September 2, 2020

Roche Molecular Systems, Inc.
Rita Hoady
Senior Manager, Regulatory Affairs
4300 Hacienda Drive
Pleasanton, California 94588-2722

Re: K202215
Trade/Device Name: cobas BKV, cobas EBV/BKV Control Kit, cobas Buffer Negative Control Kit
Regulation Number: 21 CFR 866.3183
Regulation Name: Quantitative Viral Nucleic Acid Test for Transplant Patient Management
Regulatory Class: Class II
Product Code: QMI
Dated: August 5, 2020
Received: August 6, 2020

Dear Rita Hoady:

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,

For:

Uwe Scherf, M.Sc., Ph.D.
Director
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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K202215

Device Name
cobas BKV for use on the cobas® 6800/8800 Systems

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 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, 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).

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.

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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	Rita Hoady Phone: (925) 730-8397 FAX: (925) 225-0207 Email: rita.hoady@roche.com
Date Prepared	August 5, 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	QMI: 21 CFR 866.3183
Predicate Devices	**cobas**® EBV (DEN200015)
Establishment Registration	Roche Molecular Systems, Inc. (2243471)

1. DEVICE DESCRIPTION

cobas® BKV 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.

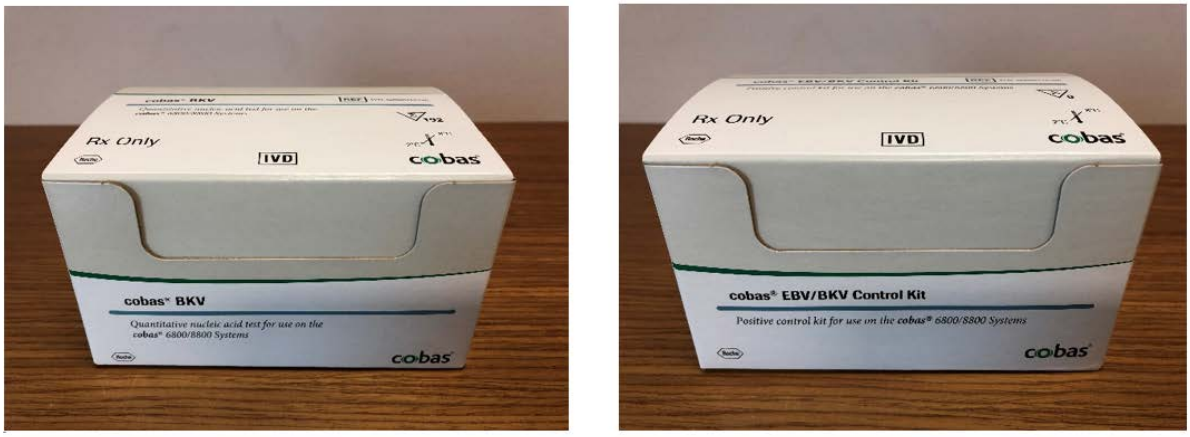
Nucleic acid from patient samples and added lambda DNA-QS molecules is simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent

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 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, 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).

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**® EBV (DEN200015) (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® EBV (DEN200015)**
Regulation Number	21 CFR 866.3183	Same
Regulation Name	Quantitative viral nucleic acid test for transplant patient management	Same
Product Code	QMI	QLX
Intended Use	**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, 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 donors of blood or blood products or human cells, tissues, and cellular and tissue-based products (HCT/Ps).	**cobas®** EBV is an in vitro nucleic acid amplification test for the quantitation of Epstein-Barr virus (EBV) DNA in human EDTA plasma on the **cobas®** 6800/8800 Systems. **cobas®** EBV is intended for use as an aid in the management of EBV in transplant patients. In patients undergoing monitoring of EBV, serial DNA measurements can be used to indicate the need for potential treatment changes and to assess response to treatment. The results from **cobas®** EBV are intended to be read and analyzed by a qualified licensed healthcare professional in conjunction with clinical signs and symptoms and relevant laboratory findings. Negative test results do not preclude EBV infection or EBV disease. Test results must not be the sole basis for patient management decisions. **cobas®** EBV is not intended for use as a screening test for donors of 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	Same
Analyte Targets	BK Virus	Epstein-Barr virus
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® EBV (DEN200015)
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 21.5 IU/mL for EDTA plasma.

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)[6], 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 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 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 2: 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 3: 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	63	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 4: Limit of Detection in EDTA Plasma, Lot 3

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

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

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

The combined results from three lots shown in Table 5 verify that – consistent with an LoD of 21.5 IU/mL – **cobas®** BKV detected BKV DNA for subgroups Ia and Ic, and subtypes II, III and IV at a concentration of 21.5 IU/mL with a ≥ 95% hit rate.

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

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

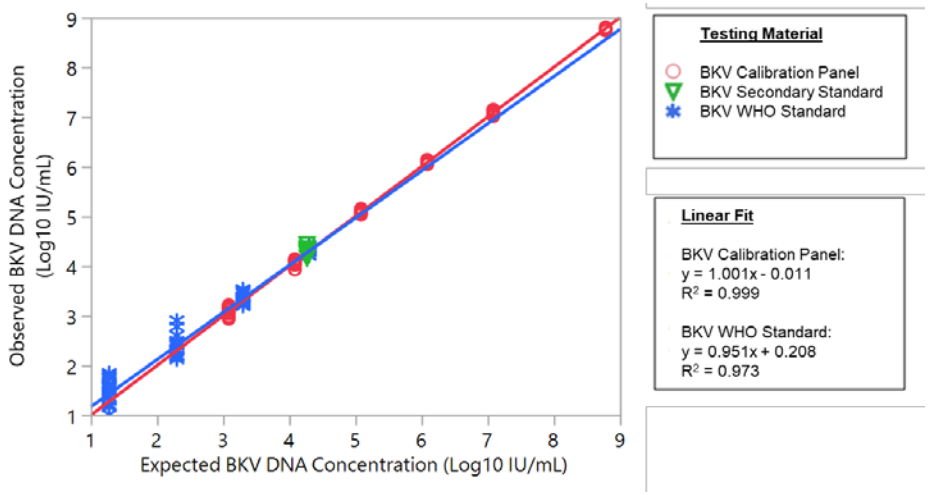
4.3. Traceability to the 1st WHO International Standard for BK Virus for Nucleic Acid Amplification Techniques (NAT)-based Assays

Several standards and controls have been used during development of this test to provide traceability to the WHO standard [the 1st WHO International Standard for BK Virus DNA (NIBSC 14/212)[6]]. The standards used during development of the test include the BKV WHO Standard, the RMS BKV Secondary Standard, and the RMS BKV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the BKV WHO Standard was from 1.90E+01 IU/mL to 2.00E+04 IU/mL (1.28-4.30 $\log_{10}$ IU/mL), the RMS BKV Secondary Standard was tested at 1.86E+04 IU/mL (4.27 $\log_{10}$ IU/mL), and the RMS BKV Calibration Panel was tested from 1.00E+03 IU/mL to 5.00E+08 IU/mL (3.00-8.70 $\log_{10}$ IU/mL).

The calibration and standardization process of **cobas**® BKV provides quantitation values for the calibration panel, the RMS BKV Secondary Standard, and the BKV WHO Standard that are

similar to the expected values with deviation of not more than 0.19 $\log_{10}$ IU/mL (Figure 2). The maximum deviation was obtained at 19.0 IU/mL (approximately LLoQ).

Figure 2: Traceability to WHO International Standard [Bivariate Fit of Observed BKV DNA Concentration (log10 IU/mL) by Expected BKV DNA Concentration (log10 IU/mL)] using cobas® BKV



4.4. 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 3.

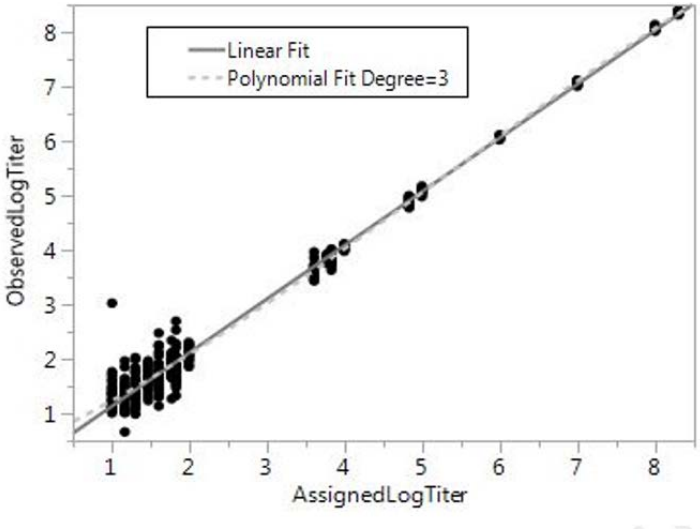
cobas® BKV was demonstrated to be linear from 1.01E+01 to 1.97E+08 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less or equal than ± 0.1 $\log_{10}$ in human EDTA plasma (see Figure 3). Across the linear range, the accuracy of the test was within ± 0.2 $\log_{10}$.

The lower limit of quantitation (LLoQ) is 21.5 IU/mL, calculated based on a goal for acceptable total analytical error (TAE) of ≤ 1.0 $\log_{10}$, where TAE = |bias| + 2 standard

deviations in alignment with the CLSI EP-17A guideline, and TAE = SQUARE ROOT(2) x 2 standard deviations based on the “difference between 2 measurements” approach.

Based on the LLoQ and the determined linear range, as well as the medical value the linear measurement range of the test was set to 21.5-1.0E+08 IU/mL. The results of calculation and claimed LLoQ are shown in Table 6.

Figure 3: Linear Range Determination in EDTA Plasma



4.5. Linearity for Subgroups Ia, Ic and subtypes II, III and IV

The dilution series used in the verification of subgroup/subtype linearity study of **cobas**® BKV consisted of eight panel members spanning the linear range of the assay. Testing was conducted with three lots of **cobas**® BKV reagent, 12 replicates per level were tested in EDTA plasma. The results of the study are presented in Table 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 $\log_{10}$.

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

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

4.6. Lower Limit of Quantitation

The analysis for LLoQ was performed with data obtained from the LoD study at concentration levels of 19.0 IU/mL, 38.0 IU/mL and 80.0 IU/mL. The LLoQ is the lowest titer within the linear range that is not lower than the LoD and meets the acceptance criteria for the Total Analytical Error (|Bias| + 2x SD) (TAE) and the difference between two measurements. The acceptance criteria for both is ≤ 1.0 log_{10} IU/mL.

The results of calculation and claimed LLoQ are shown in Table 7 the lower limit of quantitation (LLoQ) is 21.5 IU/mL.

Table 7: Lower Limit of Quantitation (LLoQ) of cobas® BKV using the 1st WHO International Standard for BK Virus (BKV) (NIBSC 14/212)

Lot	Nominal concentration (IU/mL)	log10 titer nominal	Mean log10 titer observed	SD (log10)	Absolute Bias	TAE (|Bias| + 2x SD)	Difference between Measurements in SD (= SQRT(2) x 2x SD)
1	19.0	1.28	1.39	0.25	0.11	0.61	0.71
1	38.0	1.58	1.62	0.25	0.04	0.53	0.69
1	80.0	1.90	1.89	0.26	0.01	0.52	0.73
2	19.0	1.28	1.50	0.26	0.22	0.74	0.74
2	38.0	1.58	1.76	0.21	0.18	0.60	0.59
2	80.0	1.90	2.02	0.27	0.11	0.65	0.76
3	19.0	1.28	1.47	0.27	0.19	0.72	0.75
3	38.0	1.58	1.66	0.26	0.08	0.59	0.72
3	80.0	1.90	1.91	0.19	0.00	0.38	0.53
3 Lots combined	19.0	1.28	1.45	0.26	0.18	0.69	0.73
3 Lots combined	38.0	1.58	1.68	0.24	0.10	0.57	0.67
3 Lots combined	80.0	1.90	1.94	0.24	0.04	0.52	0.68

4.7. 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. Six 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 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 5.90E+01 IU/mL to 9.83E+05 IU/mL.

Table 8: Within-laboratory Precision of cobas® BKV*

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

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

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

Nominal concen-tration Titer (IU/mL)	Nominal concen-tration Log10 titer (IU/mL)	Assigned concen-tration Titer (IU/mL)	Assigned concen-tration Log10 titer (IU/mL)	N	Instrument/ Operator %CV	Lot %CV	Day %CV	Run %CV	Within Run %CV	Total %CV
1.00E+06	6.00	9.83E+05	5.99	72	2%	5%	3%	2%	5%	8%
1.00E+05	5.00	9.83E+04	4.99	71	3%	6%	3%	0%	8%	11%
1.00E+04	4.00	9.83E+03	3.99	70	3%	7%	5%	3%	9%	13%
6.00E+03	3.78	5.90E+03	3.77	72	2%	8%	2%	1%	8%	12%
1.00E+02	2.00	9.83E+01	1.99	72	5%	8%	6%	4%	24%	26%
6.00E+01	1.78	5.90E+01	1.77	71	4%	14%	7%	15%	29%	36%

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

4.8. 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 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 $\log_{10}$ 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 10: 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 Type 1	*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	-	-

4.9. Interfering Substances

Elevated levels of triglycerides (37 mmol/L), conjugated bilirubin (0.2 g/L), unconjugated bilirubin (0.2 g/L), albumin (60 g/L), hemoglobin (2 g/L) and human DNA (2 mg/L) in samples were tested in the presence (100 IU/mL) and absence of BKV DNA. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® BKV.

In addition, drug compounds listed in Table 11 were tested at three times the C_{max} in presence (100 IU/mL) and absence of BKV DNA.

All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with **cobas**® BKV for all samples without BKV target and positive results were obtained on all of the samples with BKV target. Furthermore, the mean

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

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

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

4.10. 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%).

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 per site, 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 of three reagent lots for 5 days. One run per operator (1 run = 1 batch; 1 batch = 1 panel + 3 controls) was performed each day and 3 replicates of each panel member were included in each run. The evaluation results are summarized in Table 12.

Table 12: Attributable Percentage of Total Variance, Total Precision Standard Deviation, 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 excellent 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} copies/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%.

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 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 13).

Table 13: 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
3.8 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).
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 14).

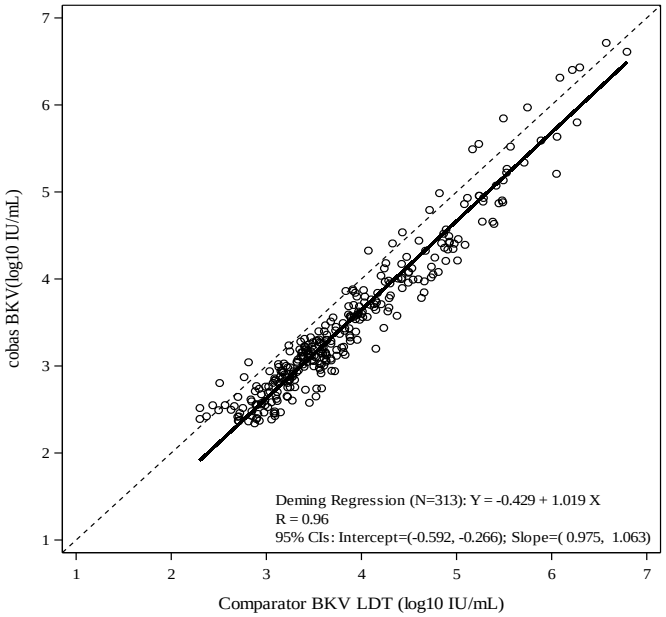
Table 14: Summary of Concordance of cobas® BKV and Comparator BKV LDT using Different Thresholds for All Samples

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 categorised as <threshold value in IU/mL.
LLoQ = lower limit of quantitation of Comparator BKV LDT (200 IU/mL = 2.3 log_{10} IU/mL).
95% confidence interval (CI) calculated by Score method assuming independence between all samples.
* Thresholds of 1000 IU/ml = 3.0 Log_{10} IU/ml and 10,000 IU/ml = 4.0 Log_{10} IU/ml.

From all samples tested with **cobas**® BKV that were BKV positive with the comparator BKV test, there were a total of 313 (133 neat and 159 diluted clinical samples from 68 transplant subjects and 21 contrived samples), which were evaluable for the correlation analysis at the three testing sites (Figure 4).

Figure 4: 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 log10 IU/mL (± 2 times analytical precision standard deviation of comparator BKV LDT). Furthermore, the mean bias was estimated at -0.357 log10 IU/mL and using the equation of the fitted line in the bias plots, the systematic difference between both assays was -0.343 log10 IU/mL and -0.362 log10 IU/mL for samples with DNA levels at 3 and 4 log10 IU/mL, respectively.

6. CONCLUSIONS

The conclusions drawn from the nonclinical and clinical studies demonstrate that the device is as safe, as effective, and performs as well as the predicate device.

7. REFERENCES

1. 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. PubMed PMID: 2227421.

2. 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. PubMed PMID: 7845459.

3. Mol CD, Arvai AS, Slupphau G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis. Cell. 1995;80:869-78. PubMed PMID: 7697717.

4. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (NY). 1992;10:413-7. PubMed PMID: 1368485.

5. Heid CA, Stevens J, Livak JK, Williams PM. Real time quantitative PCR. Genome Res. 1996;6:986-94. doi: 10.1101/gr.6.10.986. PubMed PMID: 8908518.

6. National Institute for Biological Standards and Control. 1st WHO International Standard for BK Virus DNA (NIBSC 14/212) version 2.0: Hertfordshire, England; NIBSC, 2016.