ABANALITICA®
ADVANCED BIOMEDICINE
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User Manual

REALQUALITY
RS-BKV

v.2.1

code RQ-S49

Kit for detection
and quantification of
BK virus (BKV)
by Real-Time PCR

CE

IVD

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1 PRODUCT INFORMATION

1.1 Intended use

The REALQUALITY RS-BKV kit is an IVD for detection of the DNA of *BK virus* (BKV). If used in combination with the REALQUALITY RQ-BKV STANDARD, code RQ-50-ST, it allows the quantification of the viral DNA present in the sample.
The test is based on Real-Time PCR on DNA extracted from human clinical samples.
This *in vitro* diagnostic test for detection and quantification of BKV is an auxiliary device for diagnosis and monitoring of BKV infections. It is recommended to use this kit as indicated in the instructions herein.

This manual refers to the following product:

REALQUALITY RS-BKV
Kit for detection and quantification of *BK virus* (BKV) by Real-Time PCR.

This product is in accordance with directive 98/79/EC (Annex III) on *in vitro* diagnostic medical devices (CE marking).

Contains all reagents needed for Real-Time PCR.

Code	Product	PKG
RQ-S49-48	REALQUALITY RS-BKV	48 tests
RQ-S49-96	REALQUALITY RS-BKV	96 tests

2 KIT CONTENT

BOX RG

STORE AT -30 °C TO -20 °C

DESCRIPTION	LABEL	TUBE (T) or CAP COLOR	24 tests	48 tests	96 tests
Mastermix containing PCR reagents	BKV Real time mix	Violet	1 × 540 µL	2 × 540 µL	4 × 540 µL

BOX PC

STORE AT -30 °C TO -20 °C

DESCRIPTION	LABEL	TUBE (T) or CAP COLOR	24 tests	48 tests	96 tests
Positive control BKV (DNA fragment of the BKV genome)	PC BKV	Violet	1 × 50 µL	1 × 50 µL	2 × 50 µL
Positive control BG (DNA fragment of the β-globin gene)	PC BG	Blue	1 × 50 µL	1 × 50 µL	2 × 50 µL
Internal control (DNA fragment of the β-globin gene)	IC		2 × 125 µL	4 × 125 µL	8 × 125 µL

3 STORAGE AND STABILITY OF REAGENTS

Each component of the kit must be stored at the conditions indicated on the label of each box:

Box RG	Store at -30 °C to -20 °C
Box PC	Store at -30 °C to -20 °C

If stored at the recommended temperature all reagents are stable until the expiration date on the box.

Avoid degradation of the BKV Real time mix! The mix should NOT undergo more than two freeze/thaw cycles. If performing runs with low numbers of samples, it is recommended to aliquot the reagent beforehand.
The BKV Real time mix contains fluorescent molecules and should be stored protected from direct light.

In order to avoid degradation of the positive controls and the internal control do NOT let them undergo more than three freeze/thaw cycles. If performing runs with low numbers of samples, it is recommended to aliquot the controls beforehand.

4 PRECAUTIONS FOR USE

- The kit must be used only as an IVD and be handled by qualified technicians who are trained in techniques of molecular biology applied to diagnostics.
- Before using the kit read the user manual carefully and completely.
- Keep the kit protected from heat.
- Please pay particular attention to the expiration date on the label of each box. Do not use any part of the kit past the expiration date.
- The reagents present in the kit must be considered an undividable unit. Do not use them separately or in combination with reagents from other kits or lots.
- The BKV Real time mix must be thawed at room temperature before use. Mix the solution by inverting the tube several times, then centrifuge briefly. Do NOT vortex!
- The positive controls and the internal control must be thawed at room temperature before use. Then centrifuge briefly.
- Work quickly, particularly if preparing the reactions at room temperature. If possible work on ice or on a cooling block.

In case of any doubt concerning storage conditions, box integrity or application of the method, please contact the technical support team at AB ANALITICA:

laboratorio@abanalitica.it

For nucleic acid amplification the user has to take the following precautions:

- Use filter-tips.
- In order to avoid contamination store biological samples, extracted DNA, amplification product and the internal and positive controls included in the kit separate from the BKV Real time mix.
- Set up pre- and post-PCR areas. Do not share instruments or consumables (pipettes, tips, tubes, etc.) between those areas.

- Change gloves frequently.
- Wash the bench surfaces with 5 % sodium hypochlorite.

5 SAFETY RULES

5.1 General safety rules

- Wear disposable gloves when handling reagents and clinical samples. Wash hands after the procedure.
- Do not pipet by mouth.
- No known diagnostic method can ensure the absence of infective agents. Therefore, consider all clinical samples to be potentially infectious and handle them accordingly.
- All devices that come into contact with clinical samples must be considered contaminated and disposed of as such. In case of accidental spilling of samples, clean up with 10 % sodium hypochlorite.

  Material you use to clean must be disposed of in special containers for contaminated products.
- Clinical samples, contaminated materials and products must be decontaminated before disposal.

  It is recommended to use one of the following decontamination methods:

  a) immerse for 30 minutes in a solution of 5 % sodium hypochlorite (1 volume of 5 % sodium hypochlorite solution on 10 volumes of contaminated fluid).

  b) autoclave at 121 °C for at least 2 hours (ATTENTION! Do not autoclave solutions containing sodium hypochlorite!!).

5.2 Safety rules concerning the kit

The risks for use of this kit are related to the single components.

Dangerous components: **none**.

The Material Safety Data Sheet (MSDS) of this device is available upon request.

6 MATERIAL REQUIRED, BUT NOT PROVIDED

6.1 Reagents

- Reagents for DNA extraction;
- DNAse- and RNAse-free sterile water;
- For quantitative analysis: REALQUALITY RQ-BKV STANDARD, code RQ-50-ST.

6.2 Instruments

- Laminar flow cabinets

  Use while preparing the amplification mix in order to avoid contamination. It is recommended to use a different laminar flow cabinet when adding the extracted DNA and the positive controls / quantification standards.
- Micropipettes (range: 0.5 - 10 µL, 2 - 20 µL, 10 - 100 µL, 20 - 200 µL, 100 - 1000 µL)
- Microcentrifuge (max. 12,000 – 14,000 rpm)
- Plate centrifuge (optional)
- Real-Time PCR instrument

  This kit has been validated on:

  - □ Applied Biosystems 7500 Fast / 7500 Fast Dx Real-Time PCR System (ABI 7500 Fast / 7500 Fast Dx – *Applied Biosystems*)
  - □ Applied Biosystems 7300 Real-Time PCR System (ABI 7300 – *Applied Biosystems*)
  - □ Applied Biosystems StepOne / StepOnePlus™ Real-Time PCR System (ABI StepOne / StepOnePlus – *Applied Biosystems*)
  - □ LightCycler® 480 Real-Time PCR System version II (LC 480 – *Roche*)
  - □ LightCycler® 2.0 Real-Time PCR System (LC 2.0 – *Roche*)
  - □ Dx Real-Time System (Bio-Rad Dx – *Bio-Rad*)
  - □ CFX96 Real-Time PCR Detection System (Bio-Rad CFX96 – *Bio-Rad*)

  The kit can be used on instruments that allow 25 µL of reaction volume and can read the fluorescence of the fluorophores FAM and JOE. The JOE fluorescence can also be read in the channels designated for CY3, HEX etc. Compatibility of the device with other commercially available instruments has been asserted. For further information on instrument compatibility please contact the technical support team at AB ANALITICA.

6.3 Disposables

- Talc-free disposable gloves
- Disposable sterile filter-tips (range: 0.5 - 10 µL, 2 - 20 µL, 10 - 100 µL, 20 - 200 µL, 100 - 1000 µL)
- 96-well plates for Real-Time PCR with adhesive optical film, 0.1 - 0.2 mL tubes with optical caps or glass capillaries

7 INTRODUCTION

The *BK virus* (BKV or Human Polyomavirus 1) belongs to the *Polyomaviridae* family. It was first isolated in 1971 from the urine of a renal transplant patient that had the initials “BK”. The virus is widely spread among the population with up to 90 % of adults showing seroconversion and lifelong presence of specific antibodies. Generally, the primary infection is developed during childhood and is usually asymptomatic. Only in rare cases acute respiratory infection or cystitis are observed.
The way of transmission is not yet well defined, but appears to include both aerial transmission (aerosol) and ingestion of materials contaminated with infected urine.
After the first infection, BKV remains latent in the cells of the urogenital tract and other sites (ureter, brain, spleen, B lymphocytes).
In some case the BK virus becomes reactivated. This reactivation is often asymptomatic. A relatively high frequency of reactivation has been found in pregnant women (5 - 10 %) and immunosuppressed patients. Particularly in latter patients the BKV infection is linked to different pathologic conditions such as hemorrhagic cystitis, interstitial nephritis, ureteral stenosis, disseminated vasculopathies, bladder cancer and multiple organ failure.
Interstitial nephritis linked to a BKV infection (BKVAN) was recently discovered as a prominent cause for renal dysfunction after kidney transplant, occurring in 1 - 10 % of transplant recipients. Most cases of BKVAN appear within the first year after the transplant and are due to a massive replication of the virus in the tubular epithelium.
Since the 90’s the increased use of strong immunosuppressive drugs (e.g. Tacrolimus, TAC) has brought about an increase in cases of BKVAN, many of which are confused with an acute rejection of the organ or toxicity-related tissue damage (Agha & Brennan, 2006). Episodes of acute rejection often are answered with an increase of immunosuppression which in turn results in a higher incidence of BKVAN.
Correct diagnosis of BKVAN can reduce the risk of severe renal dysfunction and organ loss that is reported for 10 - 80 % of kidney transplants (Hirsch et al., 2005). Moreover, BKVAN, in contrast to acute transplant rejection, is treated by lowering the dosage of immunosuppressive drugs. Therefore, correct diagnosis is paramount for appropriate treatment of such conditions (Vats et al., 2003).
The most common BKV associated condition (20 - 30 % of cases) in recipients of allogeneic bone marrow transplant is the late on-set hemorrhagic cystitis (HC). Viruria is present before, during and after the cystitis and can also persist after the cure. Viral load in urine and blood has been found to be

significantly higher than during epsisodes of asymptomatic reactivation of the same infection (Pavlakis et al., 2006).
Currently, the less invasive and more sensitive diagnostic method is the detection of the viral genome using the Polymerase Chain Reaction (PCR). In contrast, BKV isolation from tissue cultures is not feasible because of the slow BKV replication cycle, and serological tests are of limited value owing to the prevalence of the virus in the general population.

So far, no antiviral therapies for the BKV infection exist. Therefore, early diagnosis of the infection and systematic monitoring of the viral load are essential. Timely detection of high viral loads in kidney and bone marrow transplant patients would enable to apply the therapeutic measures necessary to prevent the development of conditions linked to BKV infection, and decreasing the risk of damage to the transplanted organ.
By now, kidney transplant programs have established protocols for BKV screening and quantitative tests are increasingly requested. In 2005, a group of experts recommended BKV screening for patients with renal transplants in the first 2 years after surgery (Hirsch et al., 2005). They proposed to use either cytological urine analysis or tests based on nucleic acid detection. Their guidelines include a BKV load cutoff above which further investigation is required: viral loads of $> 10^7$ genome copies/mL in urine or $> 10^4$ genome copies/mL in plasma that persist more than 3 weeks are considered “assumed BKVAN” and should be followed by a renal biopsy.
The Real-Time PCR allows to detect BKV in a short measure of time and to precisely monitor the viral load, thus being one of the most suited methods for the management of Polyomavirus infections.

8 TEST PRINCIPLE

The PCR method (Polymerase Chain Reaction) was the first method of DNA amplification described in literature (Saiki RK et al., 1985). It can be defined as an *in vitro* amplification reaction of a specific part of DNA (target sequence) by a thermostable DNA polymerase.
This technique was shown to be a valuable and versatile instrument of molecular biology: its application contributed to a more efficient study of new genes and their expression and has revolutionized for instance the fields of laboratory diagnostics and forensic medicine.

The Real-Time PCR represents an advancement of this basic research technology, providing the possibility to determine the number of amplified DNA molecules (amplicons) during the polymerase chain reaction (PCR).

In the system at hand monitoring the amplicons is based on primers/probes labeled with fluorescent molecules. These probes contain a reporter fluorophore and a molecule (quencher) that blocks the reporter's specific fluorescence. Fluorescent emission of the reporter is determined by its distance to the quencher. As long as a probe is not bound to a target sequence reporter and quencher are in close proximity and the reporter's fluorescence is blocked. Upon binding to a target sequence quencher and reporter become separated and the reporter can emit fluorescent light which in turn can be detected.

Typically, the main part of a Real-Time PCR run consists of 30 – 50 amplification cycles. A thermocycler equipped with a corresponding detector can record the fluorescence events at each cycle, thus monitoring the reaction in "real time".
The cycle at which the amplicon-related fluorescence becomes clearly distinguishable from the background is specific for each reaction and is correlated to the initial concentration of the target sequence. This cycle is called threshold cycle (Ct). The Ct value is used to determine the initial target concentration with the help of a standard curve. Such a standard curve is created amplifying solutions with known concentrations of the target sequence (Fig. 1).

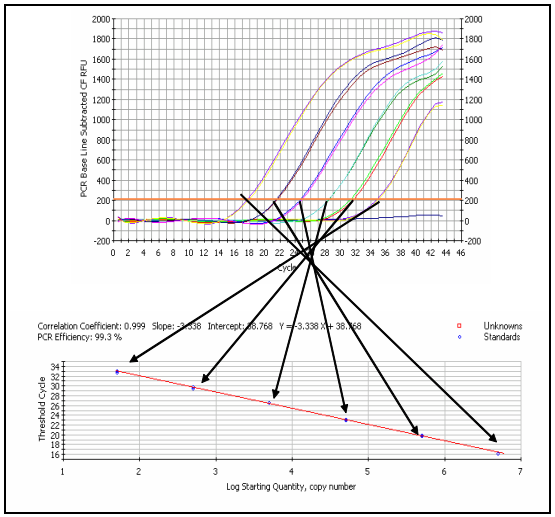

Fig. 1: Creating a standard curve using standards with known concentrations.

The main advantage of Real-Time PCR compared to conventional techniques of amplification is the possibility to perform a semi-automated amplification. This means, extra steps necessary to visualize the amplification product can be avoided and the risk of contamination by post-PCR manipulation is reduced.

9 PRODUCT DESCRIPTION

The REALQUALITY RS-BKV kit, code RQ-S49, is an IVD for detection of *BK virus* (BKV) by amplification of a fragment of the Large T antigen gene.

If used in combination with the product REALQUALITY RQ-BKV STANDARD, code RQ-50-ST, it allows the quantification of viral DNA molecules in the sample by means of a four-point standard curve (10^2 to 10^5 copies of viral DNA per reaction).

The positive controls supplied in this kit contain DNA fragments that correspond to the amplified gene region. As such, these controls are not harmful for the user.

The kit is designed to use an internal control that allows to detect inhibition of the PCR reaction, monitor the extraction process as well as identify false-negative samples. The internal control (the β-globin gene) is amplified in multiplex with the target pathogen. In cellular samples the endogenous β-globin gene is amplified. For acellular specimens the internal control is added as recombinant DNA containing the respective β-globin gene region.

The kit includes a ready-to-use mastermix that contains all reagents needed for the PCR as well as the components listed below.

- ROX™ is an inert colorant that exhibits stable fluorescent properties throughout all amplification cycles. On some Real-Time PCR instruments (Applied Biosystems, Stratagene etc.) it is used for normalization, in order to compensate differences between wells due to pipetting errors or limitations of the instrument.
- The dUTP/UNG system prevents contamination from previous amplification runs. The dUTPs are used to incorporate uracil residues into the amplification product during the amplification session. At the beginning of each new run the UNG enzyme degrades any single or double stranded DNA containing uracil. This way any amplification products from former sessions are eliminated.

10 SAMPLE COLLECTION, MANIPULATION AND PRETREATMENT

The detection of BKV is usually performed on plasma and urine, and in some cases on whole blood.
The device was tested on DNA extracted from plasma, urine and whole blood.

10.1 Blood and plasma

Sample collection should follow common routine, respecting all the usual sterility precautions (e.g. transport in sterile boxes without transport medium).
The blood must be treated with EDTA. Other anticoagulation agents, like heparin, are strong inhibitors of the Taq polymerase and may impair the PCR.
Plasma can be obtained from whole blood by centrifugation at low speed. Tubes with and without gel separators can be used.
Store fresh blood or plasma at +2 °C to +8 °C and extract the nucleic acids within 4 hours. If extraction is not feasible within 4 hours store the samples at -30 °C to -20 °C.

10.2 Urine

Urine must be collected in a sterile container and can be stored at +2 °C to +8 °C for max. 24 hours before processing.

11 PROTOCOL

11.1 DNA extraction

For DNA extraction AB ANALITICA recommends the QIAamp DNA Mini Kit or, for whole blood, the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Refer to the manufacturer's manual for instructions and protocols for the different sample types.
This IVD device can be used with DNA extracted with the most common manual and automated extraction methods.
For further information regarding the compatibility of the device with different extraction methods please contact the technical support at AB ANALITICA.

11.2 Internal control

The kit includes an internal control consisting of a recombinant DNA fragment of the β-globin gene (BG). Use of this control is recommended for analysis of acellular samples. It allows to verify the extraction procedure and detect inhibition of the PCR.
The standardization of the internal control was performed adding 10 µL of internal control to the sample and eluting in a volume of 60 µL.
If the extraction system uses a different final elution volume, adjust the volume of internal control to be added to the sample, accordingly.
For correct use of the internal control follow the instructions provided by the extraction system manufacturer.
For any further information please contact the technical support at AB ANALITICA.

11.3 Instrument programming

11.3.1 Thermal profile and fluorescence reading

Set up the following thermal profile in your instrument:

	Step	Repeats	Time	(°C)
UNG Activation	1	1	02:00	50.0
Taq Activation	2	1	10:00	95.0
Amplification cycles	3	45	00:15	95.0
			01:00	60.0 *

* Fluorescence detection step

The fluorophores to be read are:

- FAM for BKV
- JOE for BG

Select the two detection channels on your Real-Time PCR instrument:

ABI 7500 Fast / 7500 Fast Dx *
ABI 7300 *
ABI StepOne / StepOnePlus *

Name	Reporter Dye	Quencher Dye
BKV	FAM	None
BG (Internal Control)	JOE	None

LC 480

Name	Fluorophore	Filter
BKV	FAM	465 - 510
BG (Internal Control)	JOE	533 - 580

LC 2.0

Name	Fluorophore	Channel
BKV	FAM	530
BG (Internal Control)	JOE	560

Bio-Rad Dx
Bio-Rad CFX96

Name	Fluorophore
BKV	FAM
BG (Internal Control)	JOE

* For instruments that require a passive reference (e.g. Applied Biosystems, Stratagene) make sure to select *ROX* for all wells in use.

Set the final reaction volume.

11.3.2 Setup of samples/controls

Set up samples, control(s) and standards (if needed) in the instrument software. Name each sample, control and standard accordingly.
Be careful to use the same position/order for your samples, controls and standards as for your real samples.

If you want to perform a quantitative analysis, enter the concentrations of the BKV standards (10^2, 10^3, 10^4 and 10^5 viral genome copies/reaction [c/rx]).

If you prefer to obtain quantification results in *viral genome copies per mL of clinical sample* (c/mL) you need to calculate and enter the standard concentrations depending on your extraction parameters. See the table below for examples.

STANDARD		Extraction Parameters		
		Example 1 V_i = 200 µL V_e = 50 µL	**Example 2** V_i = 200 µL V_e = 60 µL	**Example 3** V_i = 200 µL V_e = 100 µL
STANDARD 1	10^2 c/rx	5×10^3 c/mL	6×10^3 c/mL	10^4 c/mL
STANDARD 2	10^3 c/rx	5×10^4 c/mL	6×10^4 c/mL	10^5 c/mL
STANDARD 3	10^4 c/rx	5×10^5 c/mL	6×10^5 c/mL	10^6 c/mL
STANDARD 4	10^5 c/rx	5×10^6 c/mL	6×10^6 c/mL	10^7 c/mL

V_i = initial volume (clinical sample volume used for extraction)
V_e = elution volume

11.4 Preparation of the reaction mix

Thaw the BKV Real time mix. After thawing homogenize the mix by inverting the tube several times. Do not vortex! Centrifuge briefly.

Work rapidly. If possible work on ice or a cooling block and in an area protected from direct light.

Note: A quantitative analysis requires the REALQUALITY RQ-BKV STANDARD, code RQ-50-ST.

Pipet 20 µL of the BKV Real time mix into the corresponding positions (wells of PCR plate, tubes, capillaries etc). Make sure to prepare sufficient positions for all samples, positive controls / quantification standards as well as the negative control.

Pipet 5 µL of extracted DNA, of negative control (sterile H_2O) or of positive controls / quantification standards into the corresponding positions.

Note: Thaw, mix and spin down the controls/standards before use.

Make sure no air bubbles remain in the wells / tubes / capillaries and centrifuge at 4000 rpm for 1 minute.

Load the samples on the instrument making sure to position/load the plate / tubes / capillaries correctly.

11.5 Analysis of results

After the PCR run is finished, view the analysis graph in logarithmic scale. Analyze the amplification results separately for BKV and the β-globin gene (BG). Proceed as follows:

11.5.1 Verify the run

Before interpreting the results of the clinical samples you need to verify the PCR run. Evaluate the controls and/or standard curve according to the tables below.

A. QUALITATIVE ANALYSIS: Evaluating the controls

	RESULT	INTERPRETATION
Positive control BG	Amplification signal in JOE	Control and PCR worked correctly
	No amplification signal in JOE	No amplification of *BG* gene **repeat the analysis**
Positive control BKV	Amplification signal in FAM	Control and PCR worked correctly
	No amplification signal in FAM	No amplification of BKV DNA **repeat the analysis**
Negative control	Amplification signal in FAM and/or JOE	Contamination **repeat the analysis**
	No amplification signal in any channel	Control and PCR worked correctly

Only if all controls worked correctly, the run is suited for analysis.

B. QUANTITATIVE ANALYSIS: Evaluating controls and standard curve

	RESULT	INTERPRETATION
Positive control BG	Amplification signal in JOE	Control and PCR worked correctly
	No amplification signal in JOE	No amplification of *BG* gene **repeat the analysis**
Negative control	Amplification signal in FAM and/or JOE	Contamination **repeat the analysis**
	No amplification signal in any channel	Control and PCR worked correctly

INSTRUMENT	STANDARD CURVE PARAMETERS
ABI 7300 ABI 7500 Fast / 7500 Fast Dx ABI StepOne / StepOnePlus Bio-Rad Dx Bio-Rad CFX96	-3.60 < slope < -3.10 R^2 > 0.99
LC 480	-3.60 < slope < -3.10
LC 2.0	1.8 < Efficiency < 2.1

The run is suited for analysis/interpretation, if all controls worked correctly and the standard curve parameters are in the specified range.

11.5.2 Interpretation of results

If the controls show the expected results, continue with the interpretation of the sample results. See the table below.

Target BG	Target BKV	INTERPRETATION
Ct (of amplification) < 34 (for whole blood: Ct < 32) §	Amplification signal	**Positive** for BKV
	No amplification signal	**Negative** for BKV
No amplification signal or Ct (of amplification) > 34 (for whole blood: Ct > 32) §	Amplification signal	* **Positive** for BKV
	No amplification signal	**Not suitable** for analysis Repeat DNA extraction

§ These Ct values refer to a DNA extraction from 200 µL of clinical sample and a final elution volume of 50 µL.

* **ATTENTION!** This assay has been optimized to favor amplification of the pathogen DNA. Therefore, the amplification signal of the control gene (β-globin, JOE fluorescence) may be delayed or absent in samples positive for BKV.

If quantification standards were included in the amplification run, the absolute number of BKV genome copies in the samples can be determined.

The exact number of viral genome copies can be determined only for results that are in the linear range of the device. See the table below for correct interpretation:

Quantification result BKV	INTERPRETATION (viral genome copies / reaction)
quantification result > 10^7 copies/reaction	more than 10^7
2.5 < quantification result < 10^7 copies/reaction	exact quantity = quantification result
quantification result < 2.5 copies/reaction	less than 2.5

The pathogen load can be calculated as *genome copies / mL of clinical sample*, if the specific extraction parameters are included in the calculation. See the table below for examples.

Extraction parameters	Quantification result BKV	INTERPRETATION (viral genome copies / mL of clinical sample)
Example 1 V_i = 200 µL V_e = 50 µL	quantification result > 5×10^8 copies/mL	more than 5×10^8
	125 < quantification result < 5×10^8 copies/mL	exact quantity = quantification result
	quantification result < 125 copies/mL	less than 125
Example 2 V_i = 200 µL V_e = 60 µL	quantification result > 6×10^8 copies/mL	more than 6×10^8
	150 < quantification result < 6×10^8 copies/mL	exact quantity = quantification result
	quantification result < 150 copies/mL	less than 150
Example 3 V_i = 200 µL V_e = 100 µL	quantification result > 10^9 copies/mL	more than 10^9
	250 < quantification result < 10^9 copies/mL	exact quantity = quantification result
	quantification result < 250 copies/mL	less than 250

V_i = initial volume (clinical sample volume used for extraction)
V_e = final elution volume

11.6 TROUBLESHOOTING

No amplification signals for positive controls / standards and samples

- *The instrument was not programmed correctly*

▶ Repeat the amplification taking care of the instrument programming. Pay particular attention to the thermal profile, the fluorophores selected and that the positions of samples in the instrument setup correspond to the actual positions/order of the samples / controls / standards.

- *The reaction mix did not work correctly*

▶ Make sure to store the BKV Real time mix at -30 °C to -20 °C. Avoid unnecessary freeze/thaw cycles. Store the mix protected from light.

▶ Do not use the product past the expiration date reported on the label.

Very weak amplification signal for positive controls / standards

- *The positive controls / standard solutions were not stored correctly and have degraded*

▶ Make sure to store the positive controls / standard solutions at -30 °C to -20 °C and do not let them undergo more than three freeze/thaw cycles.

▶ Do not use the product past the expiration date.

Amplification signal of β-globin is very delayed or absent in the extracted sample (negative for BKV)

- *The extracted DNA was not suited for PCR and the reaction was inhibited*

▶ Make sure to extract the nucleic acids correctly.

▶ If an extraction method uses wash steps with solutions containing ethanol, make sure no ethanol residue remains in the DNA extract.

▶ Use the extraction systems recommended in paragraph 11.1.

- *The clinical sample is not suited for analysis*

▶ Make sure to correctly store and pretreat the clinical sample before performing the analysis.

In case of any further problems, please contact the technical support team at AB ANALITICA:

laboratorio@abanalitica.it

fax (+39) 049-8709510

tel. (+39) 049-761698

12 DEVICE LIMITATIONS

The kit can have reduced performance if:

- The clinical sample is not suitable for this analysis,
- The DNA is not suitable for PCR (due to the presence of PCR inhibitors or to the use of an inappropriate extraction method),
- The kit was not stored correctly.

13 DEVICE PERFORMANCE

The performance reported below is verified for all instruments this device has been validated on. For further information contact the technical support team at AB ANALITICA.

13.1 Analytical specificity

The specificity of the REALQUALITY RS-BKV kit, code RQ-S49, is guaranteed by an accurate and specific selection of primers and probe and by the use of stringent amplification conditions.
Alignment of primers and probe in the most important databases showed no non-specific pairing.
In order to analyze possible cross-reactions of this assay, samples positive for potentially cross-reactive pathogens were tested with this IVD. None of the tested pathogens gave a positive result.

13.2 Analytical sensitivity: detection limit

Serial dilutions of a quantification standard, ranging from 1 to 0.05 copies of viral genome / µL, were tested in three consecutive experiments. Five microliters (5 µL) of each dilution were amplified in eight replicates per run and in multiplex with the internal control.
The results were analyzed using Probit analysis, as illustrated in Fig. 2.
The limit of the analytical sensitivity for the REALQUALITY RS-BKV kit (p = 0.05) on the ABI 7500 Fast Dx system is 0.3 viral genome copies / µL DNA extract.

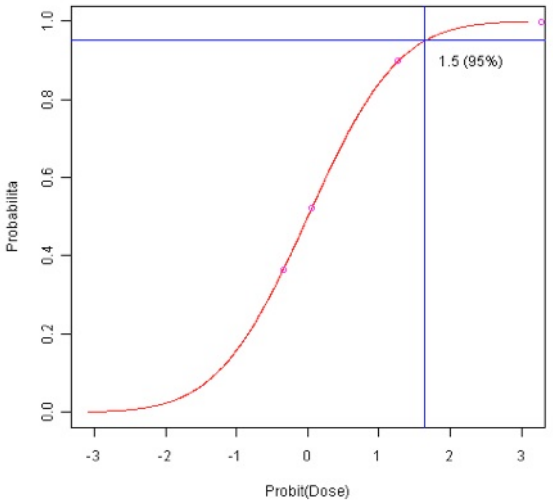

Fig. 2: Probit analysis for determination of the analytical sensitivity of the REALQUALITY RS-BKV kit (Applied Biosystens 7500 Fast Dx Real-Time PCR system). Displayed as viral genome copies / reaction.

13.3 Analytical sensitivity: linear range

The linear range of this assay was determined using a panel of dilutions of the quantification standards. Analysis was performed using linear regression. The linear range of the REALQUALITY RS-BKV kit on the ABI 7500 Fast Dx system is 2.5 to 10^7 viral genome copies / reaction.

13.4 Reproducibility

In order to determine the intra-assay variability (variability in one analysis session among replicates of the same sample) a dilution of 50 viral genome copies / µL of the quantification standard (corresponding to a final amount of 250 copies/reaction) was amplified in eight replicates in one run. The intra-assay variability coefficient of the method concerning the cycle threshold value (Ct) is 0.237 % on the ABI 7500 Fast Dx system.

In order to determine the inter-assay variability (variability over different analysis sessions of replicates of the same sample) the least concentrated quantification standard (20 viral genome copies / µL) was amplified in duplicates in three consecutive runs. For each run, the variability coefficient was calculated from the Ct of the samples.
The inter-assay variability coefficient was calculated as the average of the variability coefficients for each run. The inter-assay variability coefficient on the ABI 7500 Fast Dx system is 0.107 %.

13.5 Diagnostic specificity

A statistically significant number of samples negative for BKV were tested simultaneously with the REALQUALITY RS-BKV kit and another CE IVD device or a reference method. From the obtained results the diagnostic specificity was calculated. The diagnostic specificity of this device is 100 %.

13.6 Diagnostic sensitivity

A statistically significant number of samples positive for BKV were tested simultaneously with the REALQUALITY RS-BKV kit and another CE IVD device or a reference method. From the obtained results the diagnostic sensitivity was calculated. The diagnostic sensitivity of this device is 100 %.

13.7 Accuracy

The accuracy was calculated as the ratio of the number of correct test results to the total number of executed tests. The accuracy of the REALQUALITY RS-BKV kit is 100 %.

14 REFERENCES

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15 RELATED PRODUCTS

REALQUALITY RQ-BKV STANDARD
Ready-to-use quantification standards for quantification of *BK virus* (BKV).

This product is in accordance with directive 98/79/EC (Annex III) on *in vitro* diagnostic medical devices (CE marking).

Code	Product	PKG
RQ-50-ST	REALQUALITY RQ-BKV STANDARD	4 × 60 µL

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