ELITechGroup
EMPOWERING IVD

ELITechGroup S.p.A.
C.so Svizzera, 185
10149 Torino ITALY
Offices: Tel. +39-011 976 191 Fax +39-011 936 76 11
E. mail: emd.support@elitechgroup.com
WEB site: www.elitechgroup.com

NOTICE of CHANGE dated 22/10/2021

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«BKV ELITe MGB® Kit»
Ref. RTS175PLD

This new revision of the Instruction for Use (IFU) contains the following changes:

- *Extended Use of the product in association with «ELITe BeGenius®» instrument (REF INT040).*
- *Update of PERFORMANCE CHARACTERISTICS:*
  - *Change in Limit of Detection (LoD)*
  - *Change in Linear measuring range*
  - *Addition of Repeatability*
  - *Addition of Reproducibility*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIE REVIEW VON DIESER IFU IST KOMPATIBLE MIT DER VORIGE VERSION VON DEM TEST-KIT

ELITechGroup
EMPOWERING IVD

ELITechGroup S.p.A.
C.so Svizzera, 185
10149 Torino ITALY
Offices: Tel. +39-011 976 191 Fax +39-011 936 76 11
E. mail: emd.support@elitechgroup.com
WEB site: www.elitechgroup.com

BKV ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS175PLD CE IVD -20°C

TABLE OF CONTENTS

INTENDED USE	page 2
ASSAY PRINCIPLES	page 2
PRODUCT DESCRIPTION	page 3
MATERIALS PROVIDED IN THE PRODUCT	page 3
MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT	page 3
OTHER PRODUCTS REQUIRED	page 3
WARNINGS AND PRECAUTIONS	page 5
ELITe InGenius® and ELITe BeGenius®	page 6
SAMPLES AND CONTROLS	page 6
ELITE INGENIUS® PROCEDURE	page 8
ELITE BEGENIUS® PROCEDURE	page 15
PERFORMANCE CHARACTERISTICS	page 20
ABI 7500 Fast Dx Real-Time PCR Instrument ABI 7300 Real-Time System	page 30
SAMPLES AND CONTROLS	page 30
PROCEDURE	page 32
PERFORMANCE CHARACTERISTICS	page 40
Roche cobas z 480 analyzer	page 46
SAMPLES AND CONTROLS	page 46
PROCEDURE	page 47
PERFORMANCE CHARACTERISTICS	page 51
REFERENCES	page 53
PROCEDURE LIMITATIONS	page 54
TROUBLESHOOTING	page 55
SYMBOLS	page 57
NOTICE TO PURCHASER: LIMITED LICENSE	page 58

INTENDED USE

The **«BKV ELITe MGB® Kit»** product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of human Polyomavirus BK (BKV)** in DNA samples extracted from plasma collected in EDTA, urine collected without preservatives and cerebrospinal fluid (CSF).

The product is intended for use in the diagnosis and monitoring of BKV infections alongside clinical data of the patient and other laboratory tests outcomes.

ASSAY PRINCIPLES

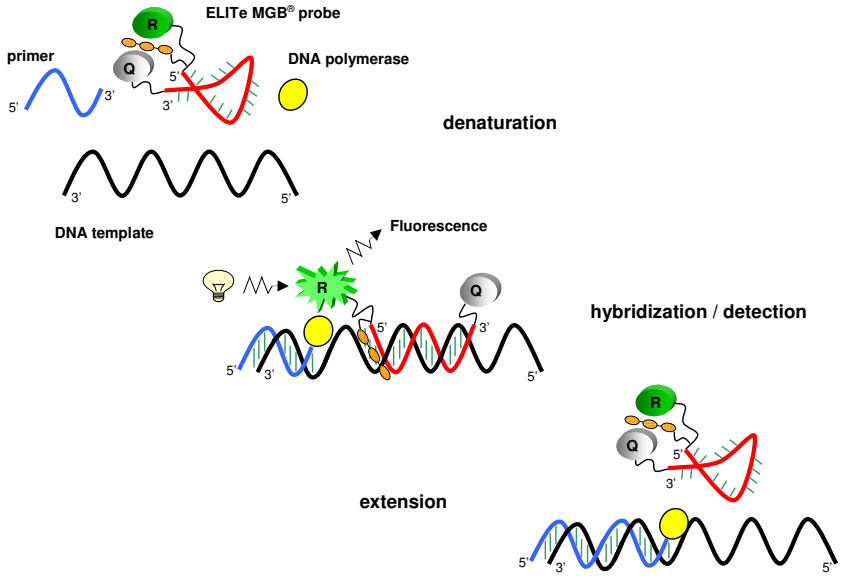
The assay consists of a real time amplification reaction with a programmable thermostat provided with a fluorescence detection optical system.

In each well, two amplification reactions are performed starting from DNA extracted from the samples being tested: a specific reaction for the region of the **Large T antigen** gene of BKV and a specific reaction for the region of the human **beta Globin** gene (Internal Control of inhibition). The BKV specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the BKV amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with AP525 fluorophore (analogous to VIC), is activated when hybridizes with the specific product of the Internal Control amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data allows detecting the presence and the titre of BKV DNA in the starting sample.

At the end of the amplification session, dissociation curve (melting curve) analysis can be carried out in order to determine the dissociation temperature (melting temperature) and to confirm the presence of the correct target or to identify the presence of mutations.

The assay is validated with the systems described in this instruction for use.

In the following picture is synthetically showed the mechanism of activation and fluorescence emission of ELITe MGB® technology probe. Note that the probe is not hydrolyzed during the amplification cycle so as it can be utilized for the dissociation curve analysis.



PRODUCT DESCRIPTION

The **«BKV ELITe MGB® Kit»** product supplies the **ready to use** complete mixture BKV Q - PCR Mix for real time amplification in a stabilising solution, **aliquoted into four disposable test tubes**. Each tube contains **540 µL** of solution, sufficient for **24 tests** (by processing at least 2 samples per session) in association with **«ELITe InGenius®»** and **«ELITe BeGenius®»** systems and **25 tests** in association with other systems.

The primers and the BKV specific probe (stabilized by MGB® group, labelled with FAM fluorophore and quenched by a non-fluorescent molecule) are specific for the region of the **Large T antigen** gene of BKV.

The primers and the Internal Control specific probe (stabilized by MGB® group, labelled with AP525 fluorophore, analogous to VIC, and quenched by a non-fluorescent molecule) are specific for the **promoter and 5' UTR** region of the **human beta Globin gene**.

The reaction mixture provides buffer, magnesium chloride, triphosphate nucleotides, AP593 fluorophore (used instead of ROX or CY5) as passive reference for fluorescence normalisation, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product, the "hot start" DNA polymerase enzyme.

The product is sufficient for **96 tests in association with «ELITe InGenius®»** and **«ELITe BeGenius®»** systems, including standards and controls.

The product is sufficient for **100 tests in association with other systems**, including standards and controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
BKV Q - PCR Mix	complete reaction mixture	**4 x 540 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.
- Programmable thermostat with optical fluorescence detection system 7300 Real Time PCR System or 7500 Fast Dx Real-Time PCR Instrument calibrated following manufacturer's instructions.
- Programmable thermostat with optical fluorescence detection system cobas z 480 analyzer, calibrated following manufacturer's instructions.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of DNA from the samples, the positive control of extraction, the positive control of the amplification, the known quantity DNA standards and the consumables are not included in this product.

For automatic sample analysis with the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) the following generic products are required: the extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200) or **«ELITe InGenius® SP 1000»** (ELITechGroup S.p.A., ref. INT033SP1000), the consumables for extraction and amplification of nucleic acids from biological samples: **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS), **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000), **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR) and **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S).

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

For automatic DNA extraction, amplification and interpretation of sample analysis, the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) and the following specific Assay protocols (ELITechGroup S.p.A.) are required:

- for the calibrators **«BKV ELITe_STD»** or **«BKV ELITe_STD_1000_100»**,
- for the positive control of amplification **«BKV ELITe_PC»** or **«BKV ELITe_PC_1000_100»**,
- for negative control of amplification **«BKV ELITe_NC»** or **«BKV ELITe_NC_1000_100»**,
- for samples analysis **«BKV ELITe_PL_200_100»**, **«BKV ELITe_PL_1000_100»** and **«BKV ELITe_U_200_100»**.

For automatic sample analysis with the instrument **«ELITe BeGenius®»** (ELITechGroup S.p.A., ref. INT040) the following generic products are validated: the extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200), the consumables for extraction and amplification of nucleic acids from biological samples **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS), **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000), **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR) and **«1000 µL Filter Tips Tecan»** (Tecan, Switzerland, ref. 30180118).

- For automatic DNA extraction, amplification and interpretation of sample analysis, the instrument **«ELITe BeGenius®»** (ELITechGroup S.p.A., ref. INT040) and the following specific Assay protocols (ELITechGroup S.p.A.), are required:
- for the calibrators **«BKV ELITe_Be_STD»**,
- for the positive control of amplification **«BKV ELITe_Be_PC»**,
- for negative control of amplification **«BKV ELITe_Be_NC»**,
- for samples analysis **«BKV ELITe_Be_PL_200_100» and «BKV ELITe_Be_U_200_100»**.

For automatic DNA extraction from samples to be analyzed, it is required the use of generic product **«ELITe STAR 200 Extraction kit»** (ELITechGroup S.p.A., ref. INT011EX), kit for extraction of nucleic acid from biological samples, with the **«ELITe STAR»** instrument (ELITechGroup S.p.A., ref. INT010).

For automatic DNA extraction and preparation of microplates for amplification of samples to be analyzed, it is required the use of generic product **«ELITe GALAXY 300 Extraction Kit»** (ELITechGroup S.p.A., ref. INT021EX), kit for extraction of DNA and RNA from non-cellular and cellular samples with the instrument **«ELITe GALAXY»** (ELITechGroup S.p.A., ref. INT020).

For automatic DNA extraction from samples to be analyzed, it is required the use of the generic products **«NucliSENS® easyMAG® Reagents»** (bioMérieux SA, ref. 280130, 280131, 280132, 280133, 280134, 280135), kits for extraction of nucleic acid from biological samples, with the instrument **«NucliSENS® easyMAG®»** (bioMérieux SA, ref. 200111).

For automatic DNA extraction from samples to be analyzed, the products **«QIAsymphony® DNA Mini Kit»** (QIAGEN GmbH, ref. 931236) and **«QIAsymphony® DSP Virus / Pathogen Midi kit»** (QIAGEN GmbH, ref. 37055), kits for extraction of nucleic acid from biological samples, with the instrument **«QIAsymphony® SP/AS»** (QIAGEN GmbH, ref. 9001297, 9001301) and related generic products are required.

For automatic DNA extraction from samples to be analyzed, the product **«MagNA Pure 24 Total NA Isolation Kit»** (Roche, ref. 07658036001), kit for extraction of nucleic acid from biological samples, with the instrument **«MagNA Pure 24 System»** (Roche, ref. 07290519001) is also validated.

When a 7300 Real-Time PCR System is used, it is recommended the use of generic product **«Q - PCR Microplates»** (ELITechGroup S.p.A., ref. RTSACC01), microplates with 0.2 mL wells and adhesive sealing sheets for real time amplification.

When a 7500 Fast Dx Real-Time PCR Instrument is used, it is recommended the use of generic product: **«Q - PCR Microplates Fast»** (ELITechGroup S.p.A., ref. RTSACC02), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

When a cobas z 480 analyzer is used, it is required the use of generic product **«AD-plate 0.3ml»** (Roche, ref. 05232724001), microplates with 0.3 mL wells and adhesive sealing sheets for real time amplification.

For the system validation (instrument and reagents lot), use the product **«BKV - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR175PLD), or the product **«BKV - ELITe Positive Control RF»** (ELITechGroup S.p.A., ref. CTR020PLD-R) specific for the use with cobas z 480 analyzer, positive control composed of plasmid DNA.

For the calculation of the system standard curve (instrument and reagents lot), use the product **«BKV ELITe Standard»** (ELITechGroup S.p.A., ref. STD175PLD), four dilutions of known quantity plasmid DNA to obtain the standard curve.

As positive control of nucleic acid extraction from non-cellular samples and inhibition control, it is required the use of generic product **«CPE - Internal Control»** (ELITechGroup S.p.A., ref. CTRCPE), a stabilised solution containing two plasmid DNAs and the genomic RNA of MS2 phage.

A conversion factor allows to express the results of the quantitative analysis in International Units of BKV of the "1st WHO International Standard for BK Virus DNA" (NIBSC code 14/212, United Kingdom).

WARNINGS AND PRECAUTIONS

This product is exclusively designed for *in-vitro* use.

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121 °C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in the work areas.
Carefully wash hands after handling samples and reagents.
Dispose of leftover reagents and waste in compliance with the regulations in force.
Carefully read all the instructions provided in the product before running the assay.
While running the assay, follow the instructions provided in the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided in the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures, such as nucleic acid extraction, amplification and detection, require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

When amplification session is manually setup, it is necessary to have available separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never introduce an amplification product in the area designated for extraction / preparation of amplification reactions.

When amplification session is manually setup, it is necessary to have available lab coats, gloves and tools which are exclusively used for the extraction / preparation of the amplification reactions and for the amplification / detection of amplification products. Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

The samples must be exclusively used for this type of analysis. Samples must be handled under a laminar airflow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, free from DNA and RNA.

The extraction products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, free from DNA and RNA.

Amplification products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination.

Warnings and precautions specific for the components

The **BKV Q - PCR Mix** must be stored at -20 °C in the dark.

The **BKV Q - PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

ELITe InGenius® and ELITe BeGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Plasma collected in EDTA

The plasma samples for nucleic acids extraction must be collected in EDTA according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples into aliquots before freezing, in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when the DNA extraction from 200 µL of plasma is carried out with the **ELITe InGenius®** and with **ELITe InGenius® Software** version **1.3** (or later equivalent versions), use the extraction protocol **BKV ELITe_PL_200_100**. This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Note: when the DNA extraction from 200 µL of plasma is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius® Software** version **2.0** (or later equivalent versions), use the extraction protocol **BKV ELITe_Be_PL_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information on how to set up and perform the extraction procedure.

Note: when the DNA extraction from 1000 µL of plasma is carried out with the **ELITe InGenius®** and with **ELITe InGenius® Software** version **1.3** (or later equivalent versions), use the extraction protocol **BKV ELITe_PL_1000_100**. This protocol processes 1000 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

The primary tube can NOT be used in association with the **BKV ELITe_PL_1000_100** assay protocol.

Urine collected without preservatives

The urine samples for nucleic acids extraction must be collected in preservative-free containers according to laboratory guidelines, transported at room temperature (+18 / +25 °C) and stored at room temperature (+18 / +25 °C) for a maximum of four hours, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

Before the analysis with this product 0.2 mL of sample has to be transferred in the Extraction tube provided with «ELITe InGenius SP 200 Consumable Set».

If possible, avoid freezing of first void urine samples. Freezing can cause precipitation of inhibitors and the loss of the DNA titre.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing.

Note: when the DNA extraction from urine is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version **1.3** (or later equivalent versions), use the extraction protocol **BKV ELITe_U_200_100**. This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Note: when the DNA extraction from 200 µL of urine is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius Software** version **2.0** (or later equivalent versions), use the extraction protocol **BKV ELITe_Be_U_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information on how to set up and perform the extraction procedure.

Interfering substances

The sample must not contain heparin, in order to prevent the problem of inhibition and the possibility of frequent invalid results.

High quantity of human genomic DNA in the DNA extracted from the sample may inhibit the amplification reaction.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Amplification calibrators and amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the Calibration curve and the amplification controls for each lot of amplification reagent:

as calibrator set, use the four concentration levels of the **BKV ELITe Standard**, in association with protocol **«BKV ELITe_STD», «BKV ELITe_STD_1000_100»** for **ELITe InGenius**, and **«BKV ELITe_Be_STD»** for **ELITe BeGenius**,
as amplification Positive Control use the **BKV - ELITe Positive Control**, in association with protocol **«BKV ELITe_PC»** or **«BKV ELITe_PC_1000_100»** for **ELITe InGenius**, and **«BKV ELITe_Be_PC»** for **ELITe BeGenius**,
as amplification Negative Control, use molecular grade water (not provided with this kit) in association with protocol **«BKV ELITe_NC»** or **«BKV ELITe_NC_1000_100»** for **ELITe InGenius**, and **«BKV ELITe_Be_NC»** for **ELITe BeGenius**.

Note: ELITe InGenius with **ELITe InGenius Software** and **ELITe BeGenius** with **ELITe BeGenius Software** allow generation of the calibration curves and the validation of amplification Controls for each lot of amplification reagent to be stored in their databases.
Calibration curves, approved and stored in the databases, will expire after **60 days**. At expiration date it is necessary to re-run the calibrator set.
Amplification validation Control results, approved and stored in the databases, will expire after **15 days**. At expiration date it is necessary to re-run the Positive and Negative Controls.

The Calibrators and amplification Controls must be retested if any of the following events occurs:
- a new lot of amplification reagents is started,
- the results of Quality Control analysis (see following paragraph) are out of specification,
- any major maintenance is performed on the instrument.

Quality controls

External quality controls shall be used in accordance with local, state, federal accrediting organizations, as applicable. External quality controls are available on the market.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification
REF RTS175PLD

ELITe InGenius® PROCEDURE

The procedure to use the **«BKV ELITe MGB® Kit»** with the system ELITe InGenius consists of three steps:

- System readiness verification
- Set up of the session
- Review and approval of results

System readiness verification

Before starting the sample analysis session, referring to the instrument documentation, it is necessary to:

- switch on the **ELITe InGenius** and select the mode "**CLOSED**";
- verify that the Calibrators (**BKV Q-PCR Standard**) have been run, approved and not expired (status). This can be checked under the "Calibration" menu in the Home page;
- verify that the amplification Controls (**BKV - Positive Control**, **BKV Negative Control**) have been run, approved and not expired (status). This can be checked under the "Control" menu in the Home page;
- choose the type of run and set up the run, following the instructions Graphical User Interface (GUI) for the session set up and using the Assay Protocols provided by ELITechGroup. These IVD protocols were specifically validated with ELITe MGB kits, matrices and ELITe InGenius instrument.

The Assay protocols available for **«BKV ELITe MGB® Kit»** are described in the table below.

Assay protocols for «BKV ELITe MGB® Kit» and ELITe InGenius

Name	Matrix	Report unitage	Characteristics
BKV ELITe_PL_200_100	Plasma	copies/mL or IU / mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
BKV ELITe_PL_1000_100	Plasma	copies/mL or IU / mL	Extraction Input Volume: 1000 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
BKV ELITe_U_200_100	Urine	copies/mL or IU / mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

If the assay protocol of interest is not in the system, contact your local ELITechGroup Customer Service.

Protocols for qualitative analysis are available on request.

Setup of the session

The **BKV ELITe MGB® Kit** in association to the **ELITe InGenius** can be used in order to perform:

A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Calibration run (PCR only),
D. Amplification run for Positive and Negative Control run (PCR only),

All the parameters needed for the session are included in the Assay protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

Note: the ELITe InGenius system can be linked to the "Location Information Server" (LIS) through which it is possible to load the work session information. Refer to the instrument user's manual for more details.

The main steps for the setup of the four types of runs are described here below.

A. Integrated run

To set up an integrated run carry out the following steps as per the **SW Graphical User Interface (GUI)**:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. Select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
5. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
6. Select the assay protocol to be used in the "Assay" column (i.e. BKV ELITe_PL_200_100).
7. Ensure that the "Protocol" displayed is: "Extract + PCR".
8. Select the sample loading position in the "Sample Position" column:
   if a primary tube is used select "Primary Tube", the Primary tube can be use only starting from 200 µL of samples;
   if a secondary tube is used select "Extraction Tube".
   Click "Next" to continue the setup.
9. Load CPE and BKV Q-PCR Mix on the Inventory Block selected by following the GUI instruction. Click "Next" button to continue the setup.
10. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click "Next" button to continue the setup.
11. Load the "PCR Cassette", the "ELITe InGenius SP 200" or "ELITe InGenius SP1000" extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click "Next" to continue the setup.
12. Close the instrument door.
13. Press "Start" to start the run.

After process completion, the **ELITe InGenius** allows to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped, identified and stored at -20 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the "PCR Cassette" containing the reaction products and the consumables must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

B. Amplification run

To set up the amplification run carry on the steps below following the GUI:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Select "Perform Run" from the "Home screen".
3. Select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
4. For each Track of interest type the "SampleID" (SID) by typing or by scanning the sample barcode.
5. Select the assay protocol to be used in the "Assay" column (i.e. BKV ELITe_PL_200_100).
6. Select "PCR Only" in the "Protocol" column.
7. Ensure the Eluted sample loading position in the "Sample Position" column is "ExtraTube (bottom row)". Click "Next" to continue the setup.
8. Load BKV Q-PCR Mix on the Inventory Block selected by following the GUI instruction. Click "Next" to continue the setup.
9. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load the PCR Cassettes and the extracted Nucleic Acid samples following the GUI instruction. Click "Next" to continue the setup.
11. Close the instrument door.
12. Press "Start" to start the run.

After process completion, the **ELITe InGenius** allows to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run

To set up the Calibration run carry on the steps below following the GUI:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw BKV Q - PCR Standard tubes (Cal1: BKV Q - PCR Standards 10^2, Cal2: BKV Q - PCR Standards 10^3, Cal3: BKV Q - PCR Standards 10^4, Cal4: BKV Q - PCR Standards 10^5). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. Select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
5. Starting from the Track of interest, select the assay protocol to be used in the "Assay" column (BKV ELITe_STD or BKV ELITe_STD_1000_100) and fill with the lot number and expiry date for the BKV Q - PCR Standard. Click "Next" button to continue the setup.
6. Load the BKV Q-PCR Mix on the Inventory Block selected by following the GUI instruction. Click "Next" to continue the setup.
7. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click "Next" to continue the setup.
8. Load the Calibrator tubes and PCR Cassettes on board, following the GUI instruction. Click "Next" to continue the setup. Take care to load the PCR Standard fluids to the correct tracks as indicated in the GUI.
9. Close the instrument door.
10. Press "Start" to start the run.

After process completion, the **ELITe InGenius** allows to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Calibrators can be removed from the instrument, capped and stored at -20 °C.

Note: At the end of the run the PCR Cassette with the reaction products and the consumables must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification
REF RTS175PLD

D. Amplification run for Positive Control and Negative Control

To set up the amplification Positive Control and Negative Control run, carry on the steps below following the GUI:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the product BKV - Positive Control, for Positive Control amplification. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water for the sessions in one Elution tube, provided with the ELITe InGenius SP Consumable Set.
4. Select "Perform Run" from the "Home screen".
5. Select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
6. For the positive control, select BKV ELITe_PC or BKV ELITe_PC_1000_100 and fill in the lot number and expiry date for the BKV Positive Control.
7. For the negative control, select BKV ELITe_NC or BKV ELITe_NC_1000_100 and fill in the lot number and expiry date for the molecular biology grade water.
8. Click "Next" to continue the setup.
9. Load BKV Q-PCR Mix on the Inventory Block selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load the amplification PCR cassette, the Positive Control and/or the Negative Control, following the GUI instruction. Click "Next" to continue the setup.
12. Close the instrument door.
13. Press "Start" to start the run.

After process completion, the **ELITe InGenius** allows to view, approve, store the results and to print and save the report.

Note: The Positive Control and the Negative Control must be run as amplification control, to set up the "Control Chart". Four Positive Control and Negative Control results, from 4 different runs are requested to set up the control chart. After that, the results of Positive Control and Negative Control are used for monitoring the amplification step. Refer to the user's manual of the instrument for more details.

Note: At the end of the run the remaining Positive Control can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the "PCR Cassettes" with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / Calibrator / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports ("Sample Report" or "Track Report").

Note: The **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible send the work session results to the laboratory data center. Refer to the instrument user's manual for more details.

The **ELITe InGenius** generates the results using the **BKV ELITe MGB® Kit** through the following procedure:

A. Validation of Calibration curve,
B. Validation of amplification Positive Control and Negative Control results,
C. Validation of sample results,
D. Sample result reporting.

A. Validation of Calibration curve

The fluorescence signals emitted by the specific BKV probe ("BKV") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocols “BKV ELITe_STD” and “BKV ELITe_STD_1000_100”.

The Calibration curve, specific for the amplification reagent lot, is stored in the database after the approval of the “Administrator” or “Analyst” personnel by following the GUI instruction. The Calibration curve, specific for the amplification reagent lot, will expire after 60 days.

Before analysing any sample, it is absolutely mandatory to generate and to approve the Calibration curve for the lot of amplification reagent used. The availability of Calibration curve results with “Approved” (Status) is shown in the “Calibration” window of the ELITe InGenius software.

Note: When the Calibration curve does not meet the acceptance criteria, the “not passed” message is shown on the “Calibration” menu and it is not possible to approve the curve. The Calibrator amplification reactions have to be repeated.

Note: When the Calibration Curve is run together with samples and its result is invalid, the entire session is invalid and the amplification of all samples must be repeated.

B. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the specific BKV probe ("BKV ") in the Positive Control and Negative Control amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocols “BKV ELITe_PC”, “BKV ELITe_PC_1000_100”, “BKV ELITe_NC” and “BKV ELITe_NC_1000_100”.

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, are stored in the database (Controls) after the approval of the “Administrator” or “Analyst” personnel by following the GUI instruction.

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire after 15 days.

Before analysing any sample and after approval of the Calibration curve, it is absolutely mandatory to generate and to approve an amplification Positive Control and Negative Control results for the lot of amplification reagent used. The availability of an amplification Positive Control and Negative Control results with “Approved” (Status) is shown in the “Controls” window of the ELITe InGenius software. If the amplification Positive Control and Negative Control results are missing, generate them as described above.

Note: When the Positive Control or Negative Control result does not meet the acceptance criteria, the “not passed” message is shown on the “Controls” menu and it is not possible to approve the result. The Positive Control or Negative Control amplification reaction has to be repeated.

Note: When the Positive Control or Negative Control is run together with samples and its result is invalid, the entire session is invalid and the amplification of all samples must be repeated.

C. Validation of Samples results

The fluorescence signals emitted by the specific BKV probe ("BKV") and by the specific Internal Control probe ("IC") in each sample amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol.

Note: Before analysing any sample, it is absolutely mandatory to generate and to approve the Calibration curve and the amplification Controls for the lot of reagent used. It is recommended, but optional, to run Positive and Negative Control together with the Calibrators. The availability of a Calibration curve and amplification Positive and Negative Control results with “Approved” (Status) is shown in the “Calibration” and “Controls” windows of the ELITe InGenius software.

Results are described in the reports generated by the instrument ("Result Display").

The Sample run is valid when the three conditions reported in the table below are met.

1) Calibration curve	**Status**
BKV Q - PCR Standard	APPROVED
2) Positive Control	**Status**
BKV - Positive Control	APPROVED
3) Negative Control	**Status**
BKV - Negative Control	APPROVED

For each Sample the calculation of the viral load is automatically performed by **ELITe InGenius software** as established by the algorithm and the assay protocol parameters.

For each Sample the calculation of the viral load is automatically performed by the system. The measure is expressed as “copies / mL” or “IU / mL” as set in the assay protocol.

The possible result messages of a Sample are listed the table below.

Result of Sample run	**Interpretation**
BKV: DNA Detected, quantity equal to XXX copies / mL or IU/mL	**BKV DNA detected** within the measurement range of the assay, quantity as shown.
BKV: DNA Detected, quantity below LLoQ copies / mL or IU/mL	**BKV DNA detected** below the lower limit of quantification of the assay
BKV: DNA Detected, quantity beyond ULoQ copies / mL or IU/mL	**BKV DNA detected** beyond the upper limit of quantification of the assay
BKV: DNA Not Detected or below LoD copies / mL or IU/mL	**BKV DNA not detected or below the Limit of Detection** of the assay.
Invalid - Retest Sample	**Not valid assay result** due to Internal Control failure (Incorrect extraction or inhibitor carry-over).

Samples not suitable for analysis are reported as “Invalid - Retest Sample” by the **ELITe InGenius software**. In this case, the Internal Control DNA was not detected due to potential problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitors carry-over in the eluate) that may lead to false negative call.

When the eluate volume is sufficient, the extracted sample can be retested by amplification run in “PCR Only” mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode.

Samples suitable for analysis in which it was not possible to detected BKV DNA are reported like: “BKV: DNA Not Detected or below LoD". In this case it cannot be excluded that the BKV DNA is present at a concentration below the limit of detection of the assay (see “performance and characteristic).

Note: The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes concerning the patient.

The Sample run results are stored in the database and, if valid, can be approved (Result Display) by “Administrator” or “Analyst” personnel by following the GUI instruction. From the Result Display” window it is possible to print and save the Sample run results as “Sample Report” and “Track Report”.

D. Samples result reporting

The sample results are stored in the database and can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the details of a sample run sorted by Sample ID (SID).

The “Track Report” shows the details of a sample run track by selected track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

ELITe BeGenius® PROCEDURE

The procedure to use the **«BKV ELITe MGB® Kit»** with the system **ELITe BeGenius** consists of three steps:

- System readiness verification
- Set up of the session
- Review and approval of results

System readiness verification

Before starting the sample analysis session, referring to the instrument documentation, it is necessary to:

- switch on the **ELITe BeGenius** and select the mode "**CLOSED**";
- verify that the Calibrators (**BKV Q-PCR Standard**) have been run, approved and not expired (status). This can be checked under the "Calibration" menu in the Home page;
- verify that the amplification Controls (**BKV - Positive Control**, **BKV Negative Control**) have been run, approved and not expired (status). This can be checked under the "Control" menu in the Home page;
- choose the type of run and set up the run, following the instructions Graphical User Interface (GUI) for the session set up and using the Assay Protocols provided by ELITechGroup. These IVD protocols were specifically validated with ELITe MGB Kits, matrices and **ELITe BeGenius** instrument.

The Assay protocols available for **«BKV ELITe MGB® Kit»** are described in the table below.

Assay protocols for «BKV ELITe MGB® Kit» and ELITe BeGenius

Name	Matrix	Report unitage	Characteristics
BKV ELITe_Be_PL_200_100	Plasma	copies/mL or IU / mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
BKV ELITe_Be_U_200_100	Urine	copies/mL or IU / mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

If the assay protocol of interest is not in the system, contact your local ELITechGroup Customer Service.

Setup of the session

The **BKV ELITe MGB Kit** in association to the **ELITe BeGenius** can be used in order to perform:

A. Sample run, (EXTR + PCR),
B. Amplification run (PCR only),
C. Calibration run (PCR only),
D. Positive and Negative Control run (PCR only).

All the parameters needed for the session are included in the Assay protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

Note: The **ELITe BeGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible to load the work session information. Refer to the instrument user's manual for more details.

The main steps for the setup of the four types of runs are described here below.

A. Sample run

To set up the integrated run, carry out the steps below following the **GUI**:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw a sufficient number of CPE tubes for the session. Each new tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. Remove all the Racks from the "Cooler Unit" and place them on the preparation table.
5. Select the "run mode": "Extract + PCR".
6. Load the samples into the cooling area starting from the L5 Sample Rack.
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.

Note: If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the sample ID.

8. Check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
9. Select the assay protocol to be used in the "Assay" column (i.e. BKV ELITe_Be_PL_200_100). Click "Next" to continue the setup.
10. If a second extraction has to be performed, repeat steps 6 to 9 using the L4 Sample Rack.
11. Load the barcoded eluate tubes into cooling area starting from L3 Elution Rack.

Note: Elution tubes can be labelled to improve traceability.

12. Insert the L3 Elution Rack into the "Cooler Unit". Click "Next" to continue the setup.
13. Repeat steps 11 and 12 using the L2 Reagent/Elution Rack.
14. Load CPE and BKV Q-PCR Mix into the into cooling area.
15. Insert the L1 Reagent Rack into the "Cooler Unit". Click "Next" to continue the setup.
16. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
17. Load the PCR Rack with "PCR Cassette" in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
18. Load the Extraction Rack with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables by following the GUI instruction. Click "Next" to continue the setup.
19. Close the instrument door.
20. Press "Start" to start the run.

After process completion, the **ELITe BeGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped, identified and stored at -20 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the "PCR Cassette" with the reaction products and the consumables must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

B. Amplification run

To set up the amplification run, with eluted samples, carry out the steps below following the GUI:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home screen”.
3. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
4. Select the “run mode”: “PCR Only”.
5. Load the samples into the cooling area starting from the L3 Elution Rack.
6. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
7. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
8. Select the assay protocol to be used in the “Assay” column (e.g. BKV ELITe_Be_PL_200_100). Click “Next” to continue the setup.
9. Load BKV Q-PCR Mix into the cooling area.
10. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
11. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
12. Load the PCR Rack with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
13. Close the instrument door.
14. Press “Start” to start the run.

After process completion, the **ELITe BeGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped, identified and stored at -20 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the “PCR Cassette” with the reaction products must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

C. Calibration run

To set up the Calibration run, with the Q-PCR Standards, carry out the steps below following the GUI:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the BKV Q - PCR Standard tubes (Cal1: BKV Q-PCR Standards 10^2, Cal2: BKV Q-PCR Standards 10^3, Cal3: BKV Q-PCR Standards 10^4, Cal4: BKV Q-PCR Standards 10^5). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home screen”.
4. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
5. Select the “run mode”: “PCR Only”.
6. Load the Calibrator tubes into the L3 Elution Rack.
7. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
8. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
9. Select the assay protocol to be used in the “Assay” column (BKV ELITe_Be_STD). Click “Next” button to continue the setup.
10. Load BKV Q-PCR Mix into the L2 Reagent/Elution Rack.
11. Insert the L2 Reagent/Elution Rack into the “Cooler Unit”. Click “Next” to continue the setup.
12. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
13. Load the PCR Rack with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
14. Close the instrument door.
15. Press “Start” to start the run.

After process completion, the **ELITe BeGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Calibrators can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Q-PCR Standards.

Note: At the end of the run the “PCR Cassette” with the reaction products must be removed from the instrument and disposed of without producing environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

D. Positive Control and Negative Control run

To set up the Positive Control and Negative Control run, carry out the steps below following the GUI:

1. Thaw a sufficient number of BKV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the product BKV - ELITe Positive Control, for Positive Control amplification. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of the molecular biology grade water (as Negative Control) for the sessions in one Elution tube, provided with the ELITe InGenius SP Consumable Set.
4. Select “Perform Run” from the “Home screen”.
5. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
6. Select the “run mode”: “PCR Only”.
7. Load the Positive Control and Negative Control tubes into the L3 Elution Rack.
8. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
9. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
10. Select the assay protocol to be used in the “Assay” column (BKV ELITe_Be_PC and BKV ELITe_Be_NC). Click “Next” button to continue the setup.
11. Load BKV Q-PCR Mix into the L2 Reagent/Elution Rack.
12. Insert the L2 Reagent/Elution Rack into the “Cooler Unit”. Click “Next” to continue the setup.
13. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
14. Load the PCR Rack with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
15. Close the instrument door.
16. Press “Start” to start the run.

After process completion, the **ELITe BeGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Positive Control can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Positive Controls.

Note: At the end of the run the “PCR Cassettes” with the reaction products must be removed from the instrument and disposed of without producing environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

Review and approval of results

At the end of the run, the “Results Display” screen is automatically shown. In this screen the sample / Calibrator / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports (“Sample Report” or “Track Report”).

Note: The **ELITe BeGenius** system can be linked to the “Location Information Server” (LIS) through which it is possible send the work session results to the laboratory data center. Refer to the instrument user’s manual for more details.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

The **ELITe BeGenius** generates the results using the BKV ELITe MGB Kit through the following procedure:

A. Validation of Calibration curve,
B. Validation of amplification Positive Control and Negative Control results,
C. Validation of sample results,
D. Sample result reporting.

Note: Please, refer to the same **ELITe InGenius** chapters for the details.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: Limit of Detection

The analytical sensitivity of this assay, as Limit of Detection (LoD) of the DNA amplification, allows detecting the presence of about 10 copies in 20 µL of DNA added to the amplification reaction.

The LoD of this assay was tested using plasmid DNA containing amplification product whose initial concentration was measured by spectrophotometer. The plasmid DNA was diluted to a titre of 10 copies / 20 µL in human genomic DNA at a titre of 500 ng / 20 µL. This sample was tested in 24 replicates carrying out the amplification by ELITechGroup S.p.A. products on two different instruments.

The results are reported in the following table.

Samples	N	positive	negative
10 copies plasmid DNA + 500 ng of human genomic DNA	24	24	0

The analytical sensitivity of this assay used in association to different matrices and **ELITe InGenius** was verified with a panel of BKV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom) in BKV DNA - negative matrix. The panel consisted of six points around the limit concentration. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, run set up, extraction of nucleic acids, real time amplification and data interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The final results for each matrix are reported in the following tables.

Limit of Detection with ELITe InGenius (IU / mL)				
Sample volume	Matrix	95% positivity	95% confidence range	
			lower limit	upper limit
200 µL	**urine**	**142 IU / mL**	110 IU / mL	222 IU / mL
	plasma	**215 IU / mL**	168 IU / mL	319 IU / mL
1000 µL	**plasma**	**44 IU/mL**	35 IU / mL	64 IU / mL

The analytical sensitivity as copies / mL for each matrices is calculated by applying the specific conversion factor reported at page 29.

The analytical sensitivity as copies / mL is reported below.

Limit of Detection with ELITe InGenius (copies / mL)				
Sample volume	Matrix	95% positivity	95% confidence range	
			Lower limit	Upper Limit
200 µL	**urine**	**89 copies / mL**	69 copies / mL	139 copies / mL
	plasma	**165 copies / mL**	129 copies / mL	245 copies / mL
1000 µL	**plasma**	**26 copies / mL**	21 copies / mL	38 copies / mL

The calculated LoD value was verified in association to **ELITe InGenius** and **ELITe BeGenius** by testing 20 replicates of Plasma collected in EDTA and 20 replicates of Urine collected without preservatives samples spiked by BKV certified reference material (1st WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 18 out of 20 replicates give a positive result as per CLSI standard EP17-A.

The results are reported in the following tables.

Limit of Detection for Plasma and Urine samples and ELITe InGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Plasma collected in EDTA	215 IU / mL	BKV	20	18	2
Urine collected without preservatives	142 IU / mL	BKV	20	20	0

Limit of Detection for Plasma and Urine samples and ELITe BeGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Plasma collected in EDTA	215 IU / mL	BKV	20	20	0
Urine collected without preservatives	142 IU / mL	BKV	20	19	1

The LoD value for BKV target was confirmed at 215 IU / mL for Plasma collected in EDTA, at 142 IU / mL for Urine collected without preservatives.

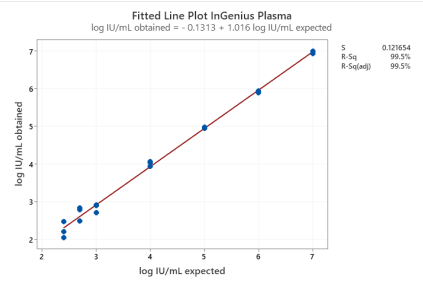
Linear measuring range and Limits of quantification

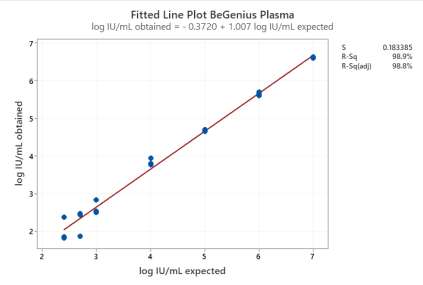
The linear measuring range of BKV ELITe MGB® Kit used in association with Plasma and Urine (sample volume 200 µL) and **ELITe InGenius** and **ELITe BeGenius** was verified using a panel of BKV dilutions. The panel was prepared by diluting the "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom) in BKV DNA - negative matrix. The panel consisted of eight dilution points (1 Log dilution steps) from 10^7 to 10^2 IU / mL. Each sample of the panel was tested in 3 replicates. The analysis of the obtained data, performed by linear regression, demonstrated that the assay shows a linear response for all the dilution levels.

For Plasma (sample volume 200 µL):

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Plasma collected in EDTA samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.995 for **ELITe InGenius** and 0.989 for **ELITe BeGenius**.

The results are reported in the following graphs.





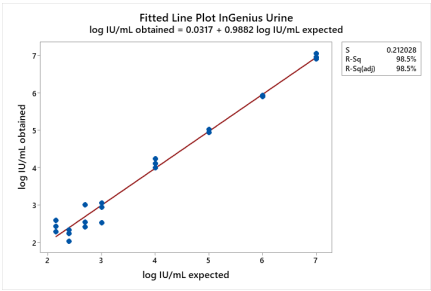
For Urine (sample volume 200 µL):

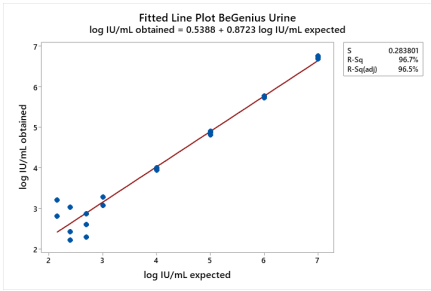
The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Urine collected without preservatives samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.985 for **ELITe InGenius** and 0.967 for **ELITe BeGenius**.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

The results are reported in the following graphs.

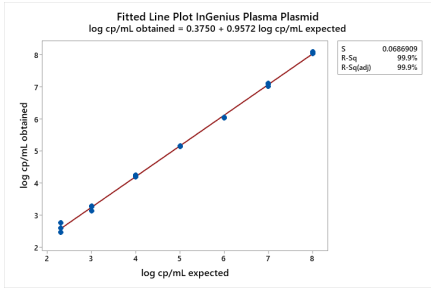


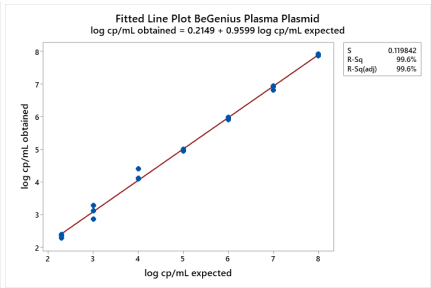


The linear measuring range of BKV ELITe MGB® Kit used in association with Plasma (sample volume 200 µL) and **ELITe InGenius** and **ELITe BeGenius** was tested over with a wider range of concentrations using a panel prepared by diluting a plasmid DNA containing the BKV amplification product in BKV DNA - negative matrix. The panel consisted of eight dilution points (1 Log dilution steps) from 10^8 to 10^2 copies / mL. Each sample of the panel was tested in 3 replicates.

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Plasma samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.999 for **ELITe InGenius** and 0.996 for **ELITe BeGenius**.

The results are reported in the following graphs.





For Plasma (sample volume 200 µL):

The Lower Limit of Quantification (LLoQ) was set at 215 IU / mL, the LoD concentration, that gives quantitative results precise (Standard Deviation equal to 0.2767 Log IU / mL for **ELITe InGenius** and 0.3012 Log IU / mL for **ELITe BeGenius**) and accurate (Bias equal to -0.0098 Log IU / mL for **ELITe InGenius** and 0.2569 Log IU / mL for **ELITe BeGenius**).

The Upper Limit of Quantification (ULoQ) was set at 130,000,000 IU / mL, the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.2159 Log IU / mL for **ELITe InGenius** and 0.3357 Log IU / mL for **ELITe BeGenius**) and accurate (Bias equal to -0.1606 Log IU / mL for **ELITe InGenius** and -0.4406 Log IU / mL for **ELITe BeGenius**).

The linear measuring range as copies / mL for Plasma is calculated by applying the specific conversion factor reported at page 29.

The final results are summarized in the following table.

Linear measuring range for Plasma samples and ELITe InGenius and ELITe BeGenius(200 µL)		
Unit of measure	lower limit	upper limit
IU / mL	**215**	**130,000,000**
copies / mL	**165**	**100,000,000**

For Urine (sample volume 200 µL):

The Lower Limit of Quantification (LLoQ) was set at 142 IU / mL, the LoD concentration that gives quantitative results precise (Standard Deviation = 0.2888 Log IU / mL for **ELITe InGenius** and Standard Deviation = 0.4031 Log IU / mL for **ELITe BeGenius**) and accurate (Bias = 0.1562 Log IU / mL for **ELITe InGenius** and Bias = -0.1668 Log IU / mL for **ELITe BeGenius**) within ±0.5 Log IU / mL.

The Upper Limit of Quantification (ULoQ) was set at 160,000,000 IU / mL the highest concentration that gives quantitative results precise (Standard Deviation = 0.2114 Log IU / mL for **ELITe InGenius** and Standard Deviation = 0.3132 Log IU / mL for **ELITe BeGenius**) and accurate (Bias = -0.3240 Log IU / mL for **ELITe InGenius** and Bias = -0.2860 Log IU / mL for **ELITe BeGenius**) within ±0.5 Log IU / mL.

The linear measuring range as copies / mL for Urine collected without preservatives is calculated by applying the specific conversion factor reported at page 29.

The final results are summarized in the following table.

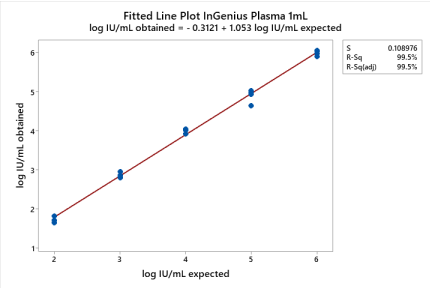
Linear measuring range for Urine samples and ELITe InGenius and ELITe BeGenius (200 µL)		
Unit of measure	lower limit	upper limit
IU / mL	**142**	**160,000,000**
copies / mL	**89**	**100,000,000**

For plasma (sample volume 1000 µL):

The linear measuring range of BKV ELITe MGB® Kit used in association with Plasma collected in EDTA (sample volume 1000 µL) and **ELITe InGenius** was verified using a panel of BKV dilutions. The panel was prepared by diluting the "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom) in BKV DNA - negative matrix. The panel consisted of five dilution points (1 Log dilution steps) from 10^6 to 10^2 IU / mL. Each sample of the panel was tested in 4 replicates.

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Plasma samples (sample volume 1000 µL) shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.995.

The results are reported in the following graph.



The linear measuring range was tested over with a wider range of concentrations by analyzing a BKV dilution panel prepared by diluting a plasmid DNA containing the amplification product in BKV DNA-negative matrix. The panel had 6 dilution steps of 1 Log from 10^9 to 10^4 copies / mL. Each panel point was tested in 4 replicates by performing the entire analysis procedure, run set up, extraction, real time amplification and interpretation of the results with ELITe InGenius and ELITechGroup S.p.A.

The analysis of the obtained data, performed with the linear regression, showed that the assay has a linear response for the panel points from 10^8 to 10^4 copies / mL. For the 10^9 copies / mL point, it was not possible to calculate a Ct value due to the very high concentration

The linear measuring range as copies / mL for Plasma collected in EDTA is calculated by applying the specific conversion factor reported at page 29.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

The final results are summarized in the following table.

Linear measuring range for Plasma samples and ELITe InGenius (1000 µL)		
Unit of measure	lower limit	upper limit
IU / mL	**100**	**170,000,000**
copies / mL	**59**	**100,000,000**

Repeatability

The Repeatability of results obtained by the product BKV ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Plasma samples collected in EDTA. The panel included one negative sample and two samples spiked by BKV certified reference material "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom) at concentration of 3 x LoD (about 645 IU / mL) and of 10 x LoD (about 2150 IU / mL).

The Intra – Session Repeatability on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, with the same lot of product, with the same instrument, by the same operator, on the same day. Samples were processed in randomized positions.

The Inter – Session Repeatability on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, with the same lot of product, with the same instrument, by the same operator, on two different days. Samples were processed in randomized positions.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe InGenius Lot U0121-047								
Sample	**BKV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	27.39	0.24	0.87
3 x LoD	8 / 8	36.66	0.45	1.23				
10 x LoD	8 / 8	34.88	0.56	1.62				

Inter – Session Repeatability ELITe InGenius Lot U0121-047								
Sample	**BKV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	27.14	0.36	1.32
3 x LoD	16 / 16	36.36	0.52	1.43				
10 x LoD	16 / 16	34.40	0.68	1.96				

In the Repeatability test on **ELITe InGenius**, the assay detected the BKV target as expected and showed low %CV of Ct values that did not exceed 2% for BKV and 1.3% for Internal Control.

The Intra – Session Repeatability on **ELITe BeGenius** was obtained through the analysis of panel samples in eight replicates, in one run per day, with the same lot of product, with the same instrument, on the same day. Samples were processed in randomized positions.

The Inter – Session Repeatability on **ELITe BeGenius** was obtained through the analysis of panel samples in eight replicates, in one run per day, with the same lot of product, with the same instrument, on two different days. Samples were processed in randomized positions.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe BeGenius Lot U0121-047								
Sample	BKV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24/24	29.92	0.41	1.37
3 x LoD	8 / 8	37.09	0.52	1.40				
10 x LoD	8 / 8	35.45	0.31	0.88				

Inter – Session Repeatability ELITe BeGenius Lot U0121-047								
Sample	BKV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	29.71	0.49	1.65
3 x LoD	16 / 16	36.68	0.71	1.94				
10 x LoD	16 / 16	34.98	0.55	1.57				

In the Repeatability test on **ELITe BeGenius**, the assay detected the BKV target as expected and showed low %CV of Ct values that did not exceed 1.9% for BKV and 1.7% for Internal Control.

Reproducibility

The Reproducibility of results obtained by the product BKV ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with BKV certified reference material "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom) at concentration of 3 x LoD (about 645 IU / mL) and of 10 x LoD (about 2150 IU / mL).

The Inter – Instrument Reproducibility on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in one run per day, in two days, with two different instruments by two different operators. Samples were processed in randomized positions on **ELITe InGenius** system in "Extract + PCR" mode.

The Inter – Batch Reproducibility on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, with two different lots and the same instrument. Samples were processed in randomized positions on **ELITe InGenius** system in "Extract + PCR" mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe InGenius								
Sample	BKV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	26.88	0.27	0.99
3 x LoD	8 / 8	36.72	0.30	0.82				
10 x LoD	8 / 8	30.89	0.41	1.33				

Inter – Batch Repeatability ELITe InGenius								
Sample	BKV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	26.83	0.34	1.26
3 x LoD	8 / 8	36.94	0.36	0.97				
10 x LoD	8 / 8	35.07	0.28	0.79				

In the Reproducibility test on **ELITe InGenius**, the assay detected the BKV target as expected and showed low %CV of Ct values that did not exceed 1.3% for BKV and 1.3%for Internal Control.

The Inter – Instrument Reproducibility on **ELITe BeGenius** was obtained through the analysis of panel samples in eight replicates, in one run per day, in two days, with two different instruments by two different operators. Samples were processed in randomized positions on **ELITe BeGenius** system in "Extract + PCR" mode.

The Inter – Batch Reproducibility on **ELITe BeGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, with two different lots and the same instrument. Samples were processed in randomized positions on **ELITe BeGenius** system in "Extract + PCR" mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Instrument Repeatability ELITe BeGenius								
Sample	BKV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	29.39	0.42	1.44
3 x LoD	8 / 8	36.87	0.58	1.56				
10 x LoD	8 / 8	34.86	0.25	0.72				

Inter – Batch Repeatability ELITe BeGenius								
Sample	BKV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	29.71	0.69	2.31
3 x LoD	8 / 8	36.81	0.66	1.80				
10 x LoD	8 / 8	35.01	0.41	1.17				

In the Reproducibility test on **ELITe BeGenius**, the assay detected the BKV target as expected and showed low %CV of Ct values that did not exceed 1.8% for BKV and 2.3 %for Internal Control.

Reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of value of a calibrated reference material, was evaluated using as reference material the calibrated panel «BKV Molecular "Q" Panel» (Qnostics, Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **«ELITe InGenius»** and ELITechGroup S.p.A. products.

The results, obtained starting from 200 µL of sample, are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius»				
Sample	Nominal titre copies/mL	Nominal titre Log_{10} copies/mL	Positive / Replicates	Mean results Log_{10} copies / mL
BKVMQP01-High	100000	5.000	2/2	5.237
BKVMQP01-Medium	10000	4.000	2/2	4.243
BKVMQP01-Low	1000	3.000	2/2	3.187
BKVMQP01-Negative	negative	-	0/2	-

All positive samples were detected as positive with a titre was within the expected value ± 0.5 Log.

The results, obtained starting from 1000 µL of sample, are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius»				
Sample	Nominal titre copies/mL	Nominal titre Log_{10} copies/mL	Positive / Replicates	Mean results Log_{10} copies / mL
BKVMQP01-High	100000	5.000	2/2	5.271
BKVMQP01-Medium	10000	4.000	2/2	4.377
BKVMQP01-Low	1000	3.000	2/2	3.120
BKVMQP01-Negative	negative	-	0/2	-

All positive samples were detected as positive with a titre was within the expected value ± 0.5 Log.

Further tests were carried out using as reference material QCMD 2014 BK Virus DNA EQA Panel (Qnostics Ltd, UK) a panel of BKV dilutions within the limit concentration. Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products.

The results obtained, starting from 200 µL of sample, are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius»				
Sample	Consensus conc. Log_{10} copies/mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} copies / mL
BKVDNA14-01	2.330	0.540	2/2	2.713
BKVDNA14-02	3.632	0.416	2/2	3.973
BKVDNA14-03	4.420	0.410	2/2	4.610
BKVDNA14-04	4.630	0.365	2/2	5.056
BKVDNA14-05	3.620	0.389	2/2	4.191
BKVDNA14-06	negative	N.A.	0/2	Not detected
BKVDNA14-07	2.788	0.544	2/2	3.159
BKVDNA14-08	3.024	0.406	2/2	3.405
BKVDNA14-09	negative	N.A.	0/2	Not detected
BKVDNA14-10	1.870	0.617	0/2	Not detected

All negative samples were correctly detected as negative and 7 out of 8 positive samples were correctly detected as positive. The sample BKDNA14-10 at 74 copies/mL was detected as negative. This can be explained because the sample titer is below the detection limit. Five (5) samples were quantified within the range defined by the EQA Consensus ± 1 Standard Deviation and 2 samples were quantitated within 2 SD.

The results obtained, starting from 1000 µL of sample, are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius»				
Sample	Consensus conc. Log_{10} copies/mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} copies / mL
BKVDNA14-01	2.330	0.540	2/2	2.794
BKVDNA14-02	3.632	0.416	2/2	4.165
BKVDNA14-03	4.420	0.410	2/2	4.684
BKVDNA14-04	4.630	0.365	2/2	5.132
BKVDNA14-05	3.620	0.389	2/2	4.118
BKVDNA14-06	negative	N.A.	0/2	Not detected
BKVDNA14-07	2.788	0.544	2/2	3.280
BKVDNA14-08	3.024	0.406	2/2	3.459
BKVDNA14-09	negative	N.A.	0/2	Not detected
BKVDNA14-10	1.870	0.617	0/2	1.914

All negative samples were correctly detected as negative and all positive samples were correctly detected as positive. Four (4) samples were quantified within the range defined by the EQA Consensus ± 1 Standard Deviation and four (4) samples were quantitated within 2 SD.

Conversion factor to International Units

The conversion factor, to convert a quantitative result from copies / mL to International Units / mL, was calculated using a panel of at least six dilutions (0.5 Log_{10} between dilutions) of calibrated reference material approved by WHO ("1st WHO international standard for BKV virus DNA", NIBSC code 14/212, United Kingdom) in different matrices tested negative for BKV DNA. Each point of the panel was tested in 16 replicates carrying out the whole analysis, extraction, amplification, detection and result interpretation with ELITe InGenius and ELITechGroup S.p.A. products.

A summary of results is shown in the tables below.

Conversion factor to International Units Plasma (200 µL), Fc = 1.3 IU / copy						
Sample			Result			Log difference
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	(ref. - test)
1,000,000	6	16	638,228	829,696	5.918	0.082
316,228	5.5	16	218,812	284,456	5.454	0.046
100,000	5	16	77,739	101,061	5.004	-0.004
31,623	4.5	16	27,458	35,695	4.552	-0.052
10,000	4	16	8,660	11,258	4.051	-0.051
3,162	3.5	16	2,436	3,167	3.501	-0.001

Conversion factor to International Units Urine, Fc = 1.6 IU / copy						
Sample			Result			Log difference
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	(ref. - test)
1,000,000	6	16	584,869	935,790	5.971	0.029
316,228	5.5	16	191,554	306,486	5.486	0.014
100,000	5	16	62,702	100,323	5.001	-0.001
31,623	4.5	16	22,267	35,627	4.551	-0.051
10,000	4	16	7,378	11,805	4.072	-0.072
3,162	3.5	16	1,886	3,018	3.479	0.021

Conversion factor to International Units Plasma (1000 µL), Fc = 1.7 IU / copy						
Sample			Result			Log difference
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	(ref. - test)
1,000,000	6	16	607,196	1,030,366	6.013	-0.013
316,228	5.5	16	196,333	333,162	5.523	-0.023
100,000	5	16	62,356	105,813	5.025	-0.025
31,623	4.5	16	19,726	33,474	4.525	-0.025
1,0000	4	16	5,663	9,610	3.983	0.017
3,162	3.5	16	1,656	2,811	3.449	0.051

The results for each matrix are reported in the following table.

Conversion to International unit with ELITe InGenius		
Sample Volume	Matrix	Fc (IU / copies)
200 µL	Plasma	1.3
200 µL	Urine	1.6
1000 µL	Plasma	1.7

The conversion factor, to convert a quantitative result from copies / mL to International Units / mL, was verified for **ELITe InGenius** and also for **ELITe BeGenius** analysing the results obtained during the Linearity test.

The target quantification precision, as Standard Deviation of Log IU/mL, was lower than 0.5 Log for both Plasma and Urine and meet the acceptance criteria for **ELITe InGenius** and **ELITe BeGenius**.

The target quantification accuracy, as difference between the Theoretical and Measured concentrations in Log IU / mL, was lower than 0.5 Log for both Plasma and Urine and meet the acceptance criteria for **ELITe InGenius** and **ELITe BeGenius.**

These results confirmed the Conversion factors calculated for each matrix with **ELITe InGenius**.

Diagnostic sensitivity: confirmation of positive samples

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analysing some clinical samples of plasma collected in EDTA and urine collected without preservatives positive for BKV DNA in association with **ELITe InGenius**. As **ELITe BeGenius** showed equivalent analytical performances to **ELITe InGenius**, it can be assumed that the results of Diagnostic sensitivity obtained in association with **ELITe InGenius** are applicable also to **ELITe BeGenius**.

The test starting from 200 µL of sample was performed on 30 plasma samples collected in EDTA positive for BKV DNA and on 30 urine samples collected without preservatives and positive for BKV DNA (tested with a real time amplification CE IVD product).

The test, starting from 1000 µL of sample, was performed on:

- 25 plasma samples collected in EDTA positive for BKV DNA (tested with a real time amplification CE IVD product).

- 30 plasma samples collected in EDTA negative for BKV DNA, that were spiked for BKV DNA adding "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom).

Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **«ELITe InGenius»**and with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Sample Volume	Type of Samples	N	positive	negative
200 µL	Plasma collected in EDTA and positive for BKV DNA	30	30	0
	Urine collected without preservatives and positive for BKV DNA	30	30	0
1000 µL	Plasma collected in EDTA and positive for BKV DNA	25	25	0
	Plasma collected in EDTA spiked for BKV DNA	30	30	0

All samples were valid for analysis and were confirmed as positive.

In these tests the total diagnostic sensitivity of the assay was equal to 100%.

Diagnostic specificity: confirmation of negative samples

The Diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analysing some clinical samples of plasma collected in EDTA and urine collected without preservatives in association with **ELITe InGenius**. As **ELITe BeGenius** showed equivalent analytical performances to **ELITe InGenius**, it can be assumed that the results of Diagnostic specificity obtained in association with **ELITe InGenius** are applicable also to **ELITe BeGenius**.

The test, starting from 200 µL of sample, was performed on:

- 30 plasma samples collected in EDTA that were negative for BKV DNA (tested with a CE IVD real time amplification product).

- 30 urine samples collected without preservatives that were negative for BKV DNA (tested with a CE IVD real time amplification product).

The test starting from 1000 µL of sample was performed on 62 plasma samples collected in EDTA negative for BKV DNA (tested with a CE IVD real time amplification product).

Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **«ELITe InGenius»**and ELITechGroup S.p.A. products.

The results are summed up in the following table.

Sample Volume	Type of Samples	N	positive	negative
200 µL	Plasma collected in EDTA and negative for BKV DNA	30	2	28
	Urine collected without preservatives and negative for BKV DNA	30	0	30
1000 µL	Plasma collected in EDTA and negative for BKV DNA	62	2	60

All urine samples were valid for analysis and confirmed negative.

All plasma samples were valid for the analysis.

For samples with 200 µL samples: twenty-eight (28) out of 30 plasma samples were confirmed to be negative for BKV - DNA, two samples were discrepant positive at low titer (respectively about 50 and 70 copies/mL) and below the limit of detection of the reference method.

For samples with 1000 µL samples: sixty (60) out of 62 plasma samples were confirmed to be negative for BKV - DNA, two samples were discrepant positive at low titer (respectively about 5 and 55 copies/mL).

In these tests, the total diagnostic specificity of the assay was equal to 97%.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File "BKV ELITe MGB® Kit", FTP 175PLD.

ABI 7500 Fast Dx Real-Time PCR Instrument
ABI 7300 Real-Time System

SAMPLES AND CONTROLS

Samples

This product must be used with **DNA extracted** from the following clinical samples: plasma collected in EDTA, urine collected without preservatives and cerebrospinal fluid.

Plasma collected in EDTA

The plasma samples for nucleic acids extraction must be collected in EDTA according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when you carry out the DNA extraction using **«EXTRAblood»** kit, please, follow the instructions for use manual: start from **200 µL** of sample, add **5 µL** of **CPE** for the internal control at the beginning of the extraction, recover the DNA with **60 µL** of elution buffer.

Note: when you carry out the DNA extraction from plasma with the **«ELITe STAR»** and with **software version 3.4.13** (or later equivalent versions), use the extraction protocol "**UUNI_E100S200_ELI**", that uses 200 µL of sample and elutes the extract in 100 µL. Samples in primary tubes can be directly loaded on **«ELITe STAR»**. A minimum volume of 700 µL is always required for each sample. Add **200 µL** of **CPE** into Proteinase-Carrier tube as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from plasma with the **«ELITe GALAXY»** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses 300 µL of sample and elutes the extract in 100 µL or 200 µL. Samples in primary tubes can be directly loaded on **«ELITe GALAXY»**. A minimum volume 400-650 µL dependent on the tube class used is always required for each sample. Add **10 µL** / **sample** of **CPE.** The CPE must be added to **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction with the instrument **«NucliSENS® easyMAG®»**, please, follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica**, recover the DNA with **100 µL** of elution buffer.

Note: when you carry out the DNA extraction from plasma with the instrument **«QIAsymphony® SP/AS»** and the kit **«QIAsymphony® DSP Virus / Pathogen Midi kit»** with **software version 3.5**, use the extraction protocol **"Virus Cell free 500_V3_DSP_default IC"** and follow these directions: the instrument is able to use a primary tube, sample volume required for the extraction is **500 µL**, it's always requested a minimum dead volume of 100 µL. Prepare the solution containing AVE buffer and RNA carrier, according to the instruction manual of the extraction kit. Add **6 µL** / **sample** of **CPE** to the solution for each requested sample. Load on the instrument, in the "internal control" slot, the tubes containing the solution, as indicated in the instruction for use manual of the kit; indicate the position where eluates will be dispensed and specify the elution volume of **85 µL**. For details on the extraction procedure follow indications in the instruction for use manual of the kit.

Urine collected without preservatives

The urine samples for nucleic acids extraction must be collected in preservative-free containers according to laboratory guidelines, transported at room temperature (+18 / +25 °C) and stored at room temperature (+18 / +25 °C) for a maximum of four hours, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

The freezing of urine samples often causes the formation of precipitates that may compromise subsequent stages of the assay: only use the supernatant for the extraction.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when you carry out the DNA extraction using **«EXTRAblood»** kit, please, follow the instructions for use manual: start from **200 µL** of sample, add **5 µL** of **CPE** for the internal control at the beginning of the extraction, recover the DNA with **60 µL** of elution buffer.

Note: when you carry out the DNA extraction from urine with the **«ELITe STAR»** and with **software version 3.4.13** (or later equivalent versions), use the extraction protocol "**UUNI_E100S200_ELI**", that uses 200 µL of sample and elutes the extract in 100 µL. Samples in primary tubes can be directly loaded on **«ELITe STAR»**. A minimum volume of 700 µL is always required for each sample. Add **200 µL** of **CPE** into Proteinase-Carrier tube as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from urine with the **«ELITe GALAXY»** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses 300 µL of sample and elutes the extract in 100 µL or 200 µL. Samples in primary tubes can be directly loaded on **«ELITe GALAXY»**. A minimum volume 400-650 µL dependent on the tube class used is always required for each sample. Add **10 µL** / **sample** of **CPE.** The CPE must be added to **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction with the instrument **«NucliSENS® easyMAG®»**, please, follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica**, recover the DNA with **100 µL** of elution buffer.

Cerebrospinal fluid

The cerebrospinal fluid samples for nucleic acids extraction must be collected according to laboratory guidelines avoiding contamination by patient blood, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of four hours, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when you carry out the DNA extraction with the instrument **«NucliSENS® easyMAG®»**, please, follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica**, recover the DNA with **100 µL** of elution buffer.

Interfering substances

The DNA extracted from the sample must not contain heparin, haemoglobin, dextran, Ficoll®, ethanol or 2-propanol in order to prevent the problem of inhibition and the possibility of frequent invalid results.

High quantity of human genomic DNA in the DNA extracted from the sample may inhibit the amplification reaction.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Amplification controls

It is absolutely mandatory to validate each amplification session with a negative control reaction and a positive control reaction.

For the negative control, use molecular biology grade water (not provided with this product) added to the reaction in place of the DNA extracted from the sample.

For the positive control, use the **«BKV - ELITe Positive Control»** product or the **«BKV ELITe Standard»** product.

PROCEDURE

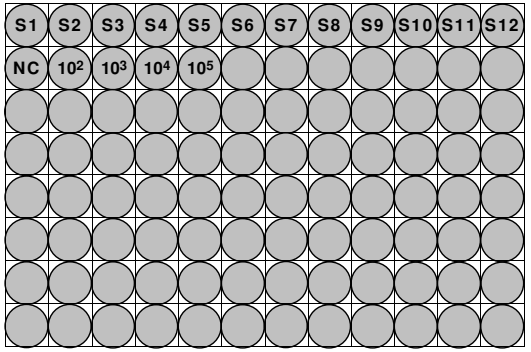
Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

When **7300 Real-Time PCR System** instrument is used:

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session;
- set (Detector Manager) the "detector" for the BKV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "BKV";
- set (Detector Manager) the "detector" for the Internal Control probe with the "reporter" = "VIC" (AP525 is analogous to VIC) and the "quencher" = "none" (non fluorescent) and call it "IC";
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

Note: In order to determine the DNA titre in the starting sample, set up a series of reactions with the **Q - PCR Standards** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard curve.**

See below, by way of example, how you can organise the quantitative analysis of 12 samples.



Legend: S1 - **S12**: Samples to be analysed; **NC**: Negative Control of amplification;
10^2: 10^2 standard copies; **10^3**: 10^3 standard copies; **10^4**: 10^4 standard copies; **10^5**: 10^5 standard copies.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:
- add to amplification stage the step (Add Step) of **extension at 72 °C**;

Note: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60 °C.

- modify timing as indicated in the table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;
- optional: add dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**.

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (fluorescence acquisition)	30 sec.
	72 °C	20 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	30 sec.
	80 °C	15 sec.

When a **7500 Fast Dx Real-Time PCR Instrument** is used:

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session and set "Run mode: Fast 7500";
- set (Detector Manager) the "detector" for the BKV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "BKV";
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "VIC" (AP525 is similar to VIC) and the "quencher" = "none" (non fluorescent) and call it "IC";
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "Cy5" (AP593 is used instead of Cy5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

Note: In order to determine the DNA titre in the starting sample, set up a series of reactions with the **Q - PCR Standards** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard curve**.

The set up of a quantitative analysis of 12 samples is shown, by way of example, in the previous paragraph describing the procedure for the **7300 Real Time PCR System** instrument.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:
- add to amplification stage the step (Add Step) of **extension at 72 °C**;

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

Note: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60 °C.

- modify timing as indicated in the table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;
- optional: add dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**.

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (fluorescence acquisition)	30 sec.
	72 °C	20 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	1 min.
	80 °C	15 sec.
Dissociation (optional)	60 °C	15 sec.

Amplification set-up
(To be performed in the extraction / preparation of the amplification reaction area)

Before starting the session, it is necessary to:
- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **BKV Q - PCR Mix** tubes required for the session, remembering that each tube is sufficient for preparing **25 reactions**. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **BKV - Positive Control** or the **BKV Q - PCR Standard** tubes. Mix them gently, spin down the content for 5 seconds and keep them on ice;
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powder-free gloves and not to damage the wells.

1. Accurately pipet **20 µL** of reaction mixture **BKV Q - PCR Mix** on the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

Note: If not all the reaction mixture is used, store the remaining volume in the dark at -20 °C for no longer than one month. Freeze and thaw the reaction mixture from a maximum of **5 TIMES**.

2. Accurately pipet, by placing into the reaction mixture, **20 µL** of **extracted DNA** from the first sample in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the sample by pipetting the **extracted DNA** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other samples of **extracted DNA**.

3. Accurately pipet, by placing into the reaction mixture, **20 µL** of **molecular biology grade water** (not provided with this product) in the well of **Amplification microplate** of the negative control of amplification, as previously established in the **Work Sheet**. Mix well the negative control by pipetting the **molecular biology grade water** three times into the reaction mixture. Avoid creating bubbles.

4. On the basis of the result required (qualitative or quantitative), one of these two options must be followed:

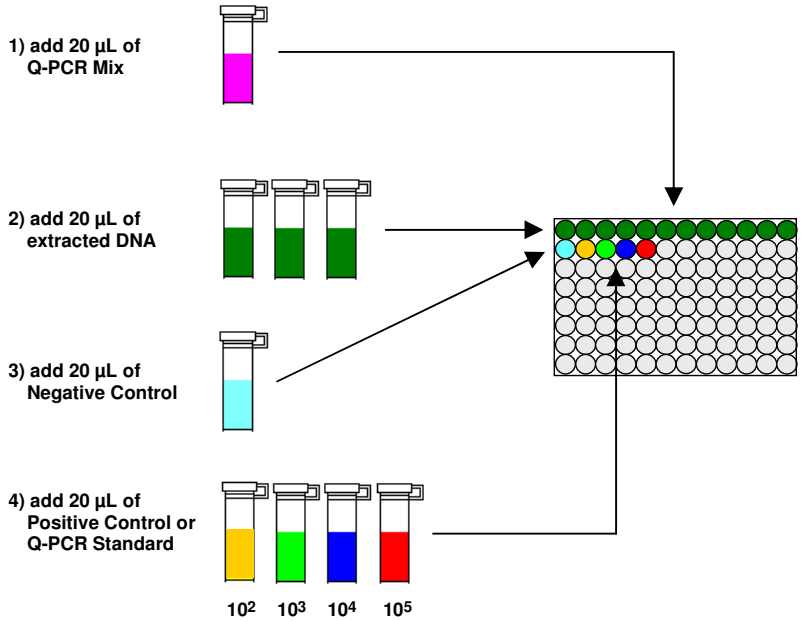
   - When a **qualitative** result of the analysis is required (detection of BKV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **BKV - Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the **BKV - Positive Control** three times into the reaction mixture. Avoid creating bubbles.

- When a **quantitative** result of the analysis is required (quantification of BKV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **BKV Q - PCR Standard 10^2** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **BKV Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the **BKV Q - PCR Standards 10^3, 10^4, 10^5**.

5. Accurately seal the **Amplification microplate** with the **Amplification Sealing Sheet**.
6. Transfer the **Amplification microplate** into the real time thermal cycler in the amplification / detection of amplification products area and start the thermal cycle for the amplification saving the session setting with an univocal and recognizable file name (e.g. "year-month-day-BKV-EGSpA ").

Note: At the end of the thermal cycle the **Amplification microplate** with the reaction products must be removed from the instrument and eliminated without producing environmental contaminations. In order to avoid the spilling of the reaction products, the **Amplification Sealing Sheet must not to be removed from the Amplification microplate**.

The following figure shows synthetically the preparation of the amplification reaction.



Note: if the preparation of the amplification is performed with the instrument **«QIAsymphony® SP/AS»**, insert the microplate containing the exctracts, the regents and the amplification microplate in the dedicated slots, using the special adaptors, then follow indications in the instruction for use manual of the setup module and the steps required by the software.

Note: if the preparation of the amplification reaction is performed with the **«ELITe GALAXY»** instrument, load the elution microplate, the complete reaction mixture and the amplification microplate as indicated in the instrument user manual and following the steps required by the GUI.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification
REF RTS175PLD

Qualitative analysis of the results

The recorded values of the fluorescence emitted by the specific BKV probe (FAM detector "BKV") and by the specific Internal Control probe (VIC detector "IC") in the amplification reactions must be analysed by the instrument software.

Before starting the analysis, referring to the instrument documentation, it is necessary to:
- set manually (Results > Amplification plot > delta Rn vs Cycle) the calculation range for the **Baseline** (**fluorescence background level**) from cycle 6 to cycle 15;

Note: In the case of a positive sample with a high titre of BKV DNA, the FAM fluorescence of the BKV specific probe may begin to increase before the cycle 15. In this case the calculation range for the **Baseline** must be adapted from cycle 6 to the cycle in which the FAM fluorescence of the sample begins to increase, as detected by the instrument software (Results > Component).

When a **7300 Real-Time PCR System** instrument is used:

- set manually the **Threshold** for the FAM detector "BKV" to **0.1**;
- set manually the **Threshold** for the VIC detector "IC" to **0.05**.

When a **7500 Fast Dx Real-Time PCR Instrument** is used:

- set manually the **Threshold** for the FAM detector "BKV" to **0.2**;
- set manually the **Threshold** for the VIC detector "IC" to **0.1**.

The values of fluorescence emitted by the specific probes in the amplification reaction and the **Threshold** value of fluorescence allow determining the **Threshold cycle (Ct)**, the cycle in which the fluorescence reached the **Threshold** value.

In the **Positive Control*** amplification reaction, the **Ct** value of BKV (Results > Report) is used to validate the amplification and the detection as described in the following table:

Positive Control reaction detector FAM "BKV"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for BKV, the target DNA was not correctly detected. This means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mix or of the positive control, degradation of the reaction mix or of the positive control, incorrect setting of the position of the positive control, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

***Note:** When this product is used for the quantification of BKV DNA, the **Q - PCR Standard** reactions were set up instead of the **Positive Control** reaction. In this case, validate the amplification and the detection by referring to the amplification reaction of **Q - PCR Standard 10^5** (**Ct ≤ 25**).

In the **Negative control** amplification reaction, the **Ct** value of BKV (Results > Report) is used to validate the amplification and the detection as described in the following table:

Negative control reaction detector FAM "BKV"	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative control** amplification reaction is different from **Ct Undetermined** for BKV, the target DNA was detected. This means that problems occurred during the amplification step (contamination) which may lead to incorrect results and false positives. The session is not valid and needs to be repeated starting from the amplification step.

In the amplification reaction of each **sample**, the **Ct** value of BKV is used to detect the target DNA, while the **Ct** value of Internal Control is used to validate extraction, amplification and detection.

Note: Verify with the instrument software (Results > Amplification plot > delta Rn vs Cycle) that the **Ct** was determined by a fast and regular increase of the fluorescence values and not by peaks or an increase of the background (irregular or high background).

This product is able to detect a minimal quantity of about 10 copies of DNA of the Large T antigen gene of BKV in the amplification reaction, corresponding to the genome Equivalents per reaction (detection limit for the product, see Performance Characteristics paragraph).

The results as **Ct** of the amplification reactions of each **sample** (Results > Report) are used as described in the following table:

Sample reaction		Sample suitability	Assay result	BKV DNA
detector FAM "BKV"	detector VIC "IC"			
Ct Undetermined	Ct > 35 or Ct Undetermined	unsuitable	invalid	-
	Ct ≤ 35	suitable	valid, negative	NOT DETECTED
Ct Determined	Ct > 35 or Ct Undetermined	suitable	valid, positive	DETECTED
	Ct ≤ 35	suitable	valid, positive	DETECTED

If the result of the amplification reaction of a sample is **Ct Undetermined** for BKV and **Ct > 35** or **Ct Undetermined** for the Internal Control, it means that it was impossible to detect efficiently the DNA for the Internal Control. In this case problems occurred during the amplification step (inefficient or absent amplification) or during the extraction step (loss of DNA during the extraction or presence of inhibitors) which may lead to incorrect results and false negatives. The sample is not suitable, the assay is invalid and it needs to be repeated starting from the extraction of a new sample.

If the result of the amplification reaction of a sample is **Ct Undetermined** for BKV and **Ct ≤ 35** for the Internal Control, it means that the BKV DNA is not detected in the DNA extracted from the sample; but it can not be excluded that the BKV DNA has a lower titre than the detection limit of the product (see the paragraph about Performance Characteristics). In this case the result could be a false negative.

The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes about the patient.

Note: When in the amplification reaction of a sample the BKV DNA is detected, the Internal Control may result as Ct > 35 or Ct Undetermined. In fact, the low efficiency amplification reaction for the Internal Control may be displaced by competition with the high efficiency amplification reaction for BKV DNA. In this case the sample is nevertheless suitable and the positive result of the assay is valid.

Quantitative analysis of the results

After carrying out the procedure for qualitative analysis of the results it is possible to perform the quantitative analysis of the results of the positive samples.

In the amplification reactions of the four **Q - PCR standards**, the **Ct** values of BKV are used to calculate the **Standard Curve** (Results > Standard Curve) for the amplification session and to validate the amplification and the detection as described in the following table:

Standard Curve detector FAM "BKV"	Acceptability range	Amplification / Detection
Correlation coefficient (R2)	0.990 ≤ R2 ≤ 1.000	CORRECT

If the **Correlation coefficient (R2)** value does not fall within the limits, this means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mixture or of the standards, degradation of the reaction mixture or of the standards, incorrect setting of the position of the standards, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification
REF RTS175PLD

The **Ct** values of BKV in the amplification reaction of each **sample** and the **Standard Curve** (Results > Standard Curve) of the amplification session are used to calculate the **Quantity** of target DNA present in the amplification reactions of the samples.

This product is able to quantify from 1,000,000 to 10 copies of DNA of the Large T antigen gene of BKV in the amplification reaction, corresponding to the genome Equivalents per reaction (linear measuring range of the product, see Performance Characteristics), as described in the following table:

Sample result detector FAM "BKV"	BKV genome Equivalents per reaction
Quantity > 1 x 10^6	MORE THAN 1,000,000
1 x 10^1 ≤ Quantity ≤ 1 x 10^6	= Quantity
Quantity < 1 x 10^1	LESS THAN 10

The results (**Quantity**) of each **sample** (Results > Report) are used to calculate the genome Equivalents (**gEq**) of BKV present in the sample used in the extraction (**Nc**) according to this formula:

$$\text{Nc (gEq)} = \frac{\text{Ve x Quantity}}{\text{Vc x Va x Ep}}$$

Where:
Vc is the quantity of the sample used in the extraction in rate to the required unit of measurement;
Ep is the efficiency of the procedure, extraction and amplification, **expressed in decimal**;
Ve is the total volume of the extraction product **expressed in µL**;
Va is the volume of the extraction product used in the amplification reaction **expressed in µL**;
Quantity is the result of the amplification reaction of the sample **expressed in gEq per reaction**.

When «**EXTRAblood**» extraction kit is used with plasma samples collected in EDTA or urine samples collected without preservatives and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for plasma, urine and «EXTRAblood»
Nc (gEq / mL) = 15 x Quantity

When **«ELITe STAR»** is used with plasma samples collected in EDTA or urine samples collected without preservatives and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for plasma, urine and «ELITe STAR»
Nc (gEq / mL) = 28 x Quantity

When **«ELITe GALAXY»** is used with plasma samples collected in EDTA or urine samples collected without preservatives and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for plasma, urine and «ELITe GALAXY»
Nc (gEq / mL) = 35 x Quantity

When «**NucliSENS® easyMAG®**» extraction system is used with plasma samples collected in EDTA, urine samples collected without preservatives or cerebrospinal fluid samples and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for plasma, urine, cerebrospinal fluid and «NucliSENS® easyMAG®»
Nc (gEq / mL) = 10 x Quantity

When «**QIAsymphony® SP/AS**» extraction system is used with plasma samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for plasma and «QIAsymphony® SP/AS»
Nc (gEq / mL) = 12 x Quantity

Calculation of the linear measuring range limits

When a particular extraction method is used, the linear measuring range limits as gEq / mL of the sample may be calculated from the linear measuring range of the amplification reaction according to this formula:

$$\text{Lower limit (gEq / mL)} = \frac{\text{Ve x 10 gEq}}{\text{Vc x Va x Ep}}$$

$$\text{Upper limit (gEq / mL)} = \frac{\text{Ve x 1,000,000 gEq}}{\text{Vc x Va x Ep}}$$

When «**EXTRAblood**» extraction kit is used with plasma samples collected in EDTA or urine samples collected without preservatives, the formula becomes:

Linear measuring range limits (gEq / mL) with «EXTRAblood»
Lower limit (gEq / mL) = 15 x 10 gEq **Upper limit (gEq / mL) = 15 x 1,000,000 gEq**
from 150 to 15,000,000 gEq / mL

When «**ELITe STAR**» is used with plasma samples collected in EDTA or urine samples collected without preservatives, the formula becomes:

Measuring range limits (gEq / mL) with «ELITe STAR»
Lower limit (gEq / mL) = 28 x 10 copies **Upper limit (gEq / mL) = 28 x 1,000,000 copies**
from 280 to 28,000,000 gEq / mL

When «**ELITe GALAXY**» is used with plasma samples collected in EDTA or urine samples collected without preservatives, the formula becomes:

Measuring range limits (gEq / mL) with «ELITe GALAXY»
Lower limit (gEq / mL) = 35 x 10 copies **Upper limit (gEq / mL) = 35 x 1,000,000 copies**
from 350 to 35,000,000 gEq / mL

When «**NucliSENS® easyMAG®**» extraction system is used with plasma samples collected in EDTA, urine samples collected without preservatives or cerebrospinal fluid samples, the formula becomes:

Linear measuring range limits (gEq / mL) with «NucliSENS® easyMAG®»
Lower limit (gEq / mL) = 10 x 10 gEq **Upper limit (gEq / mL) = 10 x 1,000,000 gEq**
from 100 to 10,000,000 gEq / mL

When «**QIAsymphony® SP/AS**» extraction system is used with plasma samples collected in EDTA, the formula becomes:

Measuring range limits (gEq / mL) with «QIAsymphony® SP/AS»
Lower limit (gEq / mL) = 12 x 10 gEq **Upper limit (gEq / mL) = 12 x 1,000,000 gEq**
from 120 to 12,000,000 gEq / mL

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: detection limit

The analytical sensitivity of this assay allows detecting the presence of about 10 target DNA molecules in the 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of the assay, as detection limit, was tested using a plasmidic DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmidic DNA was diluted to a titre of 10 copies / 20 µL in human genomic DNA at a titre of 500 ng / 20 µL. This sample was tested in 50 replicates carrying out the amplification by ELITechGroup S.p.A. products.

The final results are summed up in the following table.

Samples	**No.**	**positive**	**negative**
10 copies of plasmidic DNA + 500 ng of human genomic DNA	50	49	1

The analytical sensitivity of this assay used in association to plasma and urine samples and **«ELITe GALAXY»** was verified with a panel of BKV dilutions within the limiting concentration. The panel was prepared by diluting the JCBK11-06 sample (“QCMD 2011 JC virus and BK virus DNA EQA Panel”, Qnostics Ltd, UK) in BKV DNA - negative EDTA plasma. The viral concentrations ranged from 10 gEq / mL to 560 gEq / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The final results are reported in the following tables.

Limit of Detection for plasma samples and «ELITe GALAXY» (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**190 gEq / mL**	**122** gEq / mL	**452** gEq / mL

Limit of Detection for urine samples and «ELITe GALAXY» (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**119 gEq / mL**	**75** gEq / mL	**309** gEq / mL

Analytical sensitivity: linear measuring range

The analytical sensitivity of this assay allows the quantification from 1,000,000 to 10 molecules of target DNA in the 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of the assay, as linear measuring range, was determined using a panel of dilutions (1 log_{10} between one dilution and the next) of a plasmidic DNA containing the amplification product whose initial concentration was measured by a spectrophotometer. The dilutions from 10^7 molecules per reaction to 10^1 molecules per reaction were tested in 9 replicates carrying out the amplification by ELITechGroup S.p.A. products.

The analysis of the obtained data, performed by linear regression, demonstrated that the assay displays a linear response for all the dilutions (linear correlation coefficient greater than 0.99).

The upper limit of the linear measuring range was set at 10^6 molecules per reaction, corresponding to the genome Equivalents per reaction, within one logarithm from the highest concentration Q - PCR Standard amplification standard (10^5 molecules / 20 µL).

The lower limit of the linear measuring range was set at 10 molecules per reaction, corresponding to the genome Equivalents per reaction, within one logarithm from the lowest concentration Q - PCR Standard amplification standard (10^2 molecules / 20 µL).

The final results are summed up in the following table.

Linear measuring range (gEq / reaction)	
Upper limit	1,000,000 gEq / reaction
Lower limit	10 gEq / reaction

The linear measuring range limits as gEq / mL referring to the used extraction kit are calculated from page 29.

Analytical sensitivity: Precision and Accuracy

The precision of the assay, as the variability of results obtained with several replicates of a sample tested within the same amplification session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 30.4% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The accuracy of the assay, as the difference between the mean of results obtained with several replicates of a sample within the same amplification session and the theoretical concentration value of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 5.2% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The precision and the accuracy were determined using data obtained for the study of the linear measuring range.

Analytical sensitivity: reproducibility with panel of certified reference material

The analytical sensitivity of the assay, as reproducibility of results compared with results obtained using other assays and in different laboratories, was checked testing a panel of certified reference material.

The tests were carried out using as certified and calibrated reference material a panel of dilutions of BKV within the limit concentration ("QCMD 2009 JC Virus and BK Virus EQA Panel", Qnostics Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole analysis procedure, extraction with **«EXTRAblood»** and amplification, by ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with certified reference material and «EXTRAblood»				
Sample	Consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
JC.BK09-01	JCV-3B, 2.844	0.606	0 / 2	Not detected
JC.BK09-02	BKV-1B, 2.960	0.511	2 / 2	2.944
JC.BK09-03	JCV-2B, 2.682	0.612	0 / 2	Not detected
JC.BK09-04	Negative, NA	NA	0 / 2	Not detected
JC.BK09-05	BKV, 2.565	0.579	2 / 2	2.185
JC.BK09-06	BKV, 2.853	0.603	2 / 2	2.544
JC.BK09-07	JCV-2B, 3.801	0.628	0 / 2	Not detected
JC.BK09-08	BKV, 3.451	0.533	2 / 2	3.364
JC.BK09-09	JCV-2B, 2.234	0.697	0 / 2	Not detected
JC.BK09-10	BKV, 4.462	0.576	2 / 2	4.254
JC.BK09-11	JCV-2B, 3.142	0.663	0 / 2	Not detected
JC.BK09-12	BKV, 2.898	0.493	2 / 2	2.582

All samples were correctly detected. All the quantitative results obtained are within the range defined by the Consensus ± 1 Standard Deviation.

Further tests were carried out using as calibrated reference material a panel of dilutions of BKV within the concentration limit ("QCMD 2013 JC Virus and BK Virus EQA Panel", Qnostics Ltd, UK). Each sample was tested in duplicates carrying out the whole analysis procedure: extraction with **«ELITe STAR»** and amplification with ELITechGroup S.p.A. products.

The results in gEq/mL are reported in the following table.

Tests with calibrated reference materials and «ELITe STAR»				
Sample	Commercial assay consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
JCBK13-01	BKV-1B, 2.581	0.430	2/2	2.727
JCBK13-02	BKV-2B, 3.651	0.400	2/2	3.925
JCBK13-03	BKV-1B, 4.272	0.358	2/2	4.312
JCBK13-04	JCV-1A, 4.394	-	0/2	-
JCBK13-05	JCV-1A, 3.504	-	0/2	-
JCBK13-06	BKV-2B, 1.651	0.599	2/2	1.734
JCBK13-07	-	-	0/2	-
JCBK13-08	BKV-2B, 4.673	0.390	2/2	4.958
JCBK13-09	JCV-1A, 2.670	-	0/2	-
JCBK13-10	JCV-2B, 3.017	-	0/2	-
JCBK13-11	JCV-3A, 2.702	-	0/2	-
JCBK13-12	JCV-3A, 3.017	-	0/2	-

All negative samples were correctly detected as negatives and all positive samples were detected as positives in agreement with quantitative results defined by commercial assays consensus.

Further tests were carried out using as calibrated reference material a panel of dilutions of BKV within the concentration limit ("QCMD 2012 JC Virus and BK Virus Proficiency Panel", Qnostics Ltd, UK). Each sample was tested carrying out the whole analysis procedure: extraction and PCR Setup with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «ELITe GALAXY»				
Sample	Commercial assay consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
JCBK12-01	JCV-1A	-	0/2	-
JCBK12-02	BKV-1B-2, 3.596	0.466	2/2	4.009
JCBK12-03	BKV-1B-1, 2.543	0.445	2/2	2.905
JCBK12-04	JCV-1A	-	0/2	-
JCBK12-05	JCV-3A	-	0/2	-
JCBK12-06	Negative	-	0/2	-
JCBK12-07	BKV-1B-2, 1.729	0.573	2/2	2.346
JCBK12-08	BKV-1B-2, 4.681	0.462	2/2	5.046
JCBK12-09	JCV-1A	-	0/2	-
JCBK12-10	JCV-3A	-	0/2	-
JCBK12-11	JCV-1A	-	0/2	-
JCBK12-12	BKV-1B-1, 5.248	0.444	2/2	5.603

All negative samples were correctly detected as negatives and all positive samples were detected as positives. Four (4) samples were quantified within the range defined by the EQA Consensus ± 1 Standard Deviation and one sample was quantitated within 2 SD.

Diagnostic sensitivity: detection and quantification efficiency with different genotypes / subtypes

The diagnostic sensitivity of the assay, as detection and quantification efficiency on different genotypes / subtypes, was evaluated by comparison of sequences with nucleotide databases.

The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probe in the alignment of the sequences available in the database for the Large T antigen gene of BKV showed their conservation and absence of significant mutations.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested using some clinical samples positive for BKV DNA.

The diagnostic sensitivity was evaluated using as reference material 22 plasma samples collected in EDTA and 22 urine samples collected without preservatives, all positive for BKV DNA (tested with a CE IVD real time amplification product). Each sample was tested carrying out the whole analysis procedure, extraction with **«EXTRAblood»** and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Plasma collected in EDTA positive for BKV DNA	22	22	0
Urine collected without preservatives positive for BKV DNA	22	22	0

The diagnostic sensitivity in this test was equal to 100%.

The diagnostic sensitivity was evaluated using 30 samples of plasma collected in EDTA positive for BKV DNA, and 30 urine samples positive for BKV DNA. Each sample was used to carry out the whole analysis procedure: extraction with **«ELITe STAR»** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA positive for BKV DNA	30	29	1
Urine positive for BKV DNA	30	30	0

One plasma sample gave a negative result probably due to the low titre and to degradation of the sample.

The diagnostic sensitivity in this test was equal to 98.3 %.

The diagnostic sensitivity was evaluated using as reference material 9 plasma samples collected in EDTA from donors, positive for BKV DNA (tested with a CE IVD real time amplification product) and 30 negative plasma samples collected in EDTA that were spiked to a titre near the detection limit for BKV DNA with a calibrated and certified reference sample ("QCMD 2011 JC Virus and BK Virus EQA Panel", Qnostics Ltd, UK).

The diagnostic sensitivity was evaluated using as reference material 1 urine sample collected without preservatives, from a donor, positive for BKV DNA (tested with a CE IVD real time amplification product) and 30 negative urine samples collected without preservatives, that were spiked to a titre near the detection limit for BKV DNA with a calibrated and certified reference sample ("QCMD 2011 JC Virus and BK Virus EQA Panel", Qnostics Ltd, UK).

Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
BKV DNA positive plasma collected in EDTA	9	9	0
Plasma collected in EDTA spiked for BKV DNA	30	30	0
BKV DNA positive urine collected without preservatives	1	0	1
Urine collected without preservatives spiked for BKV DNA	30	29	1

The diagnostic sensitivity of the assay in these tests was higher than 97%.

Analytical specificity: absence of cross-reactivity with potential interfering markers

The analytical specificity of the assay, as absence of cross-reactivity with other potential interfering markers, was evaluated by comparison of sequences with nucleotide databases.

The analysis of the alignment of the sequences of the primers and of the fluorescent probe with the sequences available in databases for organisms other than BKV, including JCV complete genome, the human Polyomavirus that is most similar to BKV, showed their specificity and the absence of significant homology.

The analytical specificity of the assay, as absence of cross-reactivity with other potential interfering markers, was checked using some clinical samples negative for BKV DNA and positive for DNA of other pathogens.

The analytical specificity was checked using as reference material 18 plasma samples collected in EDTA, that were negative for BKV DNA but positive for DNA of other pathogens as JCV, HSV1 and HHV8 (tested with CE IVD amplification products). Each sample was tested carrying out the whole analysis procedure, extraction with **«EXTRAblood»** and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Plasma collected in EDTA negative for BKV DNA and positive for DNA of other pathogens	18	0	18

The analytical specificity of the assay, as absence of cross-reactivity with other potential interfering markers, was checked by testing a panel of certified reference material.

The analytical specificity was checked using as certified and calibrated reference material a panel including JCV positive samples ("QCMD 2009 JC Virus and BK Virus EQA Panel", Qnostics Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole analysis procedure, extraction and amplification by ELITechGroup S.p.A. products.

The results obtained are reported in the paragraph "Analytical sensitivity: reproducibility with panel of certified reference material".

No cross-reactivity was detected with samples positive for DNA of other pathogens.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was tested using some clinical samples negative for BKV.

The diagnostic specificity was evaluated using as reference material 22 plasma samples collected in EDTA and 22 urine samples collected without preservatives, all negative for BKV DNA (tested with a CE IVD real time amplification product). Each sample was tested carrying out the whole analysis procedure, extraction with **«EXTRAblood»** and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Plasma collected in EDTA negative for BKV DNA	22	0	22
Urine collected without preservatives negative for BKV DNA	22	0	22

The diagnostic specificity in this test was equal to 100%.

The diagnostic specificity was evaluated using 30 samples of plasma collected in EDTA and negative for BKV DNA, and 30 urine samples negative for BKV DNA. Each sample was used to carry out the whole analysis procedure: extraction with **«ELITe STAR»** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA negative for BKV DNA	30	0	30
Urine negative for BKV DNA	30	0	30

The diagnostic specificity in this test was equal to 100%.

The diagnostic specificity was evaluated using 38 plasma samples collected in EDTA that were negative for BKV DNA and 31 urine samples collected without preservatives that were negative for BKV DNA (tested with a CE IVD real time amplification product). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
BKV DNA negative plasma collected in EDTA	38	0	38
BKV DNA negative urine collected without preservatives	31	0	31

The diagnostic specificity in this test was equal to 100%.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File "BKV ELITe MGB® Kit", FTP 175PLD.

Roche cobas z 480 analyzer

SAMPLES AND CONTROLS

Samples

This product must be used with **DNA extracted** from the following clinical samples:

Plasma collected in EDTA

The plasma samples for nucleic acid extraction must be collected in EDTA according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of three days, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing.

When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when you carry out DNA extraction from plasma samples with the **"MagNA Pure 24 System"** instrument with **software version 1.0** (or equivalent later versions), use the **"Pathogen200"** extraction protocol and follow these instructions: dispense **350 µL** of sample into the MagNA Pure Tube 2.0 mL, load the tube into the instrument and begin extraction. This protocol processes 200 µL of sample, adds **CPE** 20 µL / extraction and elutes the nucleic acids into 100 µL. The **CPE** must be diluted 1:2 in ultra-pure molecular biology grade water. For details of the extraction procedure, follow the instructions contained in the kit's User Manual carefully.

Urine

Urine samples for nucleic acid extraction must be collected in preservative-free containers according to laboratory guidelines, transported at room temperature (+18 / +25 °C) and stored at room temperature (+18 / + 25 °C) for a maximum of four hours, otherwise they must be stored at +2 / +8 °C for a maximum of three days. If possible, avoid freezing of first void urine samples. Freezing can cause precipitation of inhibitors and the loss of the DNA titre.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

Note: when you carry out DNA extraction from urine samples with the **"MagNA Pure 24 System"** instrument with **software version 1.0** (or equivalent later versions), use the **"Pathogen200"** extraction protocol and follow these instructions: dispense **350 µL** of sample into the MagNA Pure Tube 2.0 mL, load the tube into the instrument and begin extraction. This protocol processes 200 µL of sample, adds **CPE** 20 µL / extraction and elutes the nucleic acids into 100 µL. The **CPE** must be diluted 1:2 in ultra-pure molecular biology grade water. For details of the extraction procedure, follow the instructions contained in the kit's User Manual carefully.

Interfering substances

The DNA extracted from the sample must not contain heparin, haemoglobin, dextran, Ficoll®, ethanol or 2-propanol in order to prevent inhibition problems and the possibility of frequent invalid results.

High quantity of human genomic DNA in the DNA extracted from the sample may inhibit the amplification reaction.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Amplification controls

It is absolutely mandatory to validate each amplification session with a negative control reaction and a positive control reaction.

For the negative control, add ultra-pure molecular biology grade water (not included in the kit) to the reaction instead of the DNA extracted from the sample.

For the positive control, use the **«BKV - ELITe Positive Control»** product or alternatively **«BKV - ELITe Positive Control RF»** product or the **«BKV ELITe Standard»** product.

Quality controls

It is recommended to validate the whole analysis procedure of each extraction and amplification session by testing Process Controls, i.e. a negative tested sample and a positive tested sample or a calibrated reference material.

PROCEDURE

Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

When **cobas z 480 analyzer (Roche)** instrument is used:

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the control computer and the real time thermal cycler. Open the dedicated software and in the main window, open an "New Experiment" session;
- set the reaction volume ("Reaction volume") to 40 µL;
- assign an identifier to each sample ("Sample editor");
- define the reaction's Thermal Cycle according to the following table:

Thermal Cycle		
Stage	**Temperatures**	**Periods**
Decontamination	50°C	2 mins.
Initial denaturation	94°C	2 mins.
Amplification and detection (45 cycles)	94°C	10 sec.
	60°C (fluorescence acquisition)	30 sec.
	72°C	20 sec.
Dissociation (optional)	95°C	15 sec.
	40°C	30 sec.
	80°C	15 sec.

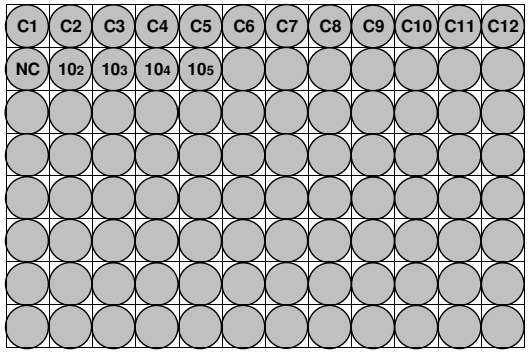
Note: fluorescence acquisition occurs individually, set Ramp Rate (°C/sec) to 4.4°C/sec.

- select the signal detection channels: "detector" for the BKV sensor with "channel FAM 465-510" and "detector" for the IC internal control sensor with "channel VIC 540-580";

Fill in the **Work Plan** attached at the end of this User Manual, transcribing this information or printing the microplate's layout. This **Work Plan** must be followed carefully when transferring the reaction mixture and samples into the wells.

Note: to determine the concentration of DNA in the source sample, you must perform a series of reactions with **Q - PCR Standard** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard Curve**.

See below, by way of example, how you can organise the quantitative analysis of 12 samples.



Legend: C1 - **C12**: Samples to be analyzed; **NC**: Negative amplification control;
10^2: Standard 10^2 copies; **10^3**: Standard 10^3 copies; **10^4**: Standard 10^4 copies; **10^5**: Standard 10^5 copies.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

Amplification set-up
(To be performed in the extraction / preparation of the amplification reaction area)

Before starting the session, it is necessary to:
- retrieve and thaw the test tubes containing the samples to be analyzed. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve and thaw the test tubes containing **BKV Q - PCR Mix** required for the session, remembering that the contents of each tube is enough to perform **25 reactions**. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve and thaw the test tubes containing **BKV – Positive Control** or alternatively **BKV - ELITe Positive Control RF** or **BKV Q - PCR Standard**. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve the **AD-plate** to be used in the session, making sure you handle it wearing dust-free gloves and do not damage the wells.

1. Without creating any bubbles and depositing it precisely on the bottom, transfer **20 µL** of reaction mixture **BKV Q - PCR Mix** into the wells on the **AD-plate** as previously established in the **Work Plan**.

Note: If not using all the reaction mixture, store any remaining mixture at -20°C for a maximum of one month. Freeze and thaw the reaction mixture a maximum of **5 TIMES**.

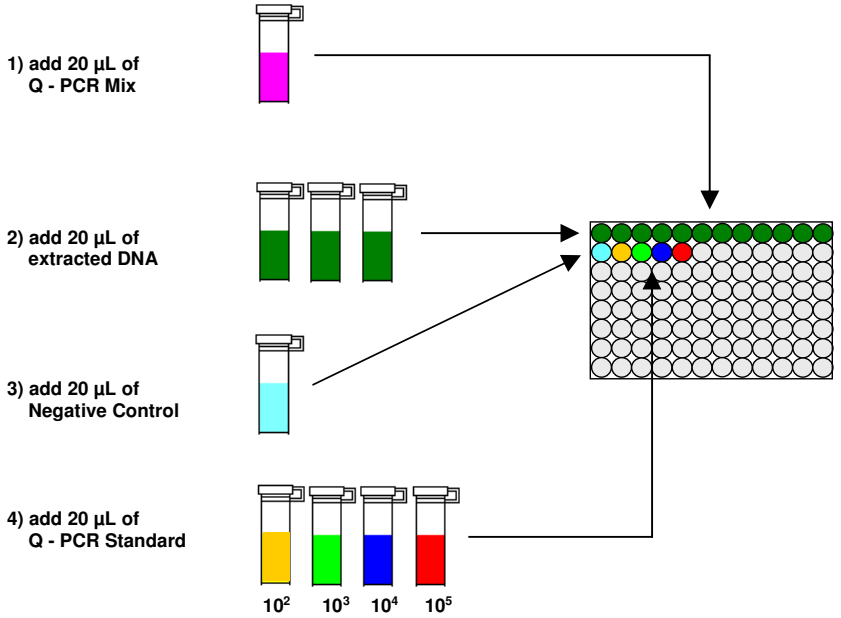
2. Depositing it precisely into the reaction mixture, transfer **20 µL** of **extracted DNA** from the first sample in the corresponding well on the **AD-plate** as previously established in the **Work Plan**. Mix the sample well by pipetting the **extracted DNA** three times into the reaction mixture. Be sure not to create any bubbles. Proceed in the same manner with all the other **extracted DNA**.
3. Depositing it precisely into the reaction mixture, transfer **20 µL** of **ultra-pure molecular biology grade water** (not supplied with the product) into the well on the **AD-plate** containing the negative amplification control as previously established in the **Work Plan**. Mix the negative control well by pipetting the **ultra-pure molecular biology grade water** three times into the reaction mixture. Be sure not to create any bubbles.
4. On the basis of the result required (qualitative or quantitative), one of these two options must be followed:

   - When a **qualitative** result is required (detection of BKV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **BKV - Positive Control** or alternatively **BKV - ELITe Positive Control RF** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the **BKV - Positive Control** three times into the reaction mixture. Avoid creating bubbles.

   - When a **quantitative** result is required (quantification of Adenovirus DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **BKV Q - PCR Standard 10^2** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **BKV Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **BKV Q - PCR Standards** (**10^3, 10^4, 10^5**).
5. Carefully seal the **AD-plate** using the **Sealing Film.**
6. Transfer the **AD-plate** into the real-time Thermal Cycler in the amplification/detection of amplification products area and start the amplification thermal cycle, saving the session settings under a unique and recognizable identifier (e.g. "year-month-day-BKV-EGSpA").

Note: At the end of the thermal cycle, the **AD-plate** and reaction products must be removed from the instrument and disposed of in a way that does not cause environmental pollution. **Never remove** the **Sealing Film from the Amplification microplate** to avoid any leakage of the reaction products.

The following figure shows synthetically the preparation of the amplification reaction.



Qualitative results analysis

The emitted fluorescence values recorded by the BKV detector and Internal Control (IC) detector during the amplification reactions must be analyzed by the instrument's software.

Select the menu " Analysis" and choose "Absolute Quant/Fit Points" (2 points)

Select the group of samples to be analyzed

In accordance with the instrument's documentation, before starting the analysis you must:
- manually enter the calculation range (Background button) for the **Background Fluorescence Level** for the FAM "BKV" detector **from cycle 2 to cycle 6**.
- manually set the **Threshold** and **Noiseband** for the FAM "BKV" detector to **0.55**;
- manually enter the calculation range (Background button) for the Background Fluorescence Level for the VIC "IC" detector **from cycle 6 to cycle 10**.
- manually set the **Threshold** and **Noiseband** for the VIC "IC" detector to **0.55.**

The fluorescence values emitted by the specific detectors in the amplification reaction and the **Threshold** and **Noiseband** fluorescence values are used to determine the **Threshold Cycle** (**Ct**), i.e. the cycle in which the fluorescence **Threshold** is reached.

The **Ct** values for BKV in the amplification reactions of the four **Q - PCR Standard** are used to calculate the **Standard Curve** (Results > Standard Curve) of that amplification session and to validate the amplification and detection as shown in the following table:

Reaction Q - PCR Standard 10^5 "BKV" detector	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

Standard Curve "BKV" detector	Acceptability range	Amplification / Detection
Correlation Coefficient (R2)	0.99 ≤ R2 ≤ 1.0	CORRECT

If the result of the amplification reaction for the **Q - PCR Standard 10^5** is **Ct > 25** or **Ct Undetermined** or if the value of the **Correlation Coefficient** (**R2**) is not within the limits, the DNA target was not correctly detected. Problems occurred during the amplification or detection stage (incorrect dispensing of the reaction mixture or standards, degradation of the reaction mixture or standards, incorrect setting of the standard positions, incorrect setting of the thermal cycle) which can cause incorrect results. The session is invalid and must be repeated from the amplification stage.

During the **Negative Control** amplification reaction, the value of **Ct** for BKV (Analysis window) is used to validate amplification and detection as shown in the following table:

Negative Control Reaction "BKV" detector	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative Control** amplification reaction is other than **Ct Undetermined** for BKV, the presence of the DNA target has been detected. Problems occurred during the amplification stage (contamination) which can lead to incorrect results and false positives. The session is invalid and must be repeated from the amplification stage.

During the amplification reactions for each **sample**, the value of **Ct** for BKV is used to detect the presence of the DNA target, whilst the value of **Ct** for the Internal Control is used to validate the extraction, amplification and detection.

Note: Check using the instrument's software (Analysis window) that the **Ct** is determined by a rapid and regular increase in fluorescence values and not by peaks or an increase of the background signal (irregular or noisy background).

Results like **Ct** from each **sample**'s amplification reactions (Analysis window) are used as shown in the following table:

Sample reaction		Sample suitability	Assay result	BKV DNA
"BKV" detector	"IC" detector			
Ct Undetermined	Ct > 35 or Ct Undetermined	not suitable	invalid	-
	Ct ≤ 35	suitable	valid, negative	NOT DETECTED
Ct Determined	Ct > 35 or Ct Undetermined	suitable	valid, positive	DETECTED
	Ct ≤ 35	suitable	valid, positive	DETECTED

If the result of a sample's amplification reaction is **Ct Undetermined** for BKV and **Ct > 35** or **Ct Undetermined** for the Internal Control, it was not possible to detect the Internal Control DNA efficiently. In this case, problems occurred during the amplification stage (inefficient or null amplification) or in the extraction stage (degraded sample DNA, sample with insufficient number of cells, loss of DNA during extraction or presence of inhibitors in the extracted DNA) which can cause incorrect results and false negatives. The sample is not suitable, the assay is not valid and must be repeated starting from the extraction of a new sample.

If the result of a sample's amplification reaction is **Ct Undetermined** for BKV and **Ct ≤ 35** for the Internal Control, the BKV DNA was not detected in the DNA extracted from the sample but it cannot be excluded that the BKV DNA is present at a concentration lower than the product's limit of detection (see Performance Characteristics). In this case, the result would constitute a false negative.

The results obtained with this assay must be interpreted by considering all the clinical data and the results of other laboratory tests connected to the patient.

Note: When BKV DNA is detected during the amplification reaction of a sample, amplification of the Internal Control can produce a result of Ct > 35 or Ct Undetermined. In fact, the low-efficiency Internal Control amplification reaction can be eliminated from the competition with the high-efficiency BKV reaction. In this case, the sample is then suitable and the positive assay result is valid.

Quantitative results analysis

After having performed the qualitative analysis procedure, you can carry out the quantitative analysis of the results relating to the positive sample.

If the result of the amplification reaction for the **Q - PCR Standard 10^5** is **Ct > 25** or **Ct Undetermined** or if the Ct values of the four Q - PCR standards don't fit regularly the standard curve the DNA target was not correctly detected. Problems occurred during the amplification or detection stage (incorrect dispensing of the reaction mixture or standards, degradation of the reaction mixture or standards, incorrect setting of the standard positions, incorrect setting of the thermal cycle) which can cause incorrect results. The session is invalid and must be repeated from the amplification stage.

The **Ct** values for BKV in the amplification reactions of each **sample** and the **Standard Curve** (**Standard Curve** button) from the amplification session are used to calculate the **Quantity** of DNA target present in the amplification reactions relating to the samples.

This product is able to quantify from 1,000,000 down to around 10 copies per reaction, from 25,000,000 to 250 copies per mL using the **MagNA Pure 24** extraction system (see Performance Characteristics), as shown in the following table:

Sample result FAM "BKV" detector	BKV copies per reaction
Quantity > 1×10^6	**GREATER THAN 1,000,000**
$1.0 \times 10^1 \leq$ Quantity $\leq 1 \times 10^6$	**= Quantity**
Quantity < 1.0×10^1	**LESS THAN 10**

The results (**Quantity**) relating to each **sample** (Analysis window) are used to calculate the **copies** of BKV present in the source sample (**Nc**) according to this formula:

$$Nc = \frac{Ve \times Quantity}{Vc \times Va \times Ep}$$

Where:
Vc is the quantity of sample used in the extraction in relation to the required unit of measure;
Ep is the efficiency of the procedure, extraction and amplification, **expressed in decimals**,
Ve is the total volume obtained from the extraction **expressed in µL**;
Va is the volume of extraction product used in the amplification reaction **expressed in µL**;
Quantity is the result of the amplification reaction relating to the sample **expressed in copies per reaction**.

When using samples of plasma collected in EDTA and urine and the **MagNA Pure 24** extraction system and the result is to be **expressed in copies / mL**, the formula becomes:

Simplified formula for plasma and urine and MagNA Pure 24
Nc (gEq / mL) = 25 x Quantity

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: limit of detection

The analytical sensitivity of this assay, as limit of detection, allows for the detection of around 10 copies in 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of this assay, as limit of detection, has been tested using a plasmid DNA containing the amplification product whose initial concentration was measured using a spectrophotometer. The plasmid DNA was diluted to a concentration of 10 copies / 20 µL in 150,000 copies of pBETAGLOBIN / 20 µL. This sample was used in 18 replicates to carry out amplification using ELITechGroup S.p.A. products.

The final results are summarized in the following table.

Samples	N	positives	negatives
10 copies of plasmid DNA + 150,000 copies of Beta-globin	18	18	0

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

Reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of value of a calibrated reference material, was evaluated using as reference material the calibrated panel «BKV Molecular "Q" Panel» (Qnostics, Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the extraction using the automatic extraction system **MAgNA Pure 24** and amplification using ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «MAgNA Pure 24»				
Sample	Nominal titre copies/mL	Nominal titre Log_{10} copies/mL	Positive / Replicates	Mean results Log_{10} copies / mL
BKVMQP01-High	100000	5.000	2/2	4.936
BKVMQP01-Medium	10000	4.000	2/2	3.899
BKVMQP01-Low	1000	3.000	2/2	2.748
BKVMQP01-Negative	negative	-	0/2	-

All negative samples were correctly detected as negative and all positive samples were detected as positive with a titre was within the expected value ± 0.5 Log.

Further tests were carried out using as reference material QCMD 2017 BK Virus DNA EQA Panel (Qnostics Ltd, UK) a panel of BKV dilutions within the limit concentration. Each sample of the panel was tested in 2 replicates carrying out the extraction using the automatic extraction system **MAgNA Pure 24** and amplification using ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «MAgNA Pure 24»			
Sample	Consensus conc. Log_{10} copies/mL	Positive / Replicates	Mean results Log_{10} copies / mL
BKVDNA17S-01	3.758	2/2	3.775
BKVDNA17S-02	3.821	2/2	3.776
BKVDNA17S-03	4.771	2/2	4.824
BKVDNA17S-04	negative	0/2	-
BKVDNA17S-05	2.736	2/2	2.748
BKVDNA17S-06	4.734	2/2	4.833
BKVDNA17S-07	3.770	2/2	3.963
BKVDNA17S-08	4.727	2/2	4.810
BKVDNA17S-09	4.637	2/2	4.488
BKVDNA17S-10	4.874	2/2	5.090

All negative samples were correctly detected as negative and all positive samples were correctly detected as positive with a titre was within the expected value ± 0.5 Log.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity was evaluated using 30 samples of plasma collected in EDTA negative for BKV DNA that were spiked for BKV DNA adding "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom) and 30 samples of urine negative for BKV DNA that were spiked for BKV DNA adding "1st WHO international standard for BKV virus DNA" (NIBSC code 14/212, United Kingdom).

Each sample was used carrying out the whole analysis procedure: extraction using the **MagNA Pure 24** automatic extraction system and amplification using ELITechGroup S.p.A. products. The results are summarized in the following table.

Samples	N	positives	negatives
Plasma collected in EDTA spiked for BKV DNA	30	30	0
Urine spiked for BKV DNA	30	30	0

All samples were confirmed positive for BKV DNA. The diagnostic sensitivity of the assay associated to plasma and urine samples was 100%.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity was evaluated using 31 samples of plasma collected in EDTA presumably negative for BKV DNA and 32 samples of urine presumably negative for BKV DNA.

Each sample was used carrying out the whole analysis procedure: extraction using the **MagNA Pure 24** automatic extraction system and amplification using ELITechGroup S.p.A. products. The results are summarized in the following table.

Samples	N	positives	negatives
Plasma collected in EDTA presumably negative for BKV DNA	31	0	31
Urine presumably negative for BKV DNA	32	0	32

All plasma and urine samples were valid at first test and confirmed negative for BKV DNA. The diagnostic specificity of the assay associated to plasma and urine samples was 100%.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File "BKV ELITe MGB® Kit", FTP 175PLD.

REFERENCES

P. Ferrante et al. (1995) *J Med Vir* 47: 219 - 225
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

PROCEDURE LIMITATIONS

Use this product only with DNA extracted from the following clinical samples: plasma collected in EDTA, urine collected without preservatives and cerebrospinal fluid.

Do not use DNA extracted from heparinized samples with this product: heparin inhibits the amplification reaction of nucleic acids and causes invalid results.

Do not use extracted DNA that is contaminated with haemoglobin, dextran, Ficoll®, ethanol or 2-propanol with this product: these substances inhibit the amplification reaction of nucleic acids and may cause invalid results.

Do not use with this product extracted DNA containing high quantity of human genomic DNA that may inhibit the amplification reaction of nucleic acids.

There are no data available concerning product performances with DNA extracted from the following clinical samples: whole blood collected in EDTA.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

The results obtained with this product depend on an adequate identification, collection, transport storage and processing of the samples. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use provided with the products for nucleic acids extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the BKV positive clinical samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations; however, the cross-contaminations can be avoided only by good laboratory practices and following carefully these instructions for use manual.

This product must be handled by qualified personnel trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of work clothes and areas that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product must be handled by qualified personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid incorrect results.

It is necessary to have separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products to prevent false positive results.

This product requires the use of special clothing and instruments for extraction / preparation of amplification reactions and for amplification / detection of amplification products to avoid false positive results.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A negative result obtained with this product means that the BKV DNA was not detected in the DNA extracted from the sample; but it can not be excluded that the BKV DNA has a lower titre than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

Results obtained with this product may sometimes be invalid due to failed internal control and require retesting, starting from extraction, that can lead to a delay in obtaining final results.

Possible polymorphisms within the region of the viral genome covered by the product primers and probe may impair detection and quantification of BKV DNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, false positive and false negative results obtained with this product. This residual risk can not be eliminated or further reduced. In some cases, as the emergency diagnosis, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

TROUBLESHOOTING

Target DNA not detected in the Positive Control or Q - PCR Standard reactions or invalid correlation coefficient of the Standard curve	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Take care when dispensing reactions into the microplate wells and comply with the work sheet. Check the volumes of reaction mixture dispensed. Check the volumes of positive control or standard dispensed.
Incorrect session setup on ELITe InGenius	Check the position of reaction mixture, positive control or standards. Check the volumes of reaction mixture, positive control or standards.
Probe degradation.	Use a new aliquot of reaction mixture.
Positive control or standard degradation.	Use a new aliquot of positive control or standard.
Instrument setting error.	Check the position settings for the positive control or standard reactions on the instrument. Check the thermal cycle settings on the instrument.
Instrument error.	Contact ELITechGroup Technical Service.

Target DNA detected in the Negative control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, negative controls, positive controls or standards into the microplate wells and comply with the work sheet.
Incorrect session setup on ELITe InGenius	Check the position of reaction mixture or negative control. Check the volumes of reaction mixture or negative control.
Error while setting the instrument.	Check the position settings of the samples, negative controls, positive controls or standards on the instrument.
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of molecular biology grade water.	Use a new aliquot of sterile water.
Contamination of the reaction mixture.	Use a new aliquot of reaction mixture.
Contamination of the extraction / preparation of amplification reactions area.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

Target and Internal Control DNA not detected in the sample reactions	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples into the microplate wells and comply with the work sheet.
Incorrect session setup on ELITe InGenius	Check the position of reaction mixture or samples. Check the volumes of reaction mixture or samples.
Internal Control degradation.	Use new aliquots of Internal Control.
Inhibition due to sample interfering substances.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction and amplification of sample.
Incorrect reagent storage.	Verify that reaction mix was not exposed to room temperature for more than 30 minutes.
Problems during extraction	Verify quality and concentration of extracted DNA.
Instrument error.	Contact ELITechGroup Technical Service.

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Take care, by pipetting three times, when mixing samples, negative controls and positive controls or standards into the reaction mixture. Avoid creating bubbles.
Baseline setting error.	Set the baseline calculation range within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g. from cycle 6 to cycle 15. Use the automatic baseline calculation by setting the "Auto Baseline" option.

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but different from that of the other samples and of the standards or positive control.	Check for detector FAM Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target DNA with a possible mutation. The target DNA of the sample should be sequenced to confirm mutation.

Error 30103 on ELITe InGenius	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - repeat the amplification of eluted sample in molecular biology grade water, in a “PCR only” session or - repeat the extraction with a dilution of the primary sample in molecular biology grade water, in a “Extract + PCR” session.

SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE	Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests.
	Attention, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

BKV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS175PLD

NOTICE TO PURCHASER: LIMITED LICENSE

This product content LTC licensed reagents.

This product is sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and LTC. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Life Technologies, Inc, 5791 Van Allen Way, Carlsbad, CA 92008.
Phone: +1(760)603-7200. Fax: +1(760)602-6500. Email: outlicensing@LTC.com.

ELITe MGB detection reagents are covered by one or more of U.S. Patents Nos. 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800 and 9,169,256 as well as applications that are currently pending and EP patent numbers 0819133, 1068358, 1144429, 1232157, 1235938, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

This limited license permits the person or legal entity to which this product has been provided to use the product, and the data generated by use of the product, only for human diagnostics. Neither ELITechGroup S.p.A. nor its licensors grants any other licenses, expressed or implied for any other purposes.

BKV ELITe MGB® kit used with Genius series platforms

Ref: RTS175PLD

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com
This document is available only in English.

A. Intended use

The BKV ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **human Polyomavirus BK**. The assay is CE-IVD validated in combination with the instruments **ELITe InGenius** and **ELITe BeGenius**.

B. Amplified sequence

	Gene	Fluorophore
Target	Large T antigen gene	FAM
Internal Control	Human beta globin gene	AP525 (VIC)

C. Validated matrix

› **Plasma** EDTA

› **Urine**

D. Kit content

BKV Q-PCR Mix
4 tubes of 540 µL

X 4

› Ready to use complete mixture
› Number of tests per kit: 96
› Freeze-thaw cycles per tube: 5
› Maximum shelf-life: 24 months
› Storage Temperature: - 20°C

E. Material required not provided in the kit

› **ELITe InGenius instrument:** INT030
› **ELITe BeGenius instrument:** INT040
› **ELITe InGenius SP200 Extraction Cartridge:** INT032SP200
› **ELITe InGenius PCR Cassette:** INT035PCR
› **ELITe InGenius SP200 Consumable Set:** INT032CS
› **CPE - Internal Control:** CTRCPE

› **BKV ELITe Standard :** STD175PLD
› **BKV - ELITe Positive Control :** CTR175PLD
› **ELITe InGenius Waste Box :** F2102-000
› **300 µL Filter Tips Axygen :** TF-350-L-R-S
› **1000 µL Filter Tips Tecan :** 30180118

F. ELITe InGenius and BeGenius protocol

› Sample volume	200 µL
› CPE Internal Control volume	10 µL
› Total eluate volume	100 µL
› PCR eluate input volume	20 µL
› BKV Q-PCR Mix volume	20 µL

› Unit of quantitative result	cp/mL or IU/mL
› Frequency of controls	15 days
› Frequency of calibration	60 days

G. Performance

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Urine	**142 IU/mL – 89 cp/mL**	**100%** 30/30*	**100%** 30/30*
Plasma	**215 IU/mL – 165 cp/mL**	**100%** 30/30*	**97%** 28/30*
Plasma (1000 µL)	**44 IU/mL – 26 cp/mL**	**100%** 55/55*	**97%** 60/62*

confirmed samples/ tested samples

Matrix	Linearity (copies/mL)	Linearity (IU/mL)	Conversion factor cp/mL to IU/mL
Urine	**89 - 100,000,000**	**142– 160,000,000**	**1.6**
Plasma	**165 - 100,000,000**	**215 – 130,000,000-**	**1.3**
Plasma (1000 µL)	**59 – 100,000,000**	**100– 170,000,000**	**1.7**

H. Reference material tested

Panel name	Provider	Qualitative results	Quantitative results
BKV Molecular "Q" Panel	Qnostics	Concordance 100% (4/4)*	Titre as expected value ± 0.5 log
QCMD 2014 BK Virus DNA EQA Panel	Qnostics	Concordance 100% (9/10)*	Titre as expected value ± 1 log

*confirmed *samples/ tested samples*

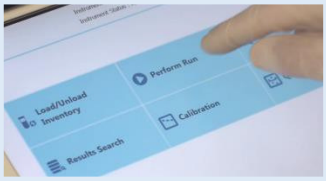
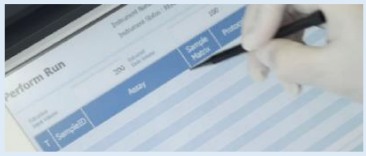
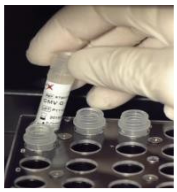
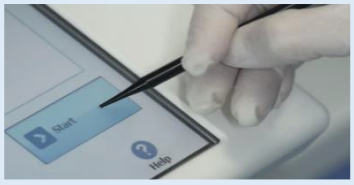
I. Procedures ELITe InGenius

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed. Three operational modes are available: complete run, or extraction only, or PCR only.

Before analysis

1. Switch on ELITe InGenius Identification with username and password Select the mode "Closed"	**2.** Verify calibrators: BKV Q-PCR standard in the "Calibration menu" Verify controls: BKV pos. and neg. controls in the "Control menu" *NB:* Both have been run, approved and not expired	**3.** Thaw the BKV Q- PCR-Mix and the CPE Internal Control tubes Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen 	**2.** Verify the extraction volumes: Input: "200 µL", elute: "100 µL" 	**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID 
4. Select the "Assay protocol" of interest 	**5.** Select the sample position: Primary tube or sonication tube 	**6.** Load the Q-PCR-Mix and the CPE Internal Control in the inventory block 
7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, sonication tube and primary sample racks 	**8.** Close the door Start the run 	**9.** View, approve and store the results 

Procedure 2 - PCR only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol "PCR only" and set the sample position "Extra tube"	**6.** Load the extracted nucleic acid tubes in the rack n°4
7. Load the PCR cassette rack Load the Q-PCR Mix in the inventory block	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol "Extraction Only" and set the sample position : Primary tube or Secondary tube	**6.** Load the CPE Internal Control in the inventory block
7. Load: Extraction cartridge, Elution tube, Tip cassette, sonication tube and primary sample racks	**8.** Close the door Start the run	**9.** Archive the eluate sample

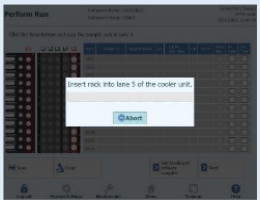
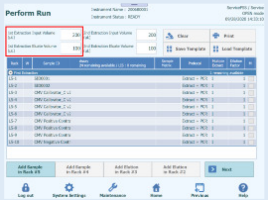
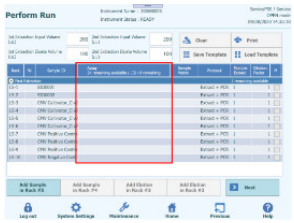
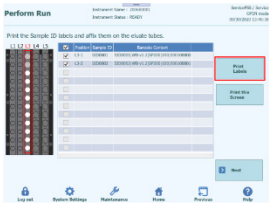
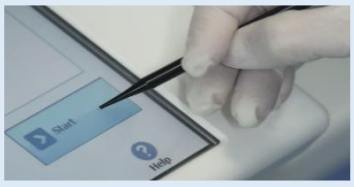
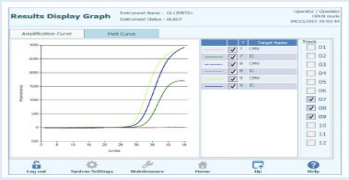
L. Procedures ELITe BeGenius

The user is guided step-by-step by the ELITe BeGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed. Three operational modes are available: complete run, or extraction only, or PCR only.

Before analysis

1. Switch on ELITe BeGenius Identification with username and password Select the mode "Closed"	**2.** Verify calibrators: BKV Q-PCR standard in the "Calibration menu" Verify controls: BKV pos. and neg. controls in the "Control menu" *NB:* Both have been run, approved and not expired	**3.** Thaw the BKV Q- PCR-Mix and the CPE Internal Control tubes Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen and then click on the run mode «Extraction and PCR» 	**2.** Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active 	**3.** Verify the extraction volumes: Input: "200 µL", Eluate: "100 µL" 
4. Select the "Assay protocol" of interest  **Note**: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area 	**6.** Load the Q-PCR-Mix and the CPE Internal Control in Reagent Rack and insert it in the cooling area 
7. Load: Filter Tips, Extraction rack, and PCR rack 	**8.** Close the door. Start the run 	**9.** View, approve and store the results 

Procedure 2 - PCR only

1. Select "Perform Run" on the touch screen and the click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid barcoded tubes in the Elution Rack and insert it in the cooling area	**3.** Select the "Assay protocol" of interest
4. Load the Q-PCR-Mix in Reagent Rack and insert it in the cooling area Load filter tips and the PCR rack	**5.** Close the door. Start the run	**6.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol "Extraction Only" in the Assay Protocol selection screen.	**6.** Load the CPE Internal Control in the Elution Rack and insert it in the cooling area
7. Load : Filter Tips and the Extraction Rack	**8.** Close the door Start the run	**9.** Archive the eluate sample

BKV ELITe MGB® kit used with ELITe InGenius®

Code: RTS175PLD

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com
This document is available only in English.

A. Intended use

The BKV ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **human Poliomavirus BKV**.
The assay is CE-IVD validated in combination with the instrument **ELITe InGenius®**.

B. Amplified sequence

	Gene	Fluorophore
Target	Large T antigen gene	FAM
Internal Control	Human beta globin gene	AP525

C. Validated matrix

Plasma EDTA

D. Kit content

BKV Q-PCR Master Mix
4 tubes of 540 µL

X 4

› Ready to use PCR Master Mix
› Number of reactions per kit: 96
› Freeze-thaw cycles per tube: 5
› Maximum shelf-life: 24 months
› Storage Temperature: - 20°C

E. Material required not provided in the kit

› **ELITe InGenius instrument:** INT030
› **ELITe InGenius SP1000** Extraction Cartridge**:** INT033SP1000
› **ELITe InGenius PCR Cassette** amplification cartridges**:** INT035PCR
› **ELITe InGenius SP200 Consumable Set** consumables for extraction**:** INT032CS

› **BKV ELITe Standard :** STD175PLD
› **BKV ELITe Positive Control:** CTR175PLD
› **CPE Internal Control:** CTRCPE
› **ELITe InGenius Waste Box:** F2102-000
› **Filter Tips 300:** TF-350-L-R-S

F. ELITe InGenius protocol

› Sample volume	1000 µL
› CPE Internal Control volume	10 µL
› Total eluate volume	100 µL
› PCR eluate input volume	20 µL
› BKV Q-PCR Mix volume	20 µL

› Unit of quantitative result	International Unit: IU/mL copies/mL
› Frequency of controls	15 days
› Frequency of calibration	60 days

G. Performance

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Plasma	**44 UI/mL** (26 copies/mL)	**100%** 55/55*	**97%** 60/62* **confirmed samples/ tested samples*

H. Reference material tested

Panel name	Provider	Qualitative results	Quantitative results
Molecular Q Panel: BKVMQP01	Qnostics	Concordance 100% (4/4)*	Titre as expected value ± 0.5 log
QCMD 2014 : BKVDNA14	Qnostics	Concordance 100% (8/8)*	Titre as expected value ± 1 log

*confirmed *samples/ tested samples*

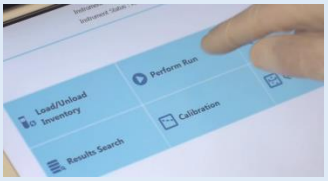
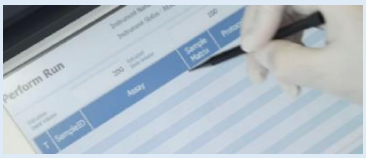
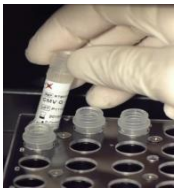
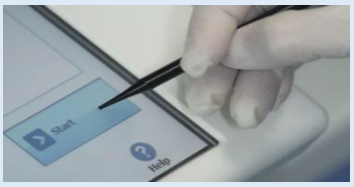
I. Procedures

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed. Three operational mode are available: complete run, or extraction only, or PCR only.

Before analysis

1. Switch on ELITe InGenius Identification with username and password Select the mode “Closed”	**2.** Verify calibrators: BKV Q-PCR standard in the “Calibration menu” Verify controls: BKV pos. and neg. controls in the “Control menu” *NB:* Both have been run, approved and not expired	**3.** Thaw the BKV Q- PCR-Mix and the CPE Internal Control tubes Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

1. Select “Perform Run” on the touch screen 	**2.** Verify the extraction volumes: Input:“1000 µL”, eluate: “100 µL” 	**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID 
4. Select the “Assay protocol” of interest 	**5.** Select the sample position: Primary tube or sonication tube 	**6.** Load the Q-PCR-Mix and the CPE Internal Control in the inventory block 
7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, sonication tube and primary sample racks 	**8.** Close the door Start the run 	**9.** View, approve and store the results 

Procedure 2 - PCR only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “PCR only” and set the sample position “Extra tube”	**6.** Load the extracted nucleic acid tubes in the rack n°4
7. Load the PCR cassette rack Load the Q-PCR Mix in the inventory block	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” and set the sample position : Primary tube or Secondary tube	**6.** Load the CPE Internal Control in the inventory block
7. Load: Extraction cartridge, Elution tube, Tip cassette, sonication tube and primary sample racks	**8.** Close the door Start the run	**9.** Archive the eluate sample

BKV ELITe MGB® Kit used with ABI PCR instrument

Code: RTS175PLD

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com
This document is available only in English.

A. Intended use

The «BKV ELITe MGB® Kit» product is a a Real-Time PCR assay for the detection and quantification of the DNA of**human Polyomavirus BK (BKV)**.

The assay is CE-IVD validated in combination with **ABI PCR thermal cyclers** (Thermo-Fisher) and the following extraction systems: **ELITe STAR** (ELITechGroup), **ELITe GALAXY** (ELITechGroup), **easyMAG** (BioMérieux) or **QIAsymphony** (Qiagen).

B. Amplified sequence

Target	Gene	Fluorophore
BKV	Large T antigen gene	FAM
Internal Control	human beta globin gene	VIC

C. Validated matrix

› **Urine** › **Plasma** EDTA › **Cerebrospinal fluid**

D. Kit content

BKV Q-PCR Master Mix
4 tubes of 540 µL

X 4

› Ready to use complete mixture
› Number of tests per kit: 100
› Freeze-thaw cycles per tube: 5
› Maximum shelf-life: 24 months
› Storage Temperature: - 20°C

E. Material required not provided in the kit

› **7500 Fast Dx and 7300 PCR Instrument**
› **ELITe STAR:** INT010
› **ELITe STAR 200 extraction kit:** INT011EX
› **ELITe GALAXY:** INT020
› **ELITe GALAXY 300 extraction kit:** INT021EX
› **BKV ELITe Positive Control:** CTR175PLD
› **BKV ELITe Standard:** STD175PLD
› **CPE Internal Control:** CTRCPE
› **easyMAG** - Generic protocol 2.0.1
› **QIAsymphony** - DNA Mini kit or DSP Virus/Pathogen Midi kit
› **Molecular biology grade water**

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
ELITe STAR - ABI	Plasma	-	**97%** (29/30)*	**100%** (30/30)*
	Urine	-	**100%** (30/30)*	**100%** (30/30)*
ELITe GALAXY - ABI	Plasma	**190 cp/mL** - L	**100%** (39/39)*	**100%** (38/38)*
	Urine	**119 cp/mL** -	**93.5%** (29/31)*	**100%** 31/31)*
easyMAG - ABI	Plasma,	-	-	-
	Urine,	-	-	-
	CSF	-	-	-
QIAsymphony - ABI	Plasma	-	-	-

**confirmed samples/tested samples*

G. Procedure

The procedure below summarized the main steps of the sample analysis with conventional PCR workflow: validated extraction systems, PCR instrument settings, PCR set-up and result interpretation.

Extraction - Validated systems

Extraction	Validated matrix	Sample volume processed	Min. sample volume	Total eluate volume	CPE Internal Control volume
ELITe Star	Plasma, Urine	200 µL	700 µL	100 µL	10 µL
ELITe Galaxy	Plasma, Urine	300 µL	400 µL	200 µL	10 µL
EasyMAG	Plasma, Urine, CSF	500 µL	-	100 µL	5 µL
QIAsymphony	Plasma	500 µL	600 µL	85 µL	6 µL

Amplification - Settings of 7500 Fast Dx and 7300 PCR instruments

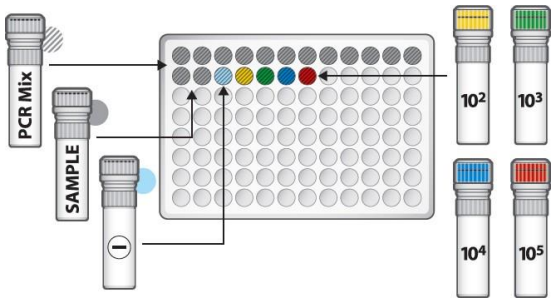
1. Switch on the thermal-cycler
2. Set "BKV" detector with "FAM" and quencher "none"
3. Set "Internal Control" detector with "VIC" and quencher "none"
4. Set passive fluorescence as "Cy5" with 7500 Fast Dx and as "ROX" with 7300 instrument
5. Set up the thermal profil as indicated. Fluorescence acquisition must be set during hybridation step at 60°C

Stage	Temperature	Timing
Decontamination	50°C	2 min
Denaturation	94°C	2 min
Amplification and detection *45 cycles*	94°C	10 sec
	60°C	30 sec
	72°C	20 sec

The melt curve analysis is optional, refer to the complete IFU

Amplification - PCR Set -up

1. Thaw BKV Q PCR-Mix and Q-PCR standard tubes
2. Mix gently and spin-down
3. Pipet **20 µL** of Q-PCR-Mix in all microplate wells in use
4. Add, **20 µL** of extracted DNA in sample wells, **20 µL** of molecular grade water in Negative Control well, and **20µL** of the 4 Q-PCR standards in standard curve wells, if quantitative, **20 µL** of the Positive Control, if qualitative. Each one has to be mixed by pipetting 3 times into the reaction mixture
5. Seal the microplate with the amplification sealing sheet
6. Transfer the microplate in the thermocycler and start



Amplification - Threshold for qualitative analysis

Instrument	BKV FAM	Internal Control VIC
7500 Fast Dx Real Time PCR	0.2	0.1
7300 Real Time PCR	0.1	0.05

Interpretation - Qualitative results

BKV Ct value	Internal Control Ct value	Interpretation
Determined	-	Positive
Undetermined	Ct ≤ 35	Negative
	Ct >35 or Undetermined	Invalid*

**Repeat the assay starting from the extraction*

Interpretation - Quantitative results

The BKV ct value obtained for each sample and the standard curve generated are used to calculate the quantity of target DNA in the reaction.

The sample quantification ranges from approximately 10 to 10^6 cp/reaction.

BKV ELITe MGB® kit used with Cobas-Z 480 PCR instruments

Code: RTS175PLD

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com
This document is available only in English.

A. Intended use

The «BKV ELITe MGB® Kit» product a Real-Time PCR assay for the detection and quantification of the DNA of **human Polyomavirus BK (BKV)**. The assay is CE-IVD validated in combination with **Cobas – Z 480 analyzer (Roche)** and the following extraction systems: **MagNA Pure 24 System**.

B. Amplified sequence

Target	Gene	Fluorophore
BKV	**Large T antigen gene**	**FAM (465 – 510)**
Internal Control	**Human beta globin gene**	**VIC (540 - 580)**

C. Validated matrix

› **Urine** › **Plasma EDTA**

D. Kit content

BKV Q-PCR Master Mix

4 tubes of 540 µL

X 4

Ready to use complete reaction mixture
Number of tests per kit: 100
Freeze and thaw cycles per tube: 5

Maximum shelf-life: 24 months
Storage temperature: -20°C

E. Material required not provided in the kit

› **Cobas – Z 480 analyzer PCR Instrument**
› **MagNA Pure 24 System = software 1.0**
› **BKV - ELITe Positive Control:** CTR175PLD
BKV – ELITe Positive Control RF: CTR175PLD-R
› **BKV ELITe Standard:** STD175PLD
› **CPE Internal Control:** CTRCPE
› **Molecular biology grade water**

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
MagNA Pure 24	Plasma	**10 cp/rxn**	**100%** (30/30)*	**100%** (31/31)*
	Urine	**10 cp/rxn**	**100%** (30/30)*	**100%** (32/32)*

**confirmed samples/tested samples*

G. Procedure

The procedure below summarized the main steps of the sample analysis with conventional PCR workflow: validated extraction systems, PCR instrument settings, PCR set-up and result interpretation.

Extraction - Validated systems

Extraction	Validated matrix	Sample volume processed	Min. sample volume	Total eluate volume	CPE Internal Control volume
MagNA Pure 24	Urine, Plasma	200 µL	350 µL	100 µL	20 µL diluted 1:2

Amplification - Settings of Cobas-Z 480 PCR instruments PCR instruments

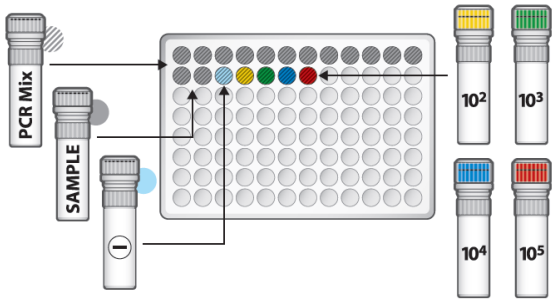
1. Switch on the thermal-cycler
2. Set "BKV" detector with "FAM (465 -510)".
3. Set "Internal Control" detector with "VIC (540 -580)".
4. Set up the thermal profile as indicated. Fluorescence acquisition must be set during hybridation step at 60°C

Stage	Temperature	Timing
Decontamination	50°C	2 min
Denaturation	94°C	2 min
Amplification and detection *45 cycles*	94°C	10 sec
	60°C	30 sec
	72°C	20 sec

The melt curve analysis is optional, refer to the complete IFU

Amplification - PCR Set-up

1. Thaw BKV - Q PCR-Mix and Q-PCR standard tubes or the Positive Control tube
2. Mix gently and spin-down
3. Pipet **20 µL** of Q-PCR-Mix in all microplate wells in use
4. Add, **20 µL** of extracted DNA in sample wells, **20 µL** of molecular grade water in Negative Control well, and **20 µL** of the 4 Q-PCR standards in standard curve wells, if quantitative, 20 µL of the Positive Control, if qualitative.
   Each one has to be mixed by pipetting 3 times into the reaction mixture
5. Seal the microplate with the amplification sealing sheet
6. Transfer the microplate in the thermocycler and start



Amplification - Threshold for qualitative analysis*

Instrument	Matrix	Background Fluorescence Level FAM	BKV FAM	Background Fluorescence Level VIC	Internal Control VIC
Cobas-Z 480 PCR instruments	Plasma	from cycle 2 to cycle 6	0.55	from cycle 6 to cycle 10	0.55
Cobas-Z 480 PCR instruments	Urine	from cycle 2 to cycle 6	0.55	from cycle 6 to cycle 10	0.55

**manually set the Threshold and Noiseband*

Interpretation - Qualitative results

BKV Ct value	Internal Control Ct value	Interpretation
Determined	-	Positive
Undetermined	Ct ≤ 35	Negative
	Ct >35 or Undetermined	Invalid*

**Repeat the assay starting from the extraction*

Interpretation - Quantitative results

The BKV Ct value obtained for each sample and the standard curve generated are used to calculate the quantity of target DNA in the reaction. The sample quantification ranges from approximately 10 to 10^6 copies/reaction or approximately from 250 to 2.5 10^7 copies/mL.