%).

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 26. 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 26 Microorganisms tested for cross-reactivity

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

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 27 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 27 Drug compounds tested for interference with the quantitation of BKV DNA by **cobas**® BKV

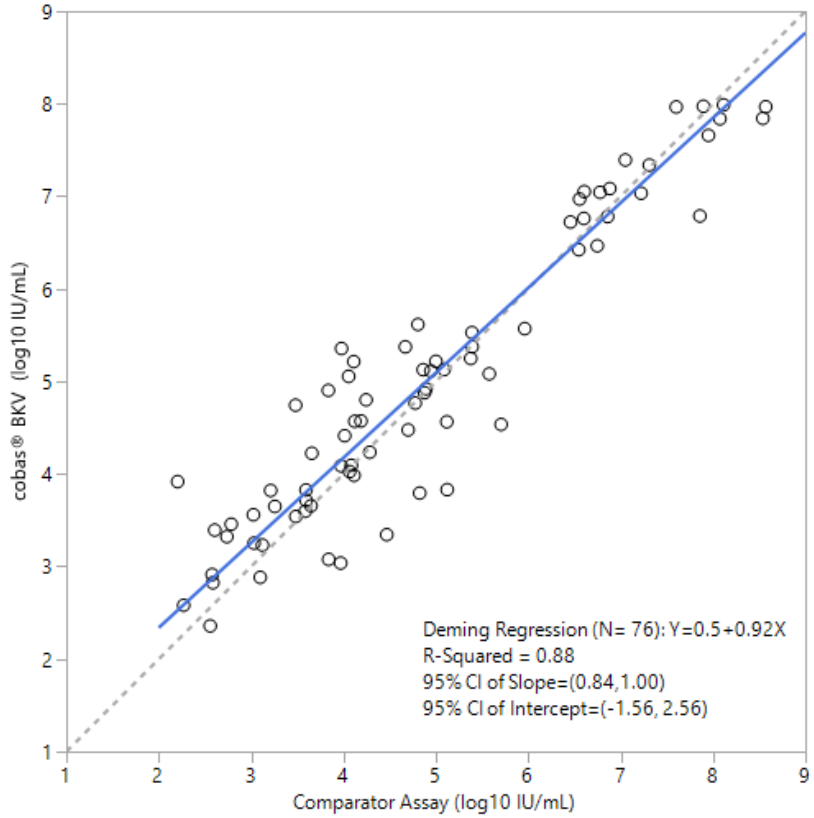
Class of drug	Active Ingredient	Concentration	Generic drug name
Antimicrobial	Clotrimazole	100 µg/mL	Gyne-Lotrimin 7
	Metronidazole	701 µmol/L	Arilin rapid Vaginal suppositories Vagi Metro Cream Nidazea Gel
Estrogen steroid hormone	Estradiol	4.41 nmol/L	Estrace
Analgesics	Phenazopyridine Hydrochloride	200 µg/mL	Azo Standard
	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
	Naproxen	2170 µmol/L	Naproxen
	Ibuprofen	2425 µmol/L	Ibuprofen
Not applicable	Talc	0.05% (w/v)	Talcum powder

Method correlation

The performance of **cobas**® BKV was assessed against a comparator assay by analyzing urine specimens from BKV-infected patients. Urine specimens within the quantitation range of both tests, were tested as single replicates. Deming regression analysis was performed.

The Deming regression results are shown in Figure 8.

Figure 8 Regression analysis of **cobas**® BKV versus comparator assay



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 sample 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

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 28.

Table 28 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

Expected BKV DNA Concentration (log_{10} IU/mL)	Observed Mean[a] BKV DNA Concentration (log_{10} IU/mL)	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 Log-normal 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

[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

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

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 BKV 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 29).

Table 29 Concordance analysis between **cobas®** BKV and the comparator LDT on BKV DNA level results for all samples

cobas® BKV (log_{10} IU/mL)	**Comparator BKV LDT (log_{10} IU/mL) Target Not Detected**	**Comparator BKV LDT (log_{10} IU/mL) < LLoQ (< 2.3)**	**Comparator BKV LDT (log_{10} IU/mL) 2.3 to < 3.0**	**Comparator BKV LDT (log_{10} IU/mL) 3.0 to < 3.7**	**Comparator BKV LDT (log_{10} IU/mL) 3.7 to 4.4**	**Comparator BKV LDT (log_{10} IU/mL) > 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%)	(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). 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 30).

Table 30 Summary of concordance of **cobas**® BKV and comparator BKV LDT using different thresholds for all samples

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 a Target Not Detected results were categorized as <threshold value in IU/mL.

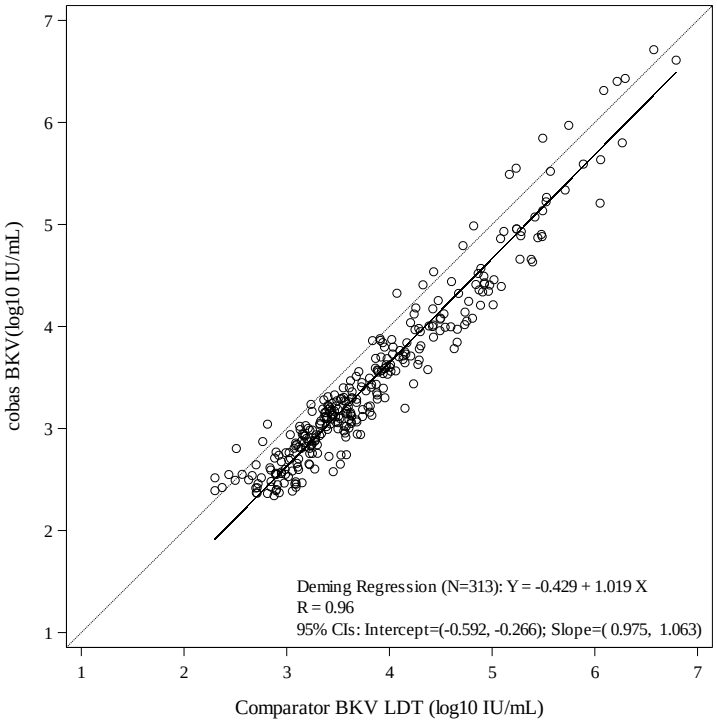
LLoQ = lower limit of quantitation of Comparator BKV LDT (200 IU/mL = 2.3 $\log_{10}$ IU/mL).

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 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)



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 31.

Table 31 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 32).

Table 32 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 33).

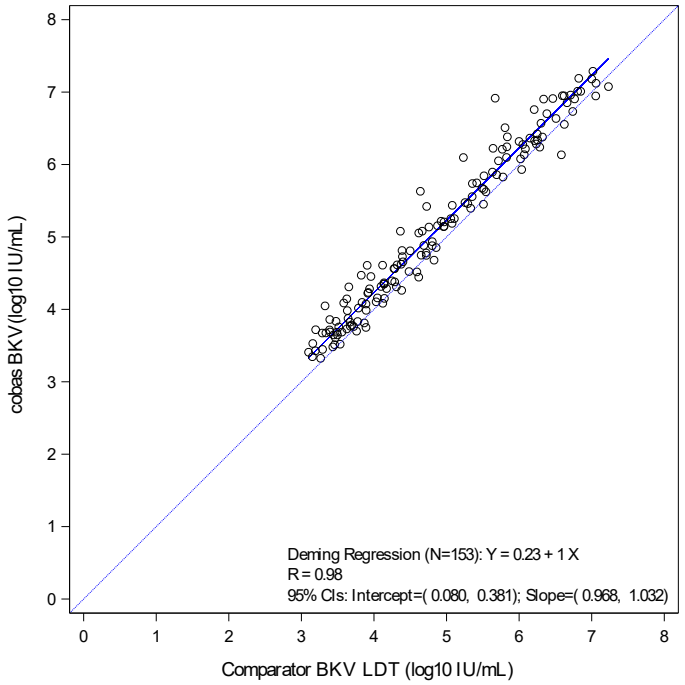
Table 33 Summary of concordance of **cobas**® BKV and comparator BKV LDT using different thresholds for all samples (stabilized urine)

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 10).

Figure 10 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.

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
Specificity	100%	100%
Subtypes detected	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 34 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 For USA: Caution: 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 importer

Table 35 Manufacturer and importer

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876, USA
www.roche.com

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

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

Copyright

CE 2797

EC REP Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

IVD

References

1. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective. Preface. *Bone Marrow Transplant*. 2009;44:453-5. PMID: 19861977.
2. Green M. Introduction: Infections in solid organ transplantation. *Am J Transplant*. 2013;13 Suppl 4:3-8. PMID: 23464993.
3. Morel V, Martin E, Francois C, et al. A Simple and Reliable Strategy for BK Virus Subtyping and Subgrouping. *J Clin Microbiol*. 2017;55:1177-85. PMID: 28151406.
4. Egli A, Infanti L, Dumoulin A, et al. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis*. 2009;199:837-46. PMID: 19434930.
5. Pinto M, Dobson S. BK and JC virus: a review. *J Infect*. 2014;68 Suppl 1:S2-8. PMID: 24119828.
6. Hirsch HH, Randhawa PS, AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation-Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant*. 2019;33:e13528. PMID: 30859620.
7. Ambalathingal GR, Francis RS, Smyth MJ, Smith C, Khanna R. BK Polyomavirus: Clinical Aspects, Immune Regulation, and Emerging Therapies. *Clin Microbiol Rev*. 2017;30:503-28. PMID: 28298471.
8. Yamada Y, Tsuchiya T, Inagaki I, Seishima M, Deguchi T. Prediction of Early BK Virus Infection in Kidney Transplant Recipients by the Number of Cells With Intranuclear Inclusion Bodies (Decoy Cells). *Transplant Direct*. 2018;4:e340. PMID: 29464201.
9. Nickeleit V, Singh HK. Polyomaviruses and disease: is there more to know than viremia and viruria? *Curr Opin Organ Transplant*. 2015;20:348-58. PMID: 25933251.
10. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene*. 1990;93:125-8. PMID: 2227421.
11. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. *Nature*. 1995;373:487-93. PMID: 7845459.
12. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis. *Cell*. 1995;80:869-78. PMID: 7697717.
13. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology (N Y)*. 1992;10:413-7. PMID: 1368485.
14. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res*. 1996;6:986-94. PMID: 8908518.
15. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. U.S. Department of Health and Human Services, Public Health Services, Centers for Disease Control and Preventions, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
16. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

17. Goetsch HE, Zhao L, Gnegy M, et al. Fate of the Urinary Tract Virus BK Human Polyomavirus in Source-Separated Urine. *Appl Environ Microbiol*. 2018;84. PMID: 29374036.

18. Boan P, Hewison C, Swaminathan R, et al. Optimal use of plasma and urine BK viral loads for screening and predicting BK nephropathy. *BMC Infect Dis*. 2016;16:342. PMID: 27448566.

Document revision

Document Revision Information	
Doc Rev. 4.0 12/2024	Added system software version 2.0 information for **cobas**® 6800/8800 systems. Added NIBSC code: 14/212 for WHO International Standard. Updated display of example results on **cobas**® 6800/8800 systems with software version 1.4. P/Ns of consumables removed, detailed information on consumables are referenced in the **cobas**® 5800 and **cobas**® 6800/8800 systems User Assistance. Removed Rx Only from front page. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.

The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed.