

Instructions for use

JCV ELITe MGB® Kit

reagents for DNA Real-Time PCR



REF RTS176PLD

UDI 08033891484514

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IVD

CHANGE HISTORY

Revision	Notice of change	Date (dd/mm/yy)
10-R	Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements. Update of the Intended use. Alignment of the Upper Limit of Quantification (ULoQ) value with the Kotton et al. guidelines 2024.	25/03/25
09	Update of the Intended Use with the description of the Applied instruments (7300 and 7500 Fast DX).	05/12/24
08	Extended use of the product in association with «ELITe BeGenius®» instrument (REF INT040) and Cerebrospinal Fluid (CSF) and urine matrices. Update of PERFORMANCE CHARACTERISTICS: <ul style="list-style-type: none"> • update LoD value for CSF and urine matrices • update of Linear measuring range for CSF and urine matrices New graphics and content setting of the IFU.	13/11/24
07	Extended use of the product on «ELITe BeGenius®» instrument (REF INT040) and Plasma matrix.	23/06/22
00-06	New product development and subsequent changes	-

NOTE

The product batches identified by the following LOT numbers are still placed on the market as per IVDD till to their expiration dates, according to Article 110 of IVDR. If you have those product batches, please contact ELITechGroup staff to request the related previous revision of IFUs.

<u>PRODUCT REF.</u>	<u>Lot Number</u>	<u>Expiry date</u>
RTS176PLD	U1023-033	31/05/2025
RTS176PLD	U0424-106	31/12/2025
RTS176PLD	U1224-058	31/12/2026

The Positive Control and the Standard product batches still placed on the market as per IVDD (identified by the LOT numbers reported in the Positive Control IFU and Standard IFU) are technically compatible with the new IVDR version of the amplification kit and can be used, until exhausted, in association with the new IVDR version of the amplification kit and in accordance with its intended use.

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1 INTENDED USE

The product **JCV ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids Real-Time PCR assay for the **detection and quantification of the DNA of Human Polyomavirus JC (JCV)** extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA, urine collected without preservatives and cerebrospinal fluid (CSF).

The product is intended for use as an aid in the diagnosis and monitoring of JCV infections in patients suspected of having or undergoing monitoring of JCV infections.

The results must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

2 ASSAY PRINCIPLE

The assay is a quantitative Real-Time PCR detecting JCV DNA, isolated from specimens and amplified using the assay reagent **JCV Q PCR Mix** that contains primers and probes with ELITe MGB technology.

The ELITe MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycle (Ct) and the melting temperatures (Tm). The **JCV** DNA quantity is calculated based on a stored calibration curve.

In the ELITe MGB probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore. Note the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

The **JCV ELITe MGB Kit** provides the assay reagent **JCV Q - PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- Large T antigen region of the JCV gene, detected in Channel JCV; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye.
- Internal Control (IC), specific for the artificial DNA sequence IC2, detected in Channel IC; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor® 525 (AP525) dye.

The **JCV Q - PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, AP593 fluorophore (analogue of ROX or Cy5) as passive reference for fluorescence normalisation, the enzyme Uracil-N-glycosidase (UNG) to inactivate contamination by the amplification product, and hot-start DNA Polymerase.

The product **JCV ELITe MGB Kit** contains sufficient reagents for **96 tests on ELITe InGenius** and **ELITe BeGenius**, with 20 uL used per reaction.

NOTE

A conversion factor to express the results of the quantitative analysis in international units of JCV, in accordance with the "1st WHO International Standard for JC virus DNA" (NIBSC code 14/114, United Kingdom), is required.

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
JCV Q - PCR Mix ref. RTS176PLD	Mixture of reagents for Real-Time PCR in tube with natural cap	4 x 540 µL	-

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~5,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA, the extraction and inhibition internal control, the amplification positive and negative controls, the DNA standards and the consumables **are not** provided with this product.

For the extraction of nucleic acids, Real-Time PCR and result interpretation of samples, the following products are required:

Table 2

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030) ELITe InGenius Software version 1.3.0.19 (or later) JCV ELITe_STD , Assay Protocol with parameters for Calibrators analysis JCV ELITe_PC , Assay Protocol with parameters for Positive Control analysis JCV ELITe_NC , Assay Protocol with parameters for Negative Control analysis JCV ELITe_U_200_100 , Assay Protocol with parameters for urine specimen analysis JCV ELITe_PL_200_100 , Assay Protocol with parameters for plasma specimen analysis JCV ELITe_CSF_200_100 , Assay Protocol with parameters for cerebrospinal fluid specimen analysis	ELITe InGenius SP200 (EG SpA, ref. INT032SP200) ELITe InGenius SP 200 Consumable Set (EG SpA, ref. INT032CS) ELITe InGenius PCR Cassette (EG SpA, ref. INT035PCR), ELITe InGenius Waste Box (EG SpA, ref. F2102-000) 300 µL Filter Tips Axygen (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only 1000 µL Filter Tips Tecan (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only CPE – Internal Control (EG SpA, ref. CTRCPE) JCV ELITe Standard (EG SpA, ref. STD176PLD) JCV – ELITe Positive Control (EG SpA, ref. CTR176PLD)
ELITe BeGenius (EG SpA ref. INT040) ELITe BeGenius Software version 2.2.1. (or later) JCV ELITe_Be_STD , Assay Protocol with parameters for Calibrators analysis JCV ELITe_Be_PC , Assay Protocol with parameters for Positive Control analysis JCV ELITe_Be_NC , Assay Protocol with parameters for Negative Control analysis JCV ELITe_Be_U_200_100 , Assay Protocol with parameters for urine specimen analysis JCV ELITe_Be_PL_200_100 , Assay Protocol with parameters for plasma specimen analysis JCV ELITe_Be_CSF_200_100 , Assay Protocol with parameters for cerebrospinal fluid specimen analysis	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) 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 infectious. 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 neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

- 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 before running the assay.
- While running the assay, follow the product instructions provided.
- Do not use the product after the indicated expiry date.
- Only use reagents provided with the product and those recommended by the manufacturer.
- Do not use reagents from different batches.
- Do not use reagents from other manufacturers.

7.2 Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to sample nucleic acid degradation or sample contamination by PCR products.

Laboratory coats, gloves and tools dedicated to work session setup are needed.

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. 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, and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. 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, and free from DNA and RNA.

The extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination.

The PCR Cassette must be handled carefully and never opened to avoid PCR product diffusion into the environment and sample and reagent contamination.

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
JCV Q PCR Mix	-20 °C or below (protected from light)	one month	up to five	up to five separate* sessions of three hours each or up to 7 consecutive hours (2 sessions of 3 hours each and the time needed to start a third session)

- with intermediate freezing

8 SPECIMENS AND CONTROLS

8.1 Specimens

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified and handled according to laboratory guidelines, and collected, transported, and stored under the following conditions:

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2° / +8° C	-20 ± 10 °C	-70 ± 15 °C
urine	Without preservatives	≤ 4 hours	≤ 4 hours	≤ 30 d	≤ 30 d
CSF	Avoid contamination with patient blood	≤ 4 hours	≤ 4 hours	≤ 30 d	≤ 30 d
plasma	EDTA	≤ 1 d	≤ 3 days	≤ 30 d	≤ 30 d

EDTA, Ethylenediaminetetraacetic acid; d, day

Even if longer storage periods at -70 ° C are possible, as extensively reported by scientific literature, their application should be evaluated internally by the end-users of this product.

It is recommended to divide the specimens into aliquots before freezing to prevent repeated freeze/thaw cycles. When using frozen samples, thaw the samples just before the extraction to avoid possible nucleic acid degradation.

To perform samples testing on the **ELITe InGenius** and **ELITe BeGenius** the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB Kits and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrices.

Table 5 Assay Protocols for JCV ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
Urine	ELITe InGenius	JCV ELITe_U_200_100	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: 10 µL
	ELITe BeGenius	JCV ELITe_Be_U_200_100	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: 10 µL
Plasma in EDTA	ELITe InGenius	JCV ELITe_PL_200_100	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: 10 µL
	ELITe BeGenius	JCV ELITe_Be_PL_200_100	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: 10 µL
CSF	ELITe InGenius	JCV ELITe_CSF_200_100	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: 10 µL
	ELITe BeGenius	JCV ELITe_Be_CSF_200_100	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: 10 µL

IU: International Units

NOTE

Verify if the primary tube and the volume of the sample are compatible with ELITe InGenius or ELITe BeGenius, following the Instruction for use of the extraction kit **ELITeInGeniusSP200** (EG SpA, ref. INT032SP200)

The volume of the sample in a primary tube varies according to the type of the tube loaded. Refer to the instructions for use of the extraction kit for more information on how to set up and perform the extraction procedure.

If required, 200 µL of sample must be transferred into an Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius).

NOTE

Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination**. Use the appropriate pipettes and follow all recommendations reported in the 7 WARNINGS AND PRECAUTIONS page 6.

Purified nucleic acids can be left at room temperature for 16 hours and stored at -20 °C or below for no longer than one month.

Refer to “Potentially Interfering Substances” in the [11 PERFORMANCE CHARACTERISTICS page 21](#) to check data concerning interfering substances.

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

8.2 PCR calibrators and controls

The Calibration curve must be generated and approved for each lot of PCR reagent.

- For the calibration curve, use the four levels of the product **JCV ELITe Standard** (not provided with this kit) with the **JCV ELITe_STD** or **JCV ELITe_Be_STD** Assay Protocols.

NOTE

The concentration of Q – PCR Standards are expressed in copies / reaction (10⁵ copies / rxn, 10⁴ copies / rxn, 10³ copies / rxn, 10² copies / rxn). Refer to “Standard Curve Uncertainty” in the 11 PERFORMANCE CHARACTERISTICS page 21.

PCR control results must be generated and approved for each lot of PCR reagent.

- For the Positive Control, use the product **JCV - ELITe Positive Control** (not provided with this kit) with the **JCV ELITe_PC** or **JCV ELITe_Be_PC** Assay Protocols,
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **JCV ELITe_NC** or **JCV ELITe_Be_NC** Assay Protocols.

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the calibration curve and PCR control validation for each lot of PCR reagent.

Calibration curves expire after **60 days**, at which time it is necessary to re-run the calibration. PCR control results expire after **15 days**, at which time it is necessary to re-run the Positive and Negative Controls.

The Calibrators and PCR controls must be re-run if any of the following events occur:

- a new lot of reagents is used,
- results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance or service is performed on the **ELITe InGenius** or **ELITe BeGenius**.

8.3 Quality controls

Verification of the extraction and PCR procedure is recommended. Archived samples or certified reference material may be used. External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

9 ELITe InGenius PROCEDURE

The procedure to use the **JCV ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

Table 6

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Calibration run (PCR Only)
		D) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Calibration curve
		2) Validation of Positive Control and Negative Control results
		3) Validation of sample results
		4) Sample result reporting

9.1 STEP 1 – Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in “**CLOSED**” mode,
- in the “Calibration” menu on the Home page, verify the Calibrators (**Q – PCR Standard**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- in the “Controls” menu on the Home page, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA.(see “Specimens and Controls”).

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

9.2 STEP 2 – Session Setup

The **JCV ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

- Sample run (Extract + PCR),
- Eluted sample run (PCR Only),
- Calibration run (PCR Only),
- Positive Control and Negative Control run (PCR Only).

All required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests**. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the four types of run follow the steps below while referring to the GUI:

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	Identify samples and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If required, transfer 200 µL of sample in an Extraction tube previously labelled. Thaw the needed CPE tubes at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	Thaw the Elution tube containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select “ Perform Run ” from the “Home” screen.	Select “ Perform Run ” from the “Home” screen.
3	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
4	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.
5	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).
6	Ensure the “Protocol” displayed is: “Extract + PCR”.	Select “PCR Only” in the “Protocol” column.
7	Select the sample loading position as “Primary tube” or “Extraction Tube” in the “Sample Position” column. Ensure the “ Dilution factor ” is “1”.	Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Ensure the “ Dilution factor ” is “1”.
8	Click “Next” to continue.	Click “Next” to continue.
9	Load CPE and the PCR Mix on the “Inventory Block” referring to the “Load List” and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	Load the PCR Mix on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.
10	Click “Next” to continue.	Click “Next” to continue.
11	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
12	Click “Next” to continue.	Click “Next” to continue.
13	Load PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted.	Load PCR Cassette and Elution tubes with samples extracted.
14	Click “Next” to continue.	Click “Next” to continue.

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
15	Close the instrument door.	Close the instrument door.
16	Press "Start".	Press "Start".
	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	Thaw the needed Q-PCR Standard tubes (Cal1: Q-PCR Standard 10 ² , Cal2: Q-PCR Standard 10 ³ , Cal3: Q-PCR Standard 10 ⁴ , Cal4: Q-PCR Standard 10 ⁵) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	Thaw Positive Control tubes at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. Prepare the Negative Control by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITE InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL.
4	For the Q-PCR Standard, assign the "Track", select the Assay Protocol (see "Specimen and Controls") in the "Assay" column and enter the reagent lot number and expiry date.	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls"). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
5	Ensure "PCR Only" is selected in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
6	Ensure the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
7	Load the PCR Mix on the "Inventory Block" referring to the Load List and enter the PCR Mix lot number, expiry date and number of reactions for each tube.	Load the PCR Mix on the "Inventory Block" referring to the "Load List" and enter the PCR Mix lot number, expiry date and number of reactions for each tube.
8	Click "Next" to continue.	Click "Next" to continue.
9	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.
10	Click "Next" to continue.	Click "Next" to continue.
11	Load the PCR Cassette and the Q-PCR Standard tubes.	Load PCR Cassette, Positive Control and Negative Control.
12	Click "Next" to continue.	Click "Next" to continue.
13	Close the instrument door.	Close the instrument door.
14	Press "Start"	Press "Start".

When the session is finished, the **ELITE InGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified and stored at -20 ± 10 °C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session); mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Q - PCR Standard** can be removed from the instrument, capped, and stored at -20 °C or below. Avoid spilling the Q - PCR Standard.

NOTE

The **Q - PCR Standard** can be used for 4 separate sessions of 2 hours each.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the Positive Control. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

9.3 STEP 3 - Review and approval of results

The **ELITe InGenius** monitors target and internal control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe InGenius** generates results with the **JCV ELITe MGB Kit** through the following procedure:

1. Validation of Calibration curve,
2. Validation of Positive Control and Negative Control results,
3. Validation of sample results,
4. Sample result reporting.

9.3.1 A. Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the target of the Calibrator reactions with the **ELITe STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

The Calibration curves, specific for the PCR reagent lot, are recorded in the database (Calibration). They can be viewed and approved by “Administrator” or “Analyst” users, following the GUI instructions.

The Calibration curve expires **after 60 days**.

NOTE

If the Calibration curve does not meet the acceptance criteria, the “Failed” message is shown on the “Calibration” screen. In this case, the results cannot be approved and the Calibrator amplification reactions must be repeated. In addition, if samples were included in the run, these are not quantified and must also be repeated to generate quantitative results.

9.3.2 Validation of amplification Positive Control and Negative Control results

The **ELITE InGenius software** interprets the PCR results for the target of the Positive Control and Negative Control reactions with the **ELITE_PC** and **ELITE_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and used to verify the system (reagents lot and instrument).

The Positive Control and Negative Control results, specific for the PCR reagent lot, are recorded in the database (Controls). They can be viewed and approved by “Administrator” or “Analyst” users following the GUI instructions.

The Positive Control and Negative Control results expire **after 15 days**.

The **ELITE InGenius software** processes the Positive Control and Negative Control results and generates Control Charts. Four approved Positive Control and Negative Control results are used to set up the initial Control Chart. For subsequent controls, the results are analyzed by the software to ensure the system performances are within the acceptance criteria, shown in the Control Chart plots. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control runs must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same run, the samples can be approved but their results are not validated. In this case, the failed Control(s) and samples must all be repeated.

9.3.3 Validation of Sample results

The **ELITE InGenius software** interprets the PCR results for the target (Channel **JCV**) and the Internal Control (Channel **IC**) with the **JCV ELITE_U_200_100**, **JCV ELITE_PL_200_100** and **JCV ELITE_CSF_200_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Results Display" screen.

The sample results can be approved when the three conditions in the table below are true.

Table 7

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

The sample results are automatically interpreted by the **ELITE InGenius software** using Assay Protocol parameters.

The possible result messages are listed in the table below.

For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

Table 8

Result of Sample run	Interpretation
JCV:DNA Detected, quantity equal to XXX copies/mL or IU/mL	JCV DNA was detected in the sample within the assay measurement range, its concentration is shown.
JCV:DNA Detected, quantity below LLoQ copies/mL or IU/mL	JCV DNA was detected in the sample, its concentration is below the assay -Lower Limit of Quantification
JCV:DNA Detected, quantity beyond ULoQ copies/mL or IU/mL	JCV DNA was detected in the sample, its concentration is above the assay Upper Limit of Quantification
JCV:DNA Not detected or below LoD copies/mL or IU/mL	JCV DNA was not detected in the sample. The sample is negative for the target DNA, or its concentration is below the assay Limit of Detection .
Invalid-Retest Sample	Not valid assay result caused by Internal Control failure (due to e.g. incorrect extraction or inhibitors carry-over). The test should be repeated.

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, extraction or PCR steps (e. g. incorrect sampling, degradation or loss of DNA during the extraction or inhibitors in the eluate), which may cause incorrect results.

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see [14 TROUBLESHOOTING page 32](#)).

Samples reported as “JCV:DNA Not detected or below LoD copies/mL or IU/mL” are suitable for analysis but JCV was not detected. In this case the sample may be either negative for JCV DNA or the JCV DNA is present at a concentration below the Limit of Detection of the assay (see [11 PERFORMANCE CHARACTERISTICS page 21](#)).

JCV DNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as “JCV: DNA Detected, quantity below LLoQ copies/mL or IU/mL” (see [11 PERFORMANCE CHARACTERISTICS page 21](#)).

JCV DNA positive samples within the Linear Measuring Range (see [11 PERFORMANCE CHARACTERISTICS page 21](#)) are detected and are reported as “JCV: DNA Detected, quantity equal to “XXX” copies / mL or IU/mL”.

JCV DNA positive samples that are above the Upper Limit of Quantification are reported as “JCV: DNA Detected, quantity beyond ULoQ copies/mL or IU/mL” and they are not suitable for quantification. If needed the sample may be diluted before extraction or PCR and retested to yield results within the Linear Measuring Range of the assay (see [11 PERFORMANCE CHARACTERISTICS page 21](#)).

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

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

9.3.4 Sample result reporting

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

The “Sample Report” shows the results details by selected sample (SID).

The “Track Report” shows the results details by selected Track.

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

10 ELITE BeGenius PROCEDURE

The procedure to use the **JCV ELITE MGB Kit** with the **ELITE BeGenius** consists of three steps:

Table 9

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Calibration run (PCR Only)
		D) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Calibration curve
		2) Validation of Positive Control and Negative Control results
		3) Validation of sample results
		4) Sample result reporting

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITE BeGenius** and login in “**CLOSED**” mode,
- in the “Calibrations” menu on the Home page, verify the Calibrators (**Q - PCR Standard**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- in the “Controls” menu on the Home page, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see “Specimens and Controls”).

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

10.2 STEP 2 – Session Setup

The **JCV ELITE MGB Kit** can be used on the **ELITE BeGenius** to perform:

- Sample run (Extract + PCR),
- Eluted sample run (PCR Only),
- Calibration run (PCR Only),
- Positive Control and Negative Control run (PCR Only).

All the required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITE BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests**. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the four types of run follow the steps below while referring to the GUI:

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	Identify samples and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If required, transfer 200 µL of sample in an 2 mL Sarstedt tube previously labelled. Thaw the needed CPE tubes at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	Thaw the Elution tube containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select " Perform Run " from the "Home" screen.	Select " Perform Run " from the "Home" screen.
3	Remove all the Racks from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode": "Extract + PCR".	Select the "Run mode": "PCR Only".
5	Load the samples into the "Sample Rack". (Note: when secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack").	Load the samples into the "Elution Rack".
6	Insert the "Sample Rack" into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert the "Sample ID" (SID) for each "Position" used. (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3) If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
9	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	When more than 12 samples are processed, repeat the procedure from point 6.	When more than 12 samples are processed, repeat the procedure from point 6.
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable
13	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3). When more than 12 samples are processed, repeat using "Lane 2" (L2).	Not applicable
14	Click "Next" to continue.	Not applicable
15	Load CPE and the PCR Mix into the "Reagent/Elution Rack".	Load the PCR Mix into "Reagent/Elution Rack".
16	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix and / or CPE enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
17	Click "Next" to continue	Click "Next" to continue.

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
18	Verify the tips in the “Tip Rack (s)” in the “Inventory Area” and replace Tip Rack(s) if necessary.	Verify the tips in the “Tip Rack (s)” in the “Inventory Area” and replace Tip Rack(s) if necessary.
19	Click “Next” to continue.	Click “Next” to continue.
20	Load the “PCR Rack” with “PCR Cassette” in the Inventory Area.	Load the “PCR Rack” with “PCR Cassette” in the Inventory Area.
21	Click “Next” to continue.	Click “Next” to continue.
22	Load the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and required extraction consumables.	Not applicable
23	Close the instrument door.	Close the instrument door.
24	Press “Start”.	Press “Start”.

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	Thaw the needed Q-PCR Standard tubes (Cal1: Q-PCR Standard 10 ² , Cal2: Q-PCR Standard 10 ³ , Cal3: Q-PCR Standard 10 ⁴ , Cal4: Q-PCR Standard 10 ⁵) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	Thaw Positive Control tubes at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. Prepare the Negative Control by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITE InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode: PCR Only".	Select the "Run mode": "PCR Only".
5	Load the Q-PCR Standard tubes into the "Elution Rack".	Load the Positive Control and Negative Control tubes into the "Elution Rack".
6	Insert the "Elution Rack" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Elution Rack" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" (200 µL) and the "Extracted Elute Volume" (100 µL).	Ensure the "Extraction Input Volume" (200 µL) and the "Extracted Elute Volume" (100 µL).
9	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	Load the PCR Mix into "Reagent/Elution Rack".	Load the PCR Mix into "Reagent/Elution Rack".
12	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
13	Click "Next" to continue.	Click "Next" to continue.
14	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.
15	Click "Next" to continue.	Click "Next" to continue.
16	Load the "PCR Rack" with " PCR Cassette " in the Inventory Area.	Load the "PCR Rack" with " PCR Cassette " in the Inventory Area.
17	Click "Next" to continue.	Click "Next" to continue.
18	Close the instrument door.	Close the instrument door.
19	Press "Start".	Press "Start".

When the session is finished, the **ELITE BeGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run, the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified, and stored at $-20 \pm 10^{\circ}\text{C}$ for no longer than one month. Avoid the spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20°C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session); mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run, the remaining **Q - PCR Standard** can be removed from the instrument, capped and stored at -20°C or below. Avoid spilling the Q - PCR Standard.

NOTE

The **Q - PCR Standard** can be used for 4 separate sessions of 2 hours each.

NOTE

At the end of the run, the remaining **Positive Control** can be removed from the instrument, capped and stored at -20°C or below. Avoid the spilling of the **Positive Control**. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run, the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

10.3 STEP 3 -Review and approval of results

The **ELITe BeGenius** monitors target and internal control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe BeGenius** generates the results with the **JCV ELITe MGB Kit** through the following procedure:

1. Validation of Calibration curve,
2. Validation of Positive Control and Negative Control results,
3. Validation of sample results,
4. Sample result reporting.

NOTE

Please, refer to the same paragraph of the **ELITe InGenius Procedure** for the details.

11 PERFORMANCE CHARACTERISTICS

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay in association to Plasma collected in EDTA and Urine was determined on the **ELITe GALAXY** instrument and was verified on the ELITe InGenius and ELITe BeGenius instruments and on the three matrices (Plasma EDTA, Urine and CSF), testing 20 negative replicates of each matrix spiked by JCV certified reference material (1st WHO International Standard, NIBSC, code 14/114, United Kingdom).

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

Table 10 Limit of Detection

Matrix	LoD (IU / mL)
Plasma collected in EDTA	275
Urine	215
cerebrospinal fluid	215

Matrix	LoD (copies / mL)
Plasma collected in EDTA	306
Urine	165
cerebrospinal fluid	239

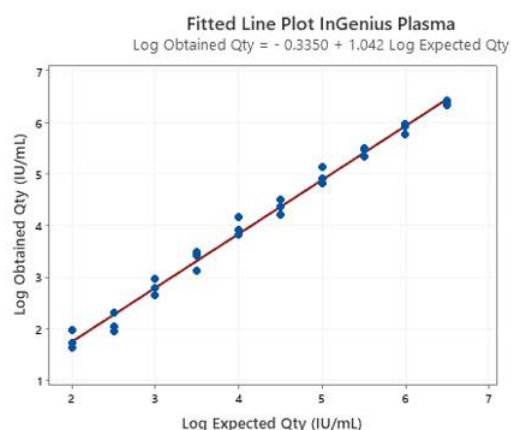
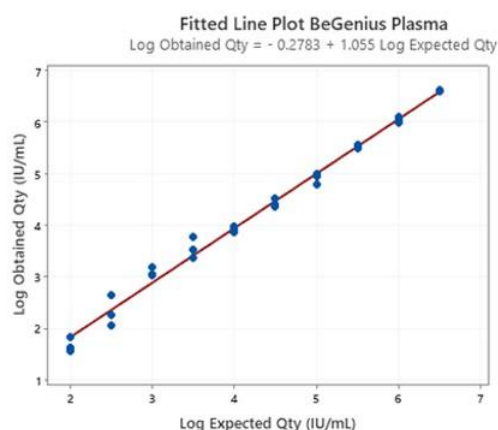
The copies / mL value for each matrix was calculated by applying the specific conversion factor reported at [11.10 Conversion factor to International Units page 27](#).

11.2 Linear measuring range and Limit of Quantification

The Linear measuring range of the assay was determined in association with plasma EDTA, urine and cerebrospinal fluid on **ELITe InGenius** and **ELITe BeGenius**, using a panel of dilutions of certified reference material (Exact Diagnostics JVC Verification Panel, Bio-Rad, code JCVP100, Italy, in association with urine and CSF; 1st WHO International Standard for JC virus DNA, NIBSC ref. 14/114, United Kingdom, in association with plasma EDTA) in JCV negative samples.

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

Plasma collected in EDTA



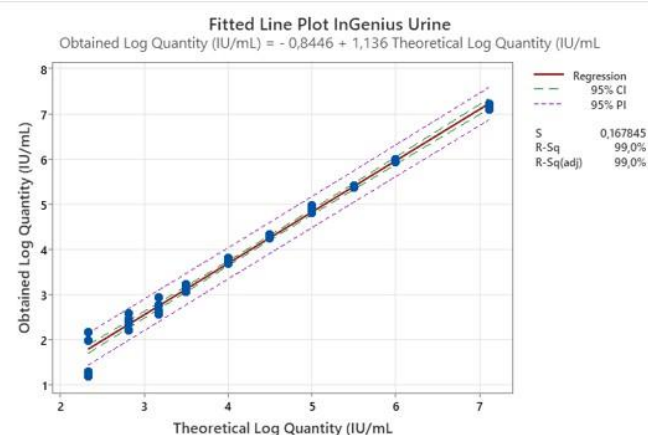
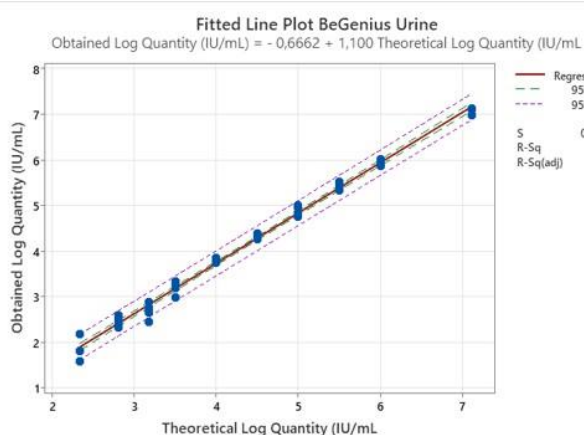
The linear measuring range as copies / mL for plasma EDTA is calculated by applying the specific conversion factor reported at paragraph [11.10 11.6 Conversion factor to International Units page 28 page 27](#)

The final results are summarized in the following table.

Table 11 Linear measuring range for plasma samples

Unit of measure	Lower Limit	Upper Limit
IU / mL	275	3,162,000
copies / mL	306	3,513,333

Urine



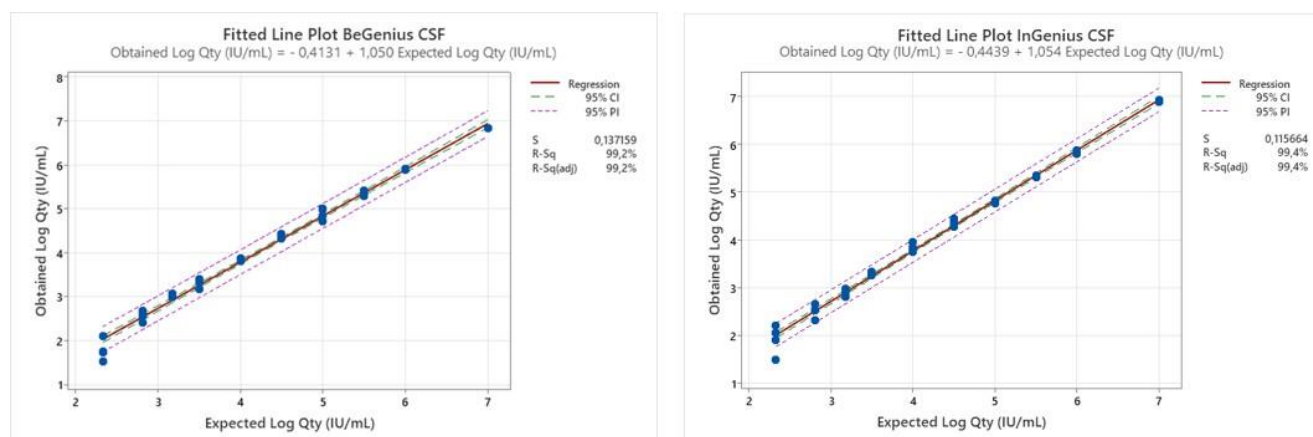
The linear measuring range as copies / mL for Urine is calculated by applying the specific conversion factor reported at [11.10 11.6 Conversion factor to International Units page 28 page 27](#)

The final results are summarized in the following table.

Table 12 Linear measuring range for urine samples

Unit of measure	Lower Limit	Upper Limit
IU / mL	215	13,000,000
copies / mL	165	10,000,000

CSF



The results are summed up in the following figure.

The linear measuring range as copies / mL for CSF is calculated by applying the specific conversion factor reported at [11.10 11.6 Conversion factor to International Units page 28 page 27](#)

The final results are summarized in the following table.

Table 13 Linear measuring range for CSF samples

Unit of measure	Lower Limit	Upper Limit
IU / mL	215	10,000,000
copies / mL	239	11,111,111

11.3 Standard Curve Uncertainty

The Uncertainty value of the Standard curve was calculated by combining the random errors (SD) of all level quantifications and multiplying for the Coverage factor $k = 2$ (Expanded Combined Uncertainty) and is equal to 0.2036 Log copies / reaction.

Table 14

Standard curve levels	Theoretical	SD	Expanded Combined Uncertainty
	Log c/rxn		
JCV Q-PCR Standard 10^5	5.0000	0.0439	0.2036
JCV Q-PCR Standard 10^4	4.0000	0.0379	
JCV Q-PCR Standard 10^3	3.0000	0.0330	
JCV Q-PCR Standard 10^2	2.0000	0.0769	

11.4 Inclusivity: Efficiency of detection and quantification efficiency on different strains

The Inclusivity of the assay, as efficiency of detection for different strain of *Polyomavirus JC*, was evaluated by *in silico* analysis of the sequences available in nucleotide databases. The analysis showed sequence conservation and absence of significant mutations. Therefore, an efficient detection for the different strains or isolates is expected.

11.5 Potential interfering markers: Cross-reactivity

The Potential cross-reactivity of unintended organisms that may be found in clinical specimens was evaluated by *in silico* analysis. The analysis showed no significant homology with other unintended organisms (viruses, bacteria, phages, fungi and invertebrates). Therefore, no cross-reactivity is expected.

11.6 Potential interfering markers: Inhibition

The potential inhibition of unintended organisms that may be found in clinical specimens was evaluated by *in silico* analysis. The analysis showed no significant homology with other unintended organisms (viruses, bacteria, phages, fungi and invertebrates). Therefore, no inhibition is expected.

11.7 Potential interfering substances: Inhibition

The potential inhibition of interfering substances (endogenous and exogenous) that might be found in clinical specimens was evaluated for the assay by analysis of a panel of substances at relevant concentration in JCV positive samples. The results, for each matrix, are reported in the following paragraphs.

Table 15 Plasma

Samples	JCV Pos. / Rep	Outcome
Azithromycin	5/5	No interference
Ganciclovir	5/5	No interference
Ribavirin	5/5	No interference
Abacavir Sulphate	5/5	No interference
Cidofovir	5/5	No interference
Cyclosporine A	5/5	No interference
Heparin	5/5	Interference*
EDTA	5/5	No interference
Bilirubin	5/5	No interference

*Even though 5/5 replicates were detected, Heparin inhibition was observed during the calculation of the bias (Heparin bias: 0.8979).

The test showed that all the substances, with the exception for heparin, do not interfere with the JCV target detection and quantification using the JCV ELITe MGB Kit on plasma samples.

Table 16 Urine

Samples	JCV Pos. / Rep	Outcome
Whole Human Blood	5/5	No interference
Phenazopyridine hydrochloride	5/5	No interference
Azithromycin	5/5	No interference
Bilirubin	5/5	No interference

The tested substances do not interfere with the JCV or Internal Control detection and quantification using the JCV ELITe MGB Kit on urine samples.

Table 17 CSF

Samples	JCV Pos. / Rep	Outcome
Ethanol	5/5	No interference
Whole Human Blood	5/5	No interference
Ganciclovir	5/5	No interference
Vancomycin	5/5	No interference
Azithromycin	5/5	No interference
Acyclovir	5/5	No interference
Ampicillin	5/5	No interference
Fluconazole	5/5	No interference
Cyclosporine A	5/5	No interference

The tested substances do not interfere with the JCV or Internal Control detection and quantification using the JCV ELITe MGB Kit on CSF samples.

11.8 Repeatability

The Intra-Session and Inter-Session Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius systems by analysis of a panel of plasma samples collected in EDTA, including one negative sample and two samples spiked by JCV certified reference material (1st WHO International Standard, NIBSC).

An example of Intra-Session Repeatability (on one day) results is shown in the tables below.

Table 18 Intra – Session Repeatability on ELITe InGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	35.38	0.82	2.33	
10 x LoD	8	33.75	0.62	1.84	

Table 19 Intra – Session Repeatability on ELITe BeGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	35.61	0.60	1.68	
10 x LoD	8	33.89	0.20	0.60	

An example of Inter-Session Repeatability (on two days) results is shown in the tables below.

Table 20 Inter – Session Repeatability on ELITe InGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	16	N.A.	N.A.	N.A.	100%
3 x LoD	16	35.40	0.75	2.11	
10 x LoD	16	33.66	0.69	2.05	

Table 21 Inter – Session Repeatability on ELITe BeGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	16	N.A.	N.A.	N.A.	100%
3 x LoD	16	35.60	0.56	1.57	
10 x LoD	16	33.78	0.29	0.86	

In the Repeatability test, the JCV ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

11.9 Reproducibility

The Reproducibility of the assay was evaluated on **ELITe InGenius** and **ELITe BeGenius** by analysis of a panel of plasma samples collected in EDTA, including one negative sample and two samples spiked with JCV certified reference material “1st WHO International Standard JCV certified reference material (1st WHO International Standard, NIBSC).

A summary of Inter-Instrument Reproducibility (on two instruments) is shown in the tables below.

Table 22 Inter – Instrument Reproducibility ELITe InGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	35.88	0.24	0.66	
10 x LoD	8	33.56	0.52	1.55	

Table 23 Inter – Instrument Reproducibility ELITe BeGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	35.54	0.75	2.12	
10 x LoD	8	33.78	0.51	1.50	

A summary of Inter-batch Reproducibility (on two lots) is shown in the tables below:

Table 24 Inter – Batch Reproducibility ELITe InGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	35.76	0.35	0.98	
10 x LoD	8	33.77	0.45	1.34	

Table 25 Inter – Batch Reproducibility ELITe BeGenius

Sample	N	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	35.74	0.59	1.65	
10 x LoD	8	33.64	0.49	1.46	

In the Reproducibility test, the JCV ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

11.10 Conversion factor to International Units

The conversion factor to report the quantitative results in International Units / mL starting from copies / mL, was calculated for each matrix, using a panel of dilutions of certificated calibrated reference material (1st WHO International Standard for JCV DNA, NIBSC ref. 14/114).

The results for each matrix are summarized in the following table

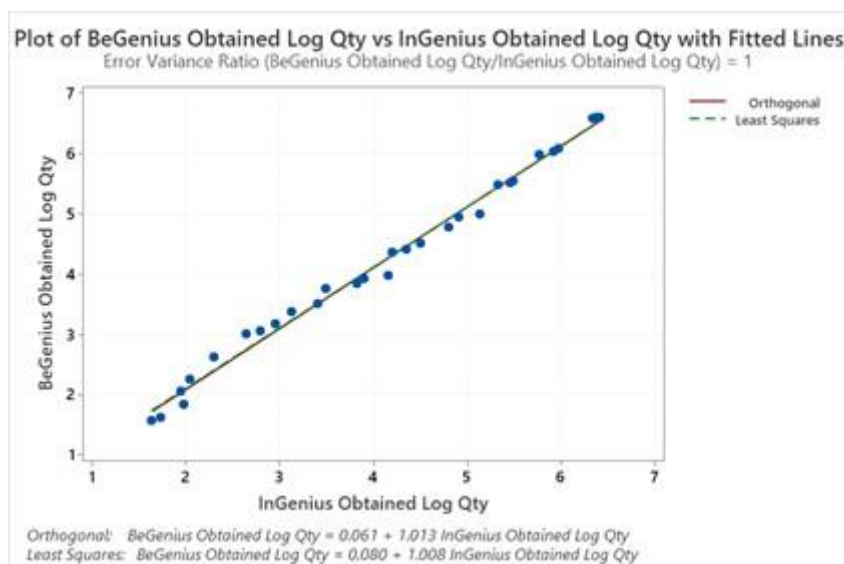
Table 26 Conversion factor to International Units with ELITe InGenius and ELITe BeGenius

Matrix	Fc (IU / copies)
urine	1.3
plasma	0.9
cerebrospinal fluid	0.9

The results obtained were analysed by orthogonal and linear regression in order to calculate the correlation.

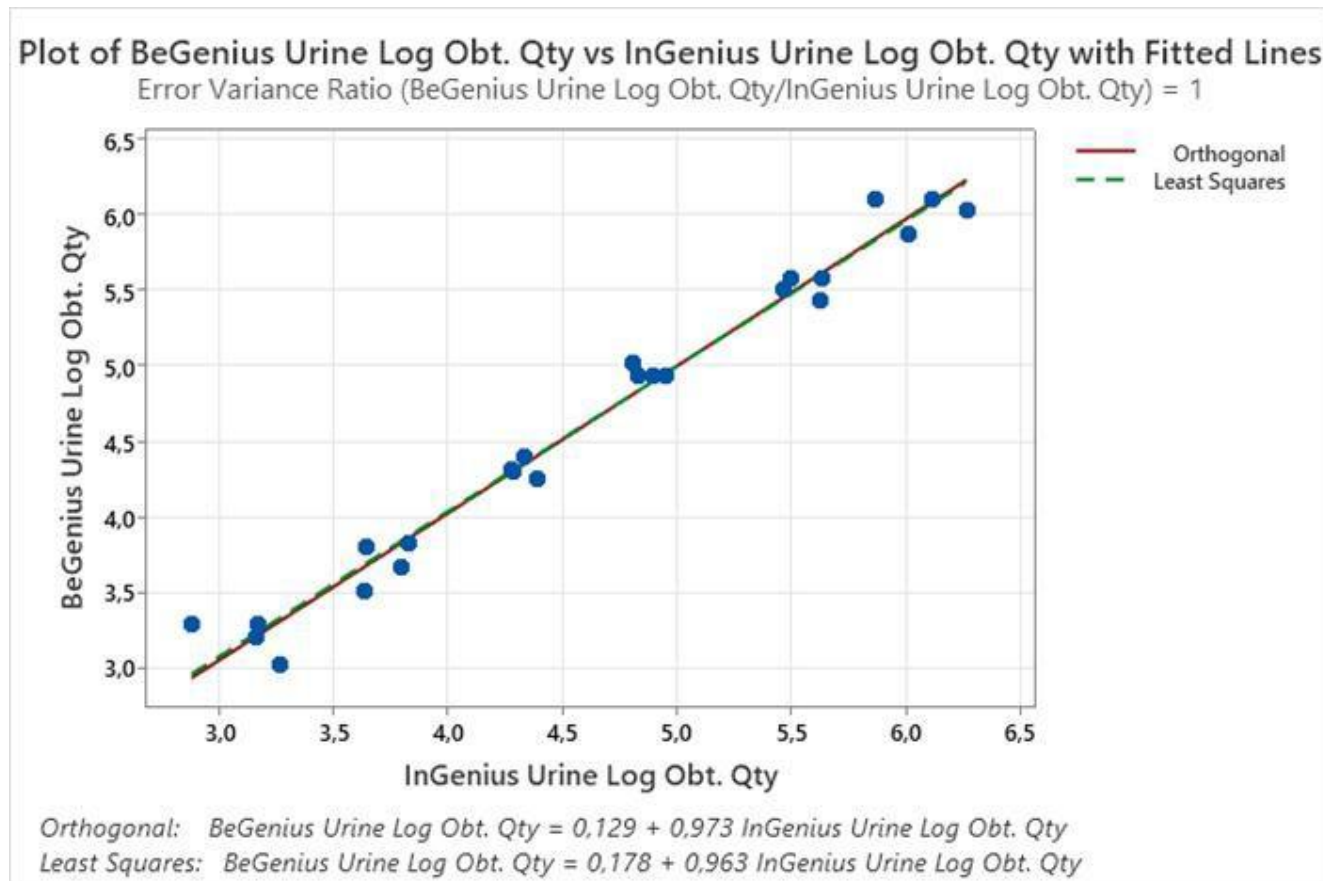
The results, for each matrix, are reported in the following paragraphs.

Plasma



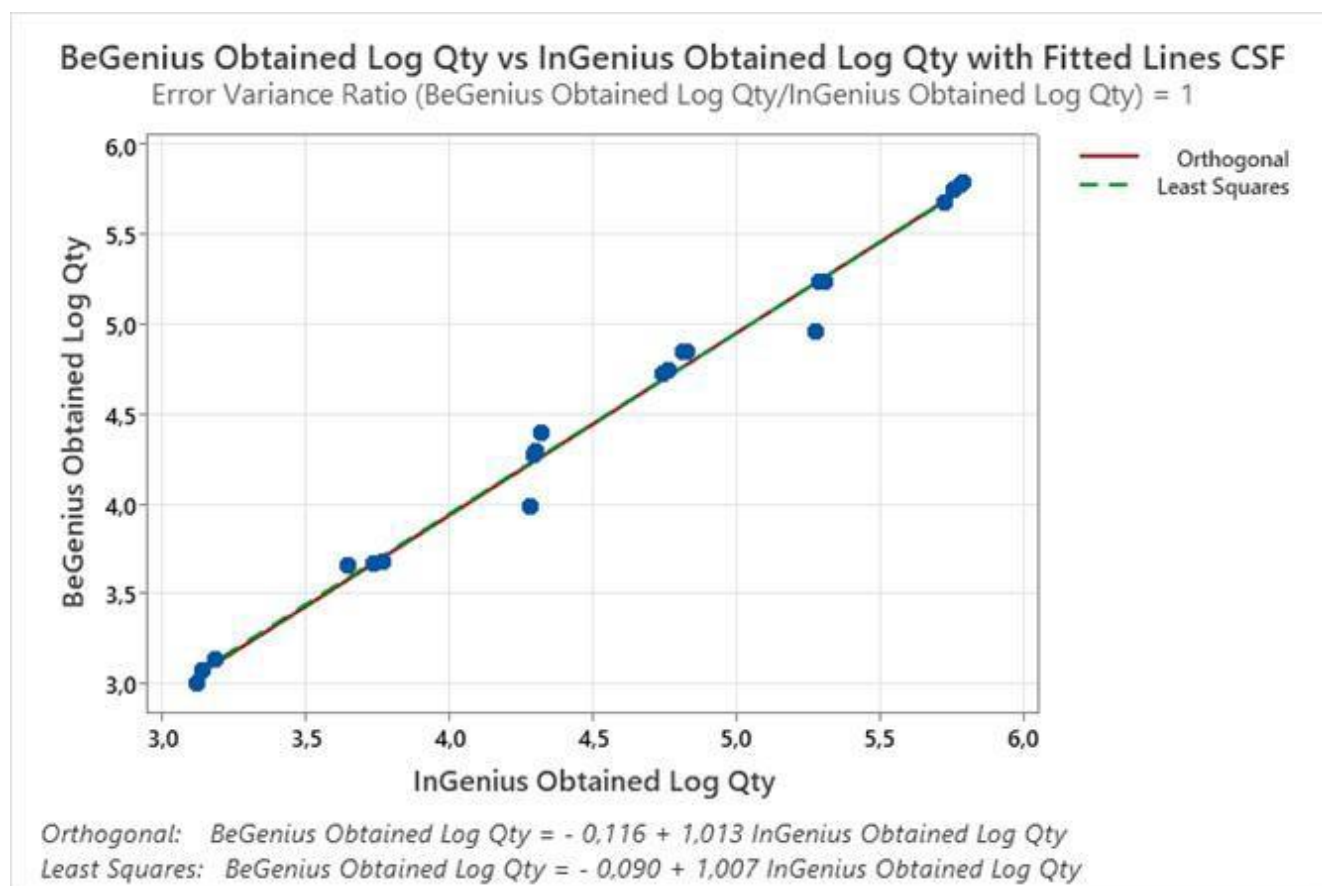
The Orthogonal Regression analysis was performed and generated an intercept equal to 0.061 (95% CI: -0.093; 0.215) and a slope equal to 1.013 (95% CI: 0.977; 1.048).

Urine



The Orthogonal Regression analysis generated an intercept equal to 0.129 (95% CI: 0.161, 0.419) and a slope equal to 0.973 (95% CI: 0.912, 1.035).

CSF



The Orthogonal Regression analysis generated an intercept equal to 0.116 (95% CI: -0.331; 0.100) and a slope equal to 1.013 (95% CI: 0.967; 1.059).

11.11 Diagnostic Sensitivity: confirmation of positive sample

The diagnostic sensitivity of the assay, as confirmation of positive samples, was evaluated in association with **ELITe InGenius** by analyzing clinical samples certified positive for JCV DNA or spiked for JCV DNA with reference material. 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 results are summed up in the following table.

Table 27

Samples	N	positive	negative	% Diagnostic sensitivity
Plasma collected in EDTA and positive for JCV DNA	1	1	0	98%
Plasma collected in EDTA and spiked for JCV DNA	50	49	1	
Urine collected without preservatives positive for JCV DNA	33	31	2	96.5%
Urine collected without preservatives spiked for JCV DNA	24	24	0	

Table 27 (continued)

Samples	N	positive	negative	% Diagnostic sensitivity
Cerebrospinal fluid positive for JCV DNA	11	11	0	100%
Cerebrospinal fluid spiked for JCV DNA	48	48	0	

11.12 Diagnostic Specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with ELITE InGenius by analyzing clinical samples certified negative or presumably negative for JCV DNA. 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 results are summed up in the following table.

Table 28

Samples	N	positive	negative	% Diagnostic Specificity
Plasma collected in EDTA negative for JCV DNA	62	1	61	98.4%
Urine collected without preservatives negative for JCV DNA	60	0	60	100%
Cerebrospinal fluid negative for JCV DNA	76	0	76	100%

The IC Ct cut-off value is set at 36 for plasma samples collected in EDTA samples,

The IC Ct cut-off value is set at 34 for urine collected without preservatives samples

The IC Ct cut-off value is set at 33 for CSF samples.

12 REFERENCES

P. Ferrante et al. (1995) J Med Vir 47: 219 – 225

K. Linnet et al. (2004) Clin. Chem. 50: 732 – 740

C. N. Kotton et al. (2024) Transplantation 00: 00 - 00

E. A. Lukhtanov et al. (2007) Nucleic Acids Res. 35: e30

13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: plasma collected in EDTA, urine collected without preservatives and cerebrospinal fluid (CSF).

Plasma collected in EDTA shall be obtained from whole blood stored at room temperature or +2 / +8 °C for no longer than 24 hours.

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 performance with DNA extracted from the following clinical samples: whole blood, suspensions of leucocytes and suspensions of granulocytes.

The results obtained with this product depend on proper 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 product.

Owing to its high analytical sensitivity, the Real-Time PCR method used in this product is sensitive to contamination from positive clinical samples, positive controls and PCR products. Cross-contamination cause false positive results. The product format is designed to limit cross-contamination. However, cross-contamination can only be avoided by good laboratory practices and following these instructions for use.

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 personal protective equipment 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 requires the use of personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques, such as extraction, PCR and detection of nucleic acids.

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 indicates that the target DNA is not detected in the DNA extracted from the sample; however it cannot be excluded that the target DNA has a lower titer than the product detection limit ([11 PERFORMANCE CHARACTERISTICS page 21](#)). In this case the result could be a false negative.

Results obtained with this product may sometimes be invalid due to failure of internal control. In this case the sample shall be retested, starting from extraction, which can lead to a delay in obtaining final results.

Possible polymorphisms, insertions or deletions within the region of the DNA targeted by the product primers and probes may impair detection and quantification of target DNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted in combination with all relevant clinical observations and laboratory results.

As with any other diagnostic medical device, there is a residual risk of obtaining invalid, or erroneous results with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient. However, this residual risk associated to the intended use of the product has been weighed against the potential benefits to the patient and it has been assessed acceptable.

14 TROUBLESHOOTING

Table 29

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	Solutions
Instrument setting error.	Check the position of PCR Mix, Q-PCR Standards and Positive Control. Check the volumes of PCR Mix, Q-PCR Standards and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 5 independent sessions (3 hours each in the Inventory Area, Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit) Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 4 independent sessions (2 hours each in the Extraction Area or in the Cooler Unit). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 30

Invalid Negative Control reaction	
Possible Causes	Solutions
Instrument setting error.	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit.	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 31

Invalid Sample reaction	
Possible Causes	Solutions
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 5 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.

Table 31 (continued)

Invalid Sample reaction	
Possible Causes	Solutions
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a "PCR Only" session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an "Extract + PCR" session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 32

Anomalous dissociation curve	
Possible causes	Solutions
Absence of a defined peak. Defined peak but T _m different from that of the other samples and that of the Standards or Positive Control.	Check for target 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 with a possible mutation. The target in the sample should be sequenced to confirm mutation.

Table 33

Error in Ct calculation	
Possible Causes	Solutions
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If significant amplification is observed in PCR plot, select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: - repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a "PCR Only" session - repeat the extraction of the sample with a 1:10 dilution in molecular biology grade water in an "Extract + PCR" session.

Table 34

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	Solutions
Sample-to-sample contamination during preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the GUI. Follow the loading sequence indicated by the software.
Laboratory environmental contamination.	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix and / or CPE.

15 SYMBOLS



Catalogue Number.



Upper limit of temperature.



Batch code.



Use by (last day of month).



in vitro diagnostic medical device.



Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.



Unique Device Identification



Contains sufficient for "N" tests.



Consult instructions for use.



Contents.



Keep away from sunlight.



Manufacturer.

16 NOTICE TO THE USERS

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and /or the patient is established. At the moment of the current revision of the IFU, no serious incident or recall with impact on product performance and safety of the device has occurred.

A "Summary of Safety and Performance" will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional. Before the notice of full functionality of Eudamed has been published, the "Summary of Safety and Performance" will be made available to the public upon request by email at emd.support@elitechgroup.com, without undue delay.

17 NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Thermo Fisher Scientific and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Thermo Fisher Scientific. 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, Thermo Fisher Scientific. Email: outlicensing@thermofisher.com.

ELITe MGB ® detection reagents are covered by one or more of U. S. Patent numbers 7319022, 7348146, 7381818, 7541454, 7671218, 7723038, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

ELITe InGenius® and ELITe BeGenius® technologies are covered by patents and pending applications.

This limited license allows the person or entity to whom the product has been provided to use the product and data generated by the use of the product, solely for human diagnostics. Neither ELITechGroup S. p. A. nor its licensors grant any other licenses, expressed or implied for any other purposes.

Appendix A JCV ELITe MGB Kit used in association with Genius series® platforms



CAUTION

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

Intended use

The product **JCV ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids Real-Time PCR assay for the **detection and quantification of the DNA of Human Polyomavirus JC (JCV)** extracted from clinical specimens.

The assay is validated in association with the **ELITE InGenius®** and **ELITE BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA, urine collected without preservatives and cerebrospinal fluid (CSF).

The product is intended for use as an aid in the diagnosis and monitoring of JCV infections in patients suspected of having or undergoing monitoring of JCV infections.

The results must be interpreted in combination with all relevant clinical observation and laboratory outcomes.


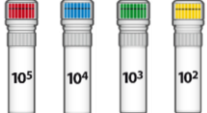

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	Large T antigen	FAM	JCV
Internal Control	IC2	AP525	IC

Validated matrix

- Plasma collected in EDTA
- Urine collected without preservatives
- Cerebrospinal fluid CSF

Kit content and related products

JCV ELITe MGB Kit	JCV ELITe Standard	JCV - ELITe Positive Control
 X 4	 X 2	 X 1
Ready-to-use PCR Mix 4 tubes of 540 µL 96 reactions per kit 5 freeze-thaw cycles	Ready-to-use 4 levels: 10 ⁵ copies/rxn, 10 ⁴ copies/rxn, 10 ³ copies/rxn, 10 ² copies/rxn 2 sets of 4 tubes of 160 µL 4 freeze-thaw cycles	Ready-to-use Positive Control 1 tube of 160 µL 4 reactions per kit 4 freeze-thaw cycles

Maximum shelf-life: **24 months**

Storage Temperature: **-20 °C**

Other products required not provided in the kit

<ul style="list-style-type: none"> • ELITe InGenius instrument: INT030. • ELITe BeGenius instrument: INT040. • ELITe InGenius SP 200: INT032SP200. • ELITe InGenius SP1000: INT033SP1000 • ELITe InGenius SP 200 Consumable Set: INT032CS. 	<ul style="list-style-type: none"> • ELITe InGenius PCR Cassette: INT035PCR. • ELITe InGenius Waste Box: F2102-000. • CPE - Internal Control: CTRCPE • 300 µL Filter Tips Axigen: TF-350-L-R-S. • 1000 µL Filter Tips Tecan: 30180118.
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ELITe InGenius and ELITe BeGenius protocol

› Sample volume	200 µL
› Total elution volume	100 µL
›CPE volume	10 µL
eluate PCR input volume	10 µL
›Q- PCR Mix volume	20 µL
› Frequency of controls	15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity	Linearity	CF cp/mL to IU/mL
plasma	275 IU / mL 306 cp/mL	98% 50/51	98.4% 61/62	275 – 3,162,000 (IU/mL) 306 – 3,513,333 (copies/mL)	0.9
urine	215 IU / mL 165 cp/mL	96.5% 55/57	100% 60/60	215 - 13,000,000 (IU/mL) 165 – 10,000,000 (copies/mL)	1.3
CSF	215 IU / mL 239 cp/mL	100% 59/59	100% 76/76	215 - 10,000,000 (IU/mL) 239 - 11,111,111 (copies/mL)	0.9

Sample preparation

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified according to laboratory guidelines, and collected, transported, and stored under the following conditions.

Table 35

Sample type	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2° / +8° C	-20 ± 10 °C	-70 ± 15 °C
urine	Preservative-free	≤ 4 h	≤ 4 h	≤ 30d	≤ 30d
plasma	EDTA	≤ 1 d	≤ 3 d	≤ 30d	≤ 30d
CSF	minimal patient blood contamination	≤ 4 h	≤ 4 h	≤ 30d	≤ 30d

CSF, cerebrospinal fluid; EDTA, Ethylenediaminetetraacetic acid; d, day, h, hours

ELITe InGenius Procedures

The user is guided step-by-step by the Graphic User Interface (GUI) of ELITe InGenius software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR) or PCR Only.

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode " CLOSED ".	2. Verify controls: Positive Control and Negative Control in the "Controls" menu. Note: Both must have been run, approved and not expired.	3. Thaw the PCR Mix and the CTRCPE tubes. Vortex gently. Spin down 5 sec.
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Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen	2. Verify the extraction volumes: Input: "200 µL", elution: "100 µL"	3. Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: JCV ELITe_U_200_100, JCV ELITe_PL_200_100 JCV ELITe_CSF_200_100 JCV ELITe_PL_1000_100	5. Select the method "Extract + PCR" and the sample position: Primary tube or Extraction Tube	6. Load the PCR Mix and the Internal Control in the Inventory Block
7. Load: PCR Cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks and primary sample racks	8. Close the door. Start the run	9. View, approve and store the results

NOTE

If an Extract Only mode is needed, refer to the instrument user's manual for procedure.

Procedure 2: PCR Only (e.g., eluates, standards, controls)

1. Select “Perform Run” on the touch screen	2. Verify the extraction volumes: Input: “200 µL”, elution: “100 µL”	3. Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the “Assay protocol” of interest: JCV ELITe_PC, JCV ELITe_NC or JCV ELITe_STD or JCV ELITe_U_200_100, JCV ELITe_PL_200_100 JCV ELITe_CSF_200_100 JCV ELITe_PL_1000_100	5. Select the method “PCR Only” and the sample position “Elution Tube”	6. Load the PCR Mix in the Inventory Block
7. Load: PCR Cassette rack and Elution tube rack with the extracted nucleic acid	8. Close the door. Start the run	9. View, approve and store the results

ELITe BeGenius Procedures

The user is guided step-by-step by the Graphic User Interface (GUI) of ELITe BeGenius software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR) or PCR Only.

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode “ CLOSED ”.	2. Verify controls: Positive Control and Negative Control in the “Controls” menu. Note: Both must have been run, approved and not expired.	3. Thaw the PCR Mix and the CTRCPE tubes. Vortex gently. Spin down 5 sec.
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Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract + PCR»	2. Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	3. Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay protocol” of interest JCV ELITe_Be_U_200_100, JCV ELITe_Be_PL_200_100 JCV ELITe_Be_CSF_200_100 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 Cooler Unit	6. Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	8. Close the door. Start the run	9. View, approve and store the results

NOTE

If an Extract Only mode is needed, refer to the instrument user’s manual for procedure.

Procedure 2: PCR Only (e.g., eluates, standards and controls)

1. Select “Perform Run” on the touch screen and then click on the run mode “PCR Only”.	2. Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit”.	3. Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”.
4. Select the “Assay Protocol” of interest: JCV ELITe_Be_PC, JCV ELITe_Be_NC or JCV ELITe_Be_STD or JCV ELITe_Be_U_200_100, JCV ELITe_Be_PL_200_100 JCV ELITe_Be_CSF_200_100	5. Load the Complete reaction mixture in the Reagent/Elution Rack and insert it in the Cooler Unit.	6. Load “PCR Rack” with “PCR Cassette”.
7. Close the door. Start the run.	8. View, approve and store the results.	



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