January 29, 2021

Roche Molecular Systems, Inc.
Raji Grewal
Sr. Regulatory Specialist
4300 Hacienda Drive
Pleasanton, California 94588-2722

Re: K203220
    Trade/Device Name: cobas BKV
    Regulation Number: 21 CFR 866.3183
    Regulation Name: Quantitative Viral Nucleic Acid Test for Transplant Patient Management
    Regulatory Class: Class II
    Product Code: QLX
    Dated: October 30, 2020
    Received: November 2, 2020

Dear Raji Grewal:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Maria Garcia, Ph.D.
Branch Chief
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
    and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number *(if known)*
K203220

Device Name
cobas® BKV

Indications for Use *(Describe)*

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

In EDTA plasma, cobas® BKV is intended for use as an aid in the management of BKV in transplant patients. In patients undergoing monitoring of BKV in EDTA plasma, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess viral response to treatment.

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

The results from cobas® BKV are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Test results must not be the sole basis for patient management decisions.

cobas® BKV is not intended for use as a screening test for blood or blood products or human cells, tissues, and cellular and tissue-based products (HCT/Ps).

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

cobas® BKV
510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

Submitter Name	Roche Molecular Systems, Inc.
Address	4300 Hacienda Drive Pleasanton, CA 94588-2722
Contact	Raji Grewal Phone: (925) 368-0246 FAX: (925) 225-0207 Email: raji.grewal@roche.com
Date Prepared	October 30, 2020
Proprietary Name	cobas® BKV for use on cobas® 6800/8800 Systems
Classification Name	Quantitative viral nucleic acid test for transplant patient management
Product Codes	QLX: 21 CFR 866.3183
Predicate Devices	cobas® BKV (K202215)
Establishment Registration	Roche Molecular Systems, Inc. (2243471)

1. DEVICE DESCRIPTION

cobas® BKV (**Figure 1**) is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800/8800 software which assigns test results for all tests as either target not detected, BKV DNA detected < LLoQ (lower limit of quantitation), BKV DNA detected > ULoQ (upper limit of quantitation), or a value in the linear range LLoQ < x < ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

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).[1-3]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 amplicons 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.[4,5]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.

Figure 1: cobas® BKV for use on cobas® 6800/8800 Systems
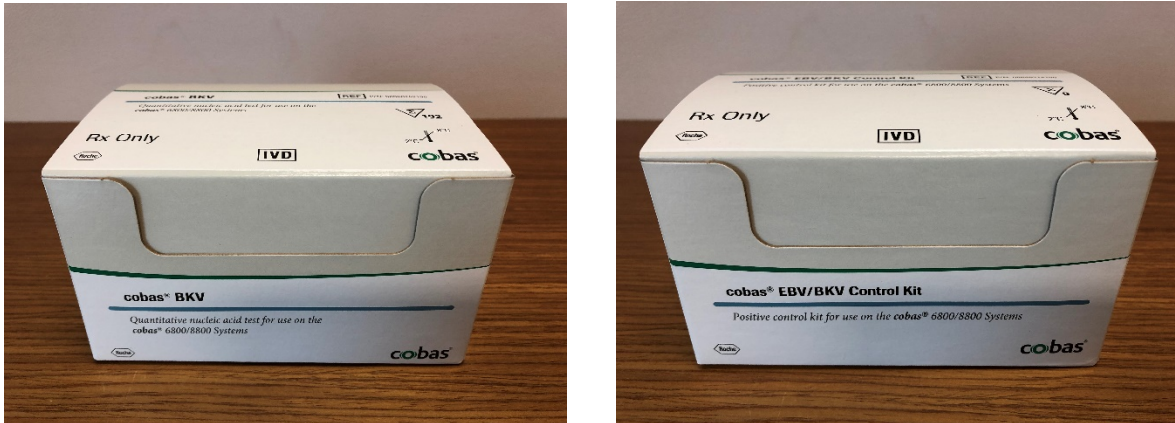
2. INDICATIONS FOR USE

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

In EDTA plasma, cobas® BKV is intended for use as an aid in the management of BKV in transplant patients. In patients undergoing monitoring of BKV in EDTA plasma, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess viral response to treatment.

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

The results from cobas® BKV are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Test results must not be the sole basis for patient management decisions.

cobas® BKV is not intended for use as a screening test for blood or blood products or human cells, tissues, and cellular and tissue-based products (HCT/Ps).

3. TECHNOLOGICAL CHARACTERISTICS

The primary technological characteristics and intended use of the RMS **cobas®** BKV for use on the **cobas®** 6800/8800 Systems are similar to the identified predicate device, **cobas®** BKV (K202215)(Table 1).

Table 1: Comparison of the cobas® BKV for use on the cobas® 6800/8800 Systems with the Predicate Device

	Submitted Device: cobas® BKV	Predicate Device: cobas® BKV (K202215)
Regulation Number	21 CFR 866.3183	Same
Regulation Name	Quantitative viral nucleic acid test for transplant patient management	Same
Product Code	QLX	Same
Intended Use	cobas® BKV is an in vitro nucleic acid amplification test for the quantitation of BK virus (BKV) DNA in human EDTA plasma and urine stabilized in cobas® PCR Media on the cobas® 6800/8800 Systems. In EDTA plasma, cobas® BKV is intended for use as an aid in the management of BKV in transplant patients. In patients undergoing monitoring of BKV in EDTA plasma, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess viral response to treatment. In urine stabilized in cobas® PCR Media, cobas® BKV is intended for use as an aid in the management of BKV in transplant patients. The results from cobas® BKV are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Test results must not be the sole basis for patient management decisions. cobas® BKV is not intended for use as a screening test for blood or blood products or human cells, tissues, and cellular and tissue-based products (HCT/Ps).	cobas® BKV is an in vitro nucleic acid amplification test for the quantitation of BK virus (BKV) DNA in human EDTA plasma on the cobas® 6800/8800 Systems. cobas® BKV is intended for use as an aid in the management of BKV in transplant patients. In patients undergoing monitoring of BKV in EDTA plasma, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess viral response to treatment. The results from cobas® BKV are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Test results must not be the sole basis for patient management decisions. cobas® BKV is not intended for use as a screening test for blood or blood products or human cells, tissues, and cellular and tissue-based products (HCT/Ps).
Conditions for use	For prescription use	Same
Sample Types	EDTA – plasma, Urine	EDTA - plasma
Analyte Targets	BK Virus	Same
Sample Preparation Procedure	Automated by cobas® 6800/8800 Systems	Same
Amplification Technology	Real-time PCR	Same
Detection Chemistry	Paired reporter and quencher fluorescence labeled probes (TaqMan Technology) using fluorescence resonance energy transfer (FRET)	Same

	Submitted Device: cobas® BKV	Predicate Device: cobas® BKV (K202215)
Controls used	Sample processing control (QS) Positive and negative control	Same
Result Analysis	Based on PCR cycle threshold analysis	Same

4. NON-CLINICAL PERFORMANCE EVALUATION

4.1. Limit of Detection (LoD)

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

4.1.1. 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 2 through Table 4. 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 2:   Limit of Detection in Urine, Lot 1

Input Titer Concentration (BKV DNA IU/mL)*	Number of Valid replicates (N)	Number of Positives (n)	Hit Rate (n/N) x100
40.0	63	63	100.0
20.0	63	63	100.0
10.0	63	60	95.2
5.0	63	47	74.6
2.5	63	25	39.7
1.25	63	26	41.3
0	63	0	0.0

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

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

Table 3:   Limit of Detection in Urine, Lot 2

Input titer Concentration (BKV DNA IU/mL)*	Number of Valid Replicates (N)	Number of Positives (n)	Hit Rate (n/N) x100
40.0	63	63	100.0
20.0	63	63	100.0
10.0	63	60	95.2
5.0	63	42	66.7
2.5	63	32	50.8
1.25	63	17	27.0
0	63	0	0.0

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

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

Table 4:   Limit of Detection in Urine, Lot 3

Input Titer Concentration (BKV DNA IU/mL)*	Number of Valid replicates (N)	Number of Positives (n)	Hit Rate (n/N) x100
40.0	63	63	100.0
20.0	63	63	100.0
10.0	63	61	96.8
5.0	63	46	73.0
2.5	63	39	61.9
1.25	63	19	30.2
0	63	0	0.0

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

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

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

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

The combined results from three lots shown in Table 5 verify that – consistent with an LoD of 12.2 IU/mL – cobas® BKV detected BKV DNA for subgroups Ia and Ic, and subtypes II, III and IV at a concentration of 12.2 IU/mL with a ≥ 95% hit rate.

Table 5:   BKV DNA Subgroups Ia, Ic and Subtypes II, III and IV Verification of Limit of Detection in Urine

Genotype	Test Concentration	Number of Valid Replicates (N)	Number of Positives (n)	Hit Rate (n/N)x100
Subgroup Ia	6.1 IU/mL	63	53	84.1%
Subgroup Ia	12.2 IU/mL	63	61	96.8%
Subgroup Ia	18.3 IU/mL	63	62	98.4%
Subgroup Ic	6.1 IU/mL	63	50	79.4%
Subgroup Ic	12.2 IU/mL	63	62	98.4%
Subgroup Ic	18.3 IU/mL	63	63	100.0%
Subtype II	6.1 IU/mL	63	56	88.9%
Subtype II	12.2 IU/mL	63	61	96.8%
Subtype II	18.3 IU/mL	63	63	100.0%

Genotype	Test Concentration	Number of Valid Replicates (N)	Number of Positives (n)	Hit Rate (n/N)x100
Subtype III	6.1 IU/mL	63	60	95.2%
Subtype III	12.2 IU/mL	63	62	98.4%
Subtype III	18.3 IU/mL	63	63	100.0%
Subtype IV	6.1 IU/mL	63	54	85.7%
Subtype IV	12.2 IU/mL	63	63	100.0%
Subtype IV	18.3 IU/mL	63	63	100.0%

4.3. 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 2.

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 log10 in pooled urine stabilized in cobas® PCR Media (see Figure 2). Across the linear range, the accuracy of the test was within ± 0.2 log10.

The lower limit of quantitation (LLoQ) was set to 200 IU/mL, to include the mean deviation between the observed vs. the assigned log10 titer (Accuracy) being equal or less than ±+/-0.3 log10, based on the upper 95% confidence interval of the worst performing lot using clinical specimen and calculated based on a goal for acceptable total analytical error (TAE) of ≤ 1.0 log10, where TAE = |bias| + 2 standard deviations in alignment with the CLSI EP-17A guideline, and TAE = SQUARE ROOT(2) x 2 standard deviations based on the "difference between 2 measurements" approach.

Based on the LLoQ and the determined linear range, as well as the medical value the linear measurement range of the test was set to 2.0E+02 IU/mLLL21 to-1.0E+08 IU/mL. The results of calculation and claimed LLoQ are shown in Table 7.

Figure 2:   Linear Range Determination in Urine
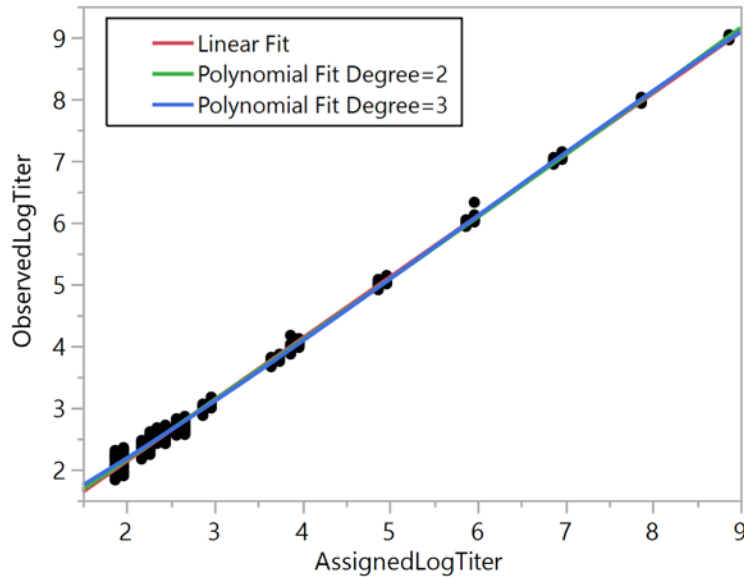
4.4.   Linearity for subgroups Ia, Ic and subtypes II, III and IV

The dilution series used in the verification of subtype/subgroup linearity study of cobas® BKV consisted of eight panel members spanning the linear range of the assay. Testing was conducted with three lots of cobas® BKV reagent, 12 replicates per level were tested in urine stabilized in cobas® PCR Media. The results of the study are presented in Table 6.

The linearity within the linear range of cobas® BKV was verified for subgroups Ia, Ic and subtypes II, III and IV. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.2 log10.

Table 6:   Linearity Verification on Subgroups Ia, Ic and Subtypes II, III and IV in Urine

Genotype	Linear Regression	Better Fitting Higher Order Model Regression	Maximum Difference Between Linear Regression and the Better Fitting Higher Order Model ($\log_{10}$ IU/mL)
Subgroup Ia	y = 0.9756016x + 0.2367214	y = -0.0081339x^3 + 0.1373308x^2 + 0.2602376x + 1.3507964	0.12
Subgroup Ic	y = 0.9773177x + 0.2051674	y = -0.005087x^3 + 0.0902742x^2 + 0.4865276x + 0.9928655	0.10
Subtype II	y = 0.9762885x + 0.2271355	y = -0.0072558x^3 + 0.1259492x^2 + 0.3032666x + 1.2971372	0.12

Genotype	Linear Regression	Better Fitting Higher Order Model Regression	Maximum Difference Between Linear Regression and the Better Fitting Higher Order Model ($\log_{10}$ IU/mL)
Subtype III	y = 0.9762129x + 0.2270439	$y = -0.0081255x^3 + 0.1370235x^2 + 0.2645895x + 1.327922$	0.12
Subtype IV	y = 0.9758502x + 0.2028958	$y = -0.0086141x^3 + 0.1427957x^2 + 0.2499516x + 1.2930774$	0.13

4.5. Lower Limit of Quantitation

The analysis for LLoQ of cobas® BKV in urine was performed with data obtained from the Linearity study at concentration levels of 100 IU/mL, 200 IU/mL and 300 IU/mL. The LLoQ was set at the concentration level of 200 IU/mL to include the mean deviation between the observed vs. the assigned log10 titer (Accuracy) being equal or less than ± 0.3 log10, based on the upper 95% confidence interval of the worst performing lot using clinical specimen. The LLoQ within the linear range meets the acceptance criterion for the Total Analytical Error (|Bias| + 2x SD) (TAE). The TAE criterion is $\leq$ 1 log10.

The results of calculation and claimed LLoQ are shown in Table 7. The LLoQ is 200 IU/mL.

Table 7: LLoQ of cobas® BKV using Clinical Sample (Urine)

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

Lot	Nominal Concentration (IU/mL)	$\log_{10}$ Titer Assigned	Mean $\log_{10}$ Titer Observed	SD ($\log_{10}$)	Absolute Bias	TAE (|Bias| + 2x SD)	Difference Between Measurements in SD (= SQRT(2) x 2x SD)
3 Lots combined	2.00E+02	2.27	2.42	0.08	0.16	0.32	0.23
3 Lots combined	1.00E+02	1.96	2.14	0.12	0.18	0.41	0.33

4.6. 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 8. The results of the variance component estimation are shown in Table 9.

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 8:   Within-laboratory Precision of cobas® BKV* (in Stabilized Urine)

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

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

Table 9: Lognormal Percent Coefficient of Variation (%CV) of cobas® BKV by Positive Panel and Contributing Components of Variance in Urine*

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

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

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

4.7. 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, TCID50/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 10. 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 log10 titer of each of the positive BKV samples containing potentially cross-reacting organisms was within ± 0.5 log10 of the mean log10 titer of the respective positive spike control.

Table 10: Microorganisms tested for cross-reactivity in urine

Bacteria	Yeast	Viruses
Bacillus cereus	Candida albicans	Herpes Simplex Virus-2
Bacillus subtilis	Candida glabrata	Human Papillomavirus 16
Chlamydia trachomatis	Candida parapsilosis	-
Corynebacterium diphteriae	Candida tropicalis	-

Bacteria	Yeast	Viruses
Enterobacter cloacae	-	-
Enterococcus faecalis	-	-
Enterococcus faecium	-	-
Escherichia coli	-	-
Klebsiella pneumoniae	-	-
Lactobacillus acidophilus	-	-
Lactobacillus crispatus	-	-
Lactobacillus jensenii	-	-
Lactobacillus vaginalis	-	-
Morganella morganii	-	-
Mycoplasma genitalium	-	-
Neisseria gonorrhoeae	-	-
Proteus mirabilis	-	-
Pseudomonas aeruginosa	-	-
Staphylococcus aureus	-	-
Staphylococcus epidermidis	-	-
Staphylococcus saphrophyticus	-	-
Streptococcus agalactiae	-	-
Streptococcus bovis	-	-
Streptococcus oralis/viridans	-	-
Streptococcus pneumoniae	-	-
Treponema pallidum	-	-
Trichomonas vaginalis	-	-
Ureaplasma urealyticum	-	-

4.8. 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 4.3 mL 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 urine 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 11 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 log10 titer of each of the positive BKV samples containing potentially interfering substances was within ± 0.5 log10 of the mean log10 titer of the respective positive spike control.

Table 11: Drug Compounds Tested for Interference with the Quantitation of BKV DNA by cobas® BKV in Urine

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

4.9.   Cross contamination

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

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

5. CLINICAL PERFORMANCE EVALUATION

5.1. Reproducibility of cobas® BKV

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 12.

Table 12: 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} copies/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, two 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.

5.2. Clinical Performance of cobas® BKV

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 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 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 13).

Table 13: **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

cobas® BKV (log₁₀ 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
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; LDT = laboratory developed test; BKV = BK virus.

Standard Deviation of Comparator BKV LDT estimated at 0.15 log₁₀ IU/mL (comparator BKV LDT validation study).

Analyte concentration of 3.3 log₁₀ IU/mL represented LLoQ + 2σ, 3.6 log₁₀ IU/mL represented LLoQ + 4σ and 3.9 log₁₀ IU/mL represented LLoQ + 6σ with a range interval of 2σ.

Paired samples evaluable for clinical concordance analysis were included in this table.

aAssumed independence between all samples.

DNA sequencing performed on representative samples from subjects with results consistently offset by more than 1 log₁₀ 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 14).

Table 14: 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 3**).

Figure 3: Correlation Between cobas® BKV and Comparator BKV LDT for All Samples: Deming Linear Regression Plot of DNA Levels ($\log_{10}$ IU/mL) (Stabilized Urine)

95% CIs: Intercept=(0.080, 0.381); Slope=(0.968, 1.032)
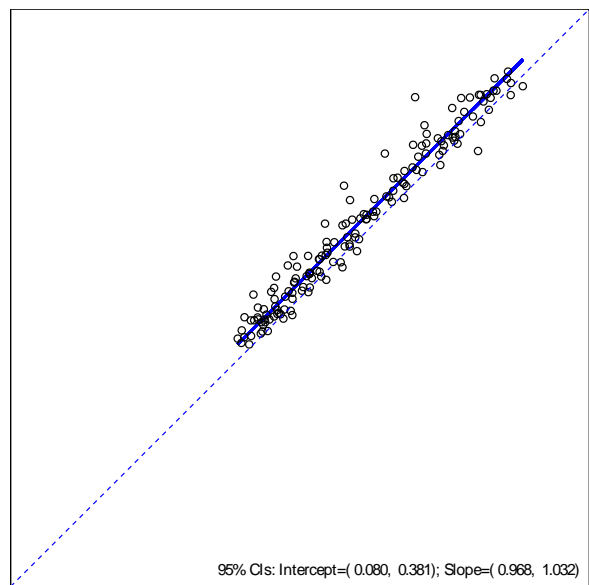
Additional bias plot analysis of DNA level differences indicated a systematic difference between both assays that is constant across the overlapping linear range. The 95% CI of the intercept of the fitted line in the bias plots was (0.168 to 0.488), which is within ±0.5 log10 IU/mL. Furthermore, the mean bias was estimated at 0.231 log10 IU/mL and using the equation of the fitted line in the bias plots, the systematic difference between both assays was -0.248 log10 IU/mL and 0.188 log10 IU/mL for samples with DNA levels at 4 and 7 log10 IU/mL, respectively.

6.   CONCLUSIONS

The results of non-clinical analytical and clinical performance studies demonstrate that cobas® BKV for use on the cobas® 6800/8800 Systems is as safe, as effective, and performs as well as the predicate device .

7.   REFERENCES

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