Instructions for use

HSV2 ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS032PLD

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IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yyyy)
19–R	Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements. Upgrade of the analytical and diagnostic performances in PERFORMANCE CHARACTERISTICS paragraph Update of the Intended use: • Validation of the products in association with ELITe InGenius (REF INT030) and ELITe BeGenius (REF INT040) instruments with Whole Blood, Plasma and CSF matrices. • Validation of the products in association with Whole Blood matrix and following instruments: ELITe GALAXY and ABI 7500 Fast Dx Real-Time PCR Instrument. **NOTE** Composition of the product remains unchanged. New graphics and content setting of the IFU.	19/05/25
18	• *Update for the use of the product for CSF matrix in association with «ELITe BeGenius®» instrument (REF INT040);* • *Internal Control Ct cut-off update in association with «ELITe InGenius®» (REF INT030) and «ELITe BeGenius®» (REF INT040)*	28/10/22
17	Update for the use of the product in association with □ELITe BeGenius □*instrument (REF INT040).* *Update of PERFORMANCE CHARACTERISTICS (pag.22):* • Change in Limit of Detection (LoD) • Addition of Linear measuring range • Addition of Repeatability • Addition of Reproducibility	28/01/22
16	Extended Use of the product with the platform Roche cobas z 480 analyzer.	15/06/2020
15	The number of tubes and the volume of Positive Control (ref. CTR032PLD) has been modified: from 4 x 65 µL to 2 x 160 µL.	28/07/17
00 — 14	New product development and succeeding changes.	-

NOTE

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

PRODUCT REF.	Lot Number	Expiry date
RTS032PLD	U0125-116	31/01/2027
RTS032PLD	U0724-004	30/06/2026
RTS032PLD	U0324-114	30/11/2025
RTS032PLD	U1123-019	31/08/2025

TABLE OF CONTENT

1 INTENDED USE

The product **HSV2 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 Herpes Simplex virus type 2 (HSV2)** 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 whole blood collected in EDTA, plasma collected in EDTA and cerebrospinal fluid (CSF).

The assay is also validated in association with the **ELITe GALAXY**, automatic extraction and PCR set-up system and **7500 Fast Dx Real-Time PCR Instrument**, Real-Time PCR platform, using human specimens of whole blood collected in EDTA.

The product is intended for use as an aid in the diagnosis and monitoring of HSV2 infections in patients suspected of having or undergoing monitoring of HSV2 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 HSV2 DNA isolated from specimens and amplified using the assay reagent **HSV2 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 cycles (Ct) and the melting temperatures (Tm). The HSV2 quantity is calculated based on a stored calibration curve.

7500 Fast Dx Real-Time PCR Instrument measures and records the increase of fluorescence emission. The subsequent data processing allows the detection and quantification of HSV2 in the primary specimen.

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 **HSV2 ELITe MGB Kit** provides the assay reagent **HSV2 Q-PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- **Specific region of gpG**, detected in Channel HSV2; the probe is stabilized by ELITe MGB technology, quenched by a non-fluorescent molecule and labelled by FAM dye.
- Internal Control, specific for **promoter and 5' UTR region** of the **human beta-globin gene,** detected in Channel **IC**; the probe is stabilized by ELITe MGB technology, quenched by the Eclipse Dark Quencher and labelled by AquaPhluor® 525 (AP525) dye.

The **HSV2 Q-PCR Mix** also contains buffer, magnesium chloride, triphosphates nucleotides, AP593 fluorophore, (used instead of ROX or Cy5 as passive reference for fluorescence normalization), the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product and the “hot start” DNA Polymerase enzyme. The product **HSV2 ELITe MGB Kit** contains sufficient reagents for **96 tests** on **ELITe InGenius** and **ELITe BeGenius with 20** µL used per reaction.

The product **HSV2 ELITe MGB Kit** contains sufficient reagents for **100 tests on other systems,** with **20** µL used per reaction.

NOTE

A conversion factor allows to express the results of the quantitative analysis in International Units of HSV2, in accordance with the “1st WHO International Standard for HSV2 DNA (NIBSC ref.17/122, United kingdom).

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
HSV2 Q-PCR Mix ref. RTS032PLD	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) **HSV2 ELITe_STD**, Assay Protocol with parameters for Calibrators analysis **HSV2 ELITe _PC**, Assay Protocol with parameters for Positive Control analysis **HSV2 ELITe _NC**, Assay Protocol with parameters for Negative Control analysis **HSV2 ELITe_WB_200_100** Assay Protocol with parameters for whole blood specimen analysis **HSV2 ELITe_PL_200_100** Assay Protocols with parameters for plasma specimen analysis **HSV2 ELITe_CSF_200_100** Assay Protocols with parameters for CSF 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) **HSV2 - ELITe Standard** (EG SpA, ref. STD032PLD) **HSV2 - ELITe Positive Control** (EG SpA, ref. CTR032PLD)
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.2.1 (or later) **HSV2 ELITe_Be_STD**, Assay Protocol with parameters for Calibrators analysis **HSV2 ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **HSV2 ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **HSV2 ELITe_Be_WB_200_100**, Assay Protocol with parameters for whole blood specimen analysis **HSV2 ELITe_Be_PL_200_100**, Assay Protocol with parameters for plasma specimen analysis **HSV2 ELITe_Be_CSF_200_100** Assay Protocols with parameters for CSF specimen analysis	
7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific, ref. 4406985) **ELITe GALAXY** (EG SpA, ref. INT020) with **software version 1.3.1** (or later). Extraction Protocol for ELITe GALAXY, xNA Extraction (Universal)	**ELITe GALAXY 300 Extraction Kit** (EG SpA, ref. INT021EX). **MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL** (Life Technologies, ref. 4346906), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification **CPE – Internal Control** (EG SpA, ref. CTRCPE) **HSV2 - ELITe Standard** (EG SpA, ref. STD032PLD) **HSV2 - ELITe Positive Control** (EG SpA, ref. CTR032PLD)

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.

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.

When the amplification session has to be performed with the 7500 Fast Dx Real-Time PCR Instrument, 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.

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)
HSV2 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 for ELITe InGenius and ELITe BeGenius

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:

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ± 10 °C**	**-70 ± 15 °C**
Whole blood	EDTA	≤ 1 d	≤ 3 d	≤ 30 d	≤ 30 d
Plasma	EDTA	≤ 1 d	≤ 3 d	≤ 30 d	≤ 30 d
CSF	Avoid patient blood contamination	≤ 4 hours	≤ 4 hours	≤ 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 the **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 4

Specimen	Instrument	Assay Protocol name	Report	Characteristics
Whole blood in EDTA	ELITe InGenius	HSV2 ELITe_WB_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	HSV2 ELITe_Be_WB_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Plasma in EDTA	ELITe InGenius	HSV2 ELITe_PL_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	HSV2 ELITe_Be_PL_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
CSF	ELITe InGenius	HSV2 ELITe_CSF_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	HSV2 ELITe_Be_CSF_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 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 **ELITe InGenius SP200** (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" section.

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 WITH ELITe InGenius and ELITe BeGenius page 22 section to check data concerning interfering substances.

NOTE

Do not use samples collected in heparin, which is a known reverse transcription and PCR inhibitor.

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 **HSV2 ELITe Standard** (not provided with this kit) with the **HSV2 ELITe_STD** or **HSV2 ELITe_Be_STD** Assay Protocols.

NOTE

The concentration of Q – PCR Standards are expressed in copies / reaction (10^5 copies / rxn, 10^4 copies / rxn, 10^3 copies / rxn, 10^2 copies /rxn). Refer to "Standard Curve Uncertainty" in the 11 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 22 section.

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

- For the Positive Control, use the product **HSV2 - ELITe Positive Control** (not provided with this kit) with the **HSV2 ELITe_PC** or **HSV2 ELITe_Be_PC** Assay Protocols.
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **HSV2 ELITe_NC** or **HSV2 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** instruments.

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 **HSV2 ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

Table 5

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 that 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 8 "Specimens and Controls" page 8).

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 **HSV2 ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

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).

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.
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 **HSV2 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 **HSV2**) and the Internal Control (Channel **IC**) with the **HSV2 ELITe_WB_200_100** or **HSV2 ELITe_PL_200_100** or **HSV2 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.

1) Calibration Curve	**Status**
HSV2 Q-PCR Standard	APPROVED
2) Positive Control	**Status**
HSV2 Positive Control	APPROVED
3) Negative Control	**Status**
HSV2 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.

Result of sample run	**Interpretation**
HSV2:DNA Detected, quantity equal to XXX copies/mL or IU/mL	**HSV2 DNA was detected** in the sample within the assay measurement range, its concentration is shown.
HSV2:DNA Detected, quantity below “LLoQ” copies/mL or IU/mL	**HSV2 DNA was detected** in the sample, its concentration is below the assay Lower Limit of Quantification

Result of sample run	Interpretation
HSV2:DNA Detected, quantity beyond "ULoQ" copies/mL or IU/mL	**HSV2 DNA was detected** in the sample, its concentration is above the assay Upper Limit of Quantification
HSV2:DNA Not Detected or below "LoD" copies/mL or IU/mL	**HSV2 DNA was not detected** in the sample. The sample is negative for HSV2 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 17 TROUBLESHOOTING page 41).

Samples reported as "HSV2:DNA Not Detected or below "LoD" copies/mL or IU/mL" are suitable for analysis but HSV2 was not detected. In this case the sample may be either negative for HSV2 DNA or the HSV2 DNA is present at a concentration below the Limit of Detection of the assay (see 11 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 22).

HSV2 DNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as "HSV2:DNA Detected, quantity below "LLoQ" copies/mL or IU/mL" (see 11 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 22).

HSV2 DNA positive samples within the Linear Measuring Range are detected and are reported as "HSV2:DNA Detected, quantity equal to "XXX" copies/mL or IU/mL" (see 11 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 22).

HSV2 DNA positive samples that are above the Upper Limit of Quantification are reported as "HSV2:DNA Detected, quantity beyond "ULoQ" copies/mL or IU/mL" (see11 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 22), 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.

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 **HSV2 ELITe MGB Kit** with the **ELITe BeGenius** 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

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 **HSV2 ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

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).

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 a 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 ± 10 °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 **HSV2 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 WITH ELITe InGenius and ELITe BeGenius

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay in association to whole blood collected in EDTA was determined on the **ELITe BeGenius** instrument, by testing a panel of HSV2 negative matrix spiked with reference material of HSV2 (1st WHO International Standard for HSV2 DNA, NIBSC ref. 17/122, United Kingdom). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

The results for whole blood collected in EDTA are reported in the following tables.

Table 7 Limit of Detection with ELITe InGenius and ELITe BeGenius (IU / mL)

Matrix	LoD	95% confidence range	
		lower limit	upper limit
whole blood EDTA	33	23	63

The results obtained confirmed the claimed concentration for the target of HSV2 ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius for whole blood collected in EDTA matrix.

The analytical sensitivity as copies/mL for whole blood collected in EDTA matrix is calculated by applying the specific conversion factor reported at paragraph 11.10 Conversion factor to International Units page 29

The analytical sensitivity as copies / mL is reported below.

Table 8 Limit of Detection with ELITe InGenius and ELITe BeGenius (copies / mL)

Matrix	LoD	95% confidence range	
		lower limit	upper limit
whole blood EDTA	165	115	315

The Limit of Detection (LoD) of the assay in association to plasma collected in EDTA and cerebrospinal fluid matrices was determined on the ELITE GALAXY and 7500 FAST DX instruments, and it was verified on **ELITe InGenius** and **ELITe BeGenius** instruments by testing 20 replicates of samples spiked with HSV2 reference material (1st WHO International Standard for HSV2 DNA, NIBSC ref.17/122, United Kingdom).

The results for plasma collected in EDTA and CSF matrices are reported in the following tables.

Table 9 Limit of Detection with ELITe InGenius and ELITe BeGenius (IU / mL)

Matrix	LoD
plasma EDTA	12
CSF	24

The results obtained confirmed the claimed concentration for the target of HSV2 ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius for plasma collected in EDTA and CSF.

The analytical sensitivity as copies/mL for plasma collected in EDTA matrix and CSF is calculated by applying the specific conversion factor reported at paragraph 11.10 Conversion factor to International Units page 29

The analytical sensitivity as copies / mL is reported below.

Table 10 Limit of Detection with ELITe InGenius and ELITe BeGenius (copies / mL)

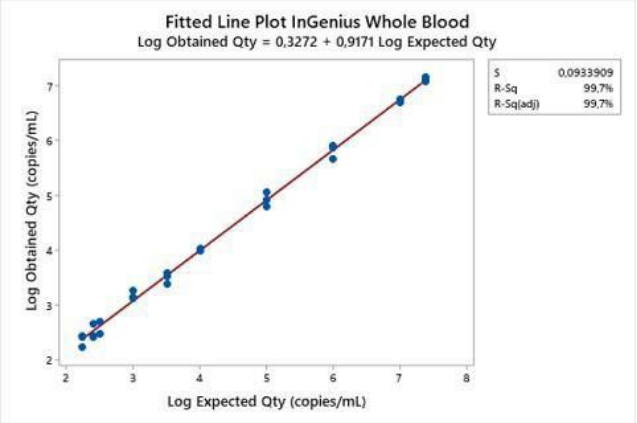
Matrix	LoD
plasma EDTA	119
CSF	119

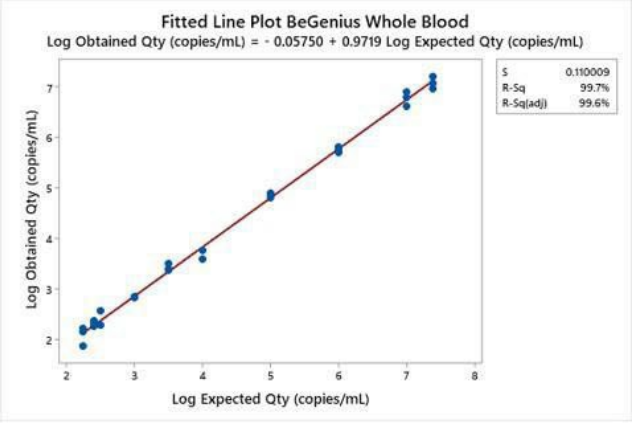
11.2 Linear measuring range and Limits of quantification

The linear measuring range of the assay was determined in association with whole blood collected in EDTA, plasma collected in EDTA and cerebrospinal fluid samples on **ELITe InGenius** and **ELITe BeGenius** using a panel of HSV2 reference material (Herpes Simplex Virus Type 2 Culture Fluid Heat Inactivated, ZeptoMetrix in association with whole blood EDTA and CSF; 1st WHO International Standard for HSV2 DNA, NIBSC ref.17/122, United Kingdom, in association with plasma EDTA) in HSV2 DNA - negative matrices.

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

Whole Blood:





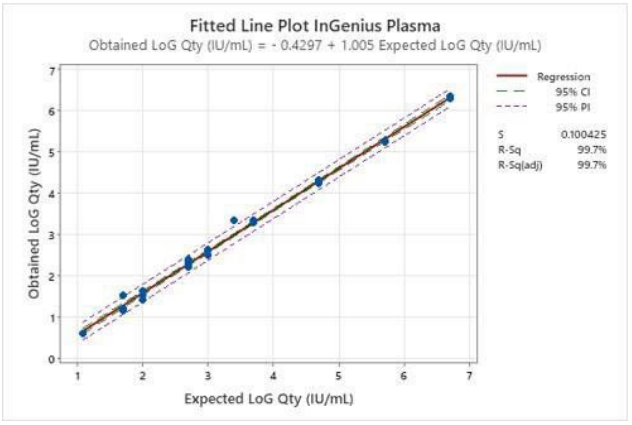
The linear measuring range as copies / mL for whole blood EDTA is calculated by applying the specific conversion factor reported at paragraph 11.10 Conversion factor to International Units page 29

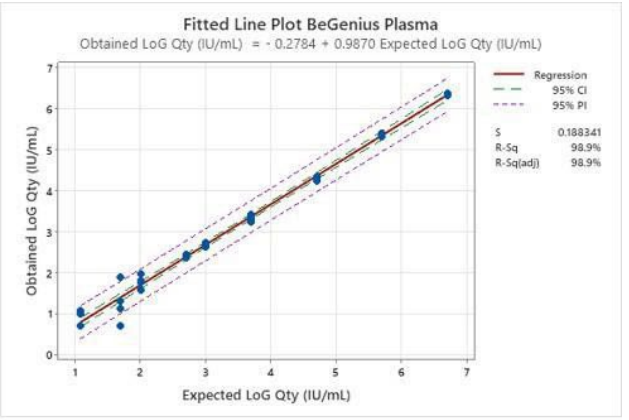
The final results are summarized in the following table.

Table 11 Linear measuring range for whole blood samples and ELITe InGenius and ELITe BeGenius

Unit	Lower limit	Upper limit
IU / mL	33	5,000,000
copies / mL	165	25,000,000

Plasma:





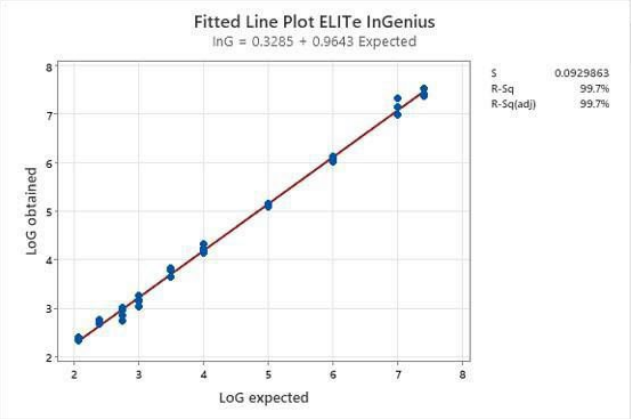
The linear measuring range as copies / mL for plasma collected in EDTA is calculated by applying the specific conversion factor reported at paragraph 11.10 Conversion factor to International Units page 29

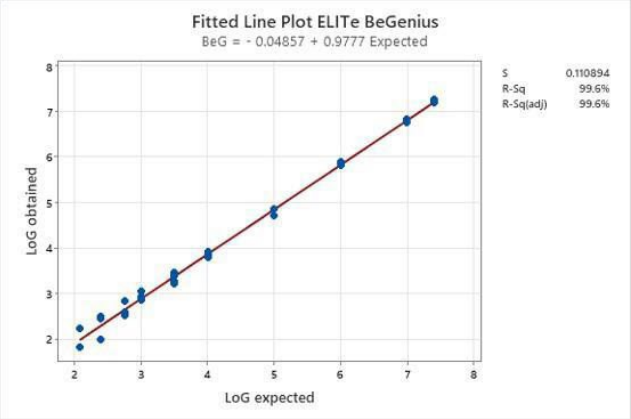
The final results are summarized in the following table.

Table 12 Linear measuring range for plasma samples and ELITe InGenius and ELITe BeGenius

Unit	Lower limit	Upper limit
IU / mL	12	2,500,000
copies / mL	119	25,000,000

CSF:





The linear measuring range as copies / mL for CSF is calculated by applying the specific conversion factor reported at paragraph 11.10 Conversion factor to International Units page 29

The final results are summarized in the following table.

Table 13 Linear measuring range for CSF samples and ELITe InGenius and ELITe BeGenius

Unit	Lower limit	Upper limit
IU / mL	24	5,000,000
copies / mL	119	25,000,000

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 0.3977 Log copies / reaction.

Table 14

Standard curve levels	Obtained	SD	Expanded Combined Uncertainty
	Log c/rxn		
HSV2 Q - PCR Standard 10^5	5.0262	0.0882	0.3977
HSV2 Q - PCR Standard 10^4	3.9339	0.1056	
HSV2 Q - PCR Standard 10^3	2.9061	0.1192	
HSV2 Q - PCR Standard 10^2	1.8536	0.0801	

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

The inclusivity of the assay, as efficiency of detection for different genotypes of HSV2 was evaluated by *in silico* analysis of the sequences available in nucleotide databases. The analysis showed sequence conservation and absence of significant mutations. So, 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, protozoa and fungi). Therefore, no cross-reactivity should be 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, protozoa and fungi). Therefore, no inhibition should be 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 HSV2 positive samples.

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

Table 15 Whole Blood

Samples	HSV2 Pos. / Rep	Outcome
Azithromycin	5/5	No interference
Ganciclovir	5/5	No interference
Ethanol	5/5	No interference
Ampicillin	5/5	No interference
Fluconazole	5/5	No interference
Cyclosporine A	5/5	No interference
Acyclovir	5/5	No interference

Table 15 Whole Blood (continued)

Samples	HSV2 Pos. / Rep	Outcome
Vancomycin	5/5	No interference
Heparin	5/5	No interference
EDTA	5/5	No interference

The tested substances do not interfere with the HSV2 or Internal Control amplification using the HSV2 ELITe MGB Kit on whole blood EDTA samples.

Table 16 Plasma

Samples	HSV2 Pos. / Rep	Outcome
Panel 1 EDTA Plasma	5/5	No interference
Panel 2 Haemolytic Bloow Low	5/5	No interference
Panel 3 Haemolytic Bloow Mid	5/5	No interference
Panel 4 Haemolytic Bloow High	5/5	No interference
Panel 5 Heparinized Plasma	5/5	Interference (Internal Control)
Panel 6 Lipemic Plasma	5/5	No interference
Panel 7 Icteric Plasma	5/5	No interference

The test showed that all the substances, with the exception for heparin, do not interfere with the HSV2 target or Internal Control detection and quantification using the HSV2 ELITe MGB Kit on plasma samples.

Table 17 CSF

Samples	HSV2 Pos. / Rep	Outcome
Azithromycin	5/5	No interference
Ganciclovir	5/5	No interference
Ethanol	5/5	No interference
Ampicillin	5/5	No interference
Fluconazole	5/5	No interference
Cyclosporine A	5/5	No interference
Acyclovir	5/5	No interference
Vancomycin	5/5	No interference
Human Whole Blood	5/5	No interference

The tested substances do not interfere with the HSV2 or Internal Control amplification using the HSV2 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 by analysis of a panel of whole blood samples collected in EDTA, including one negative sample and two samples spiked by HSV2 certified reference material (Herpes Simplex Virus Type 2 Culture Fluid Heat Inactivated, ZeptoMetrix).

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	HSV2				
	N	Mean Ct	SD Ct	% CV Ct	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	35.65	0.87	2.45	100%
10 x LoD	8	33.96	0.20	0.58	100%

Table 19 Intra – Session Repeatability on ELITe BeGenius

Sample	HSV2				
	N	Mean Ct	SD Ct	% CV Ct	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	37.60	0.69	1.84	100%
10 x LoD	8	35.57	1.07	3.01	100%

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	HSV2 ELITe MGB Kit - Days 1-2				
	N	Mean Ct	SD Ct	% CV Ct	% Agreement
Negative	16	-	-	-	100%
3 x LoD	16	35.87	0.71	1.99	100%
10 x LoD	16	33.92	0.22	0.66	100%

Table 21 Inter – Session Repeatability on ELITe BeGenius

Sample	HSV2 ELITe MGB Kit - Days 1-2				
	N	Mean Ct	SD Ct	% CV Ct	% Agreement
Negative	16	-	-	-	100%
3 x LoD	16	37.59	0.66	1.75	100%
10 x LoD	16	35.41	0.84	2.37	100%

In the Repeatability test, the HSV2 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 whole blood samples collected in EDTA negative or spiked with HSV2 reference material (Herpes Simplex Virus Type 2 Culture Fluid Heat Inactivated, ZeptoMetrix).

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

Table 22 Inter–Instrument Reproducibility on ELITe InGenius

Sample	HSV2				
	N	Mean Ct	SD Ct	% CV Ct	%Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.33	0.55	1.51	100%
10 x LoD	8	34.46	0.29	0.86	100%

Table 23 Inter–Instrument Reproducibility on ELITe BeGenius

Sample	HSV2				
	N	Mean Ct	SD Ct	% CV Ct	%Agreement
Negative	8	-	-	-	100%
3 x LoD	8	37.24	0.51	1.37	100%
10 x LoD	8	35.01	0.64	1.83	100%

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

Table 24 Inter–Batch Reproducibility on ELITe InGenius

Sample	HSV2				
	N	Mean Ct	SD Ct	% CV Ct	%Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.45	0.46	1.26	100%
10 x LoD	8	34.66	0.26	0.76	100%

Table 25 Inter–Batch Reproducibility on ELITe BeGenius

Sample	HSV2				
	N	Mean Ct	SD Ct	% CV Ct	%Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.90	0.58	1.59	100%
10 x LoD	8	35.17	0.49	1.39	100%

In the Reproducibility test, the HSV2 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 the certified calibrated reference material (1st WHO International Standard for HSV2 DNA, NIBSC ref.17/122, United Kingdom).

The results for each matrix are summarized in the following table

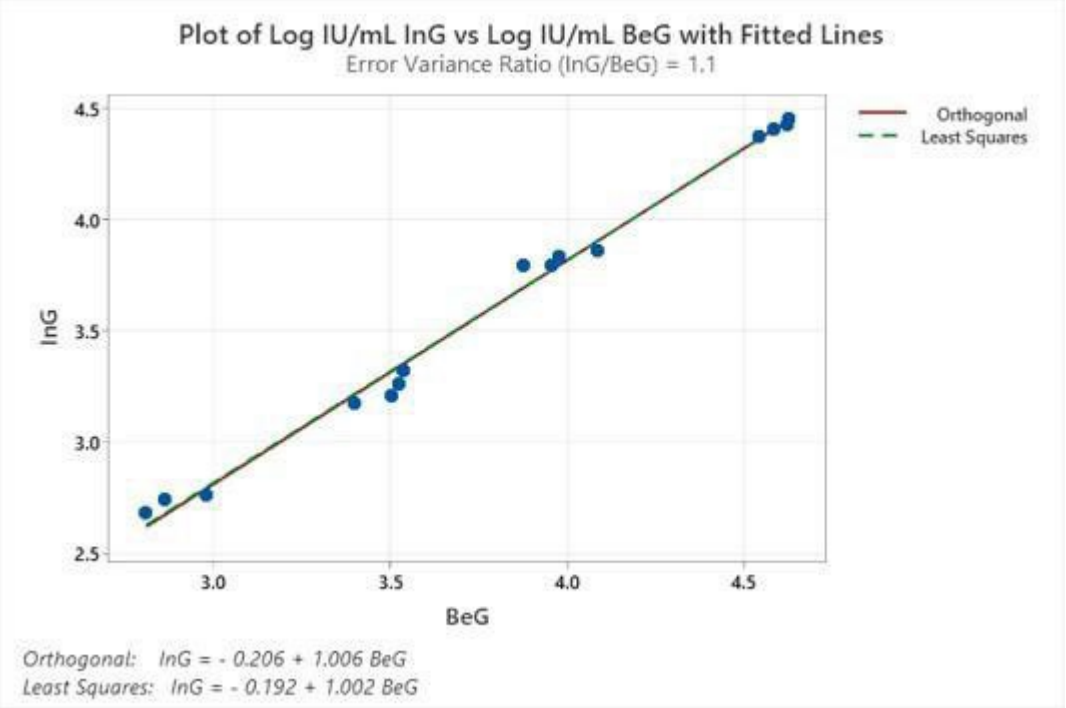
Table 26 Conversion factor to International Units with ELITe InGenius

Matrix	Fc (IU / copies)
whole bood EDTA	0.2
plasma EDTA	0.1
CSF	0.2

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

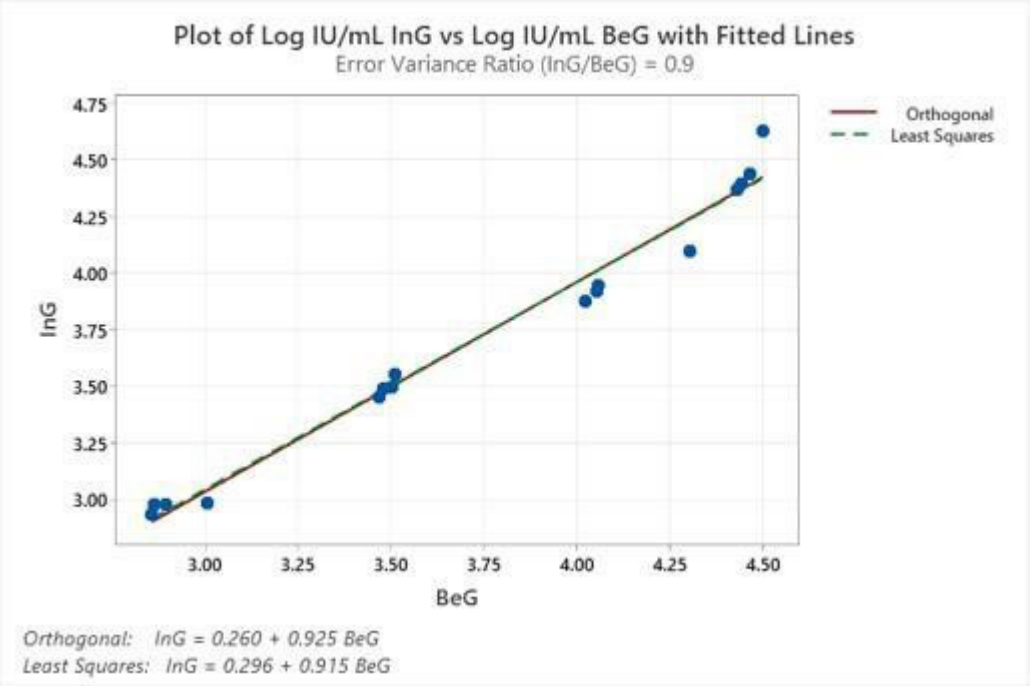
The results, for each matrix, are reported in the following paragraphs.

Whole Blood EDTA



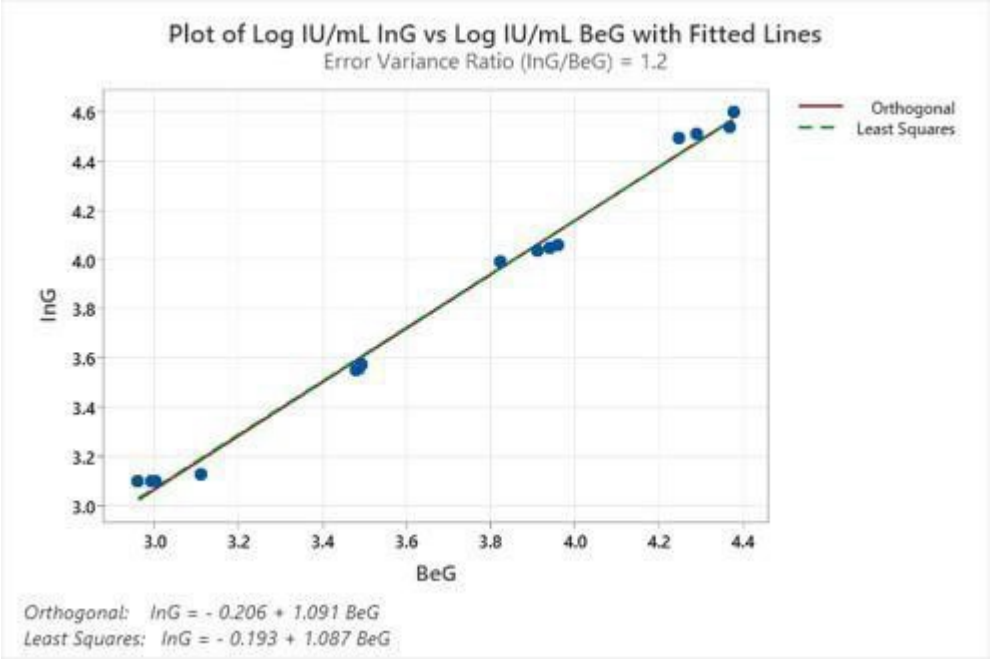
The Orthogonal Regression analysis generated an intercept equal to -0.206 (95% CI: -0.3970, 0.0128) and a slope equal to 1.006 (95% CI: 0.9487, 1.0555).

Plasma



The Orthogonal Regression analysis generated an intercept equal to 0.260 (95% CI: 0.006, 0.586) and a slope equal to 0.925 (95% CI: 0.8388, 0.9920).

CSF



The Orthogonal Regression analysis generated an intercept equal to -0.206 (95% CI:-0.3918, 0.0056) and a slope equal to 1.091 (95% CI: 1.0341, 1.1407).

11.11 Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative samples, was evaluated in association with **ELITe InGenius** analysing clinical samples of whole blood collected in EDTA, plasma collected in EDTA and CSF, certified negative or presumably negative for the target. As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Table 27 Diagnostic Specificity

Samples	N	Positive	Negative	% Diagnostic Specificity
Whole blood collected in EDTA and negative for HSV2 DNA	125	0	125	100
Plasma collected in EDTA and negative for HSV2 DNA	92	0	92	100
Cerebrospinal fluid negative for HSV2 DNA	67	0	67	100

The IC Ct cut-off value is set at 35for whole blood samples collected in EDTA, plasma collected in EDTA and CSF samples when tested with ELITe InGenius and ELITe BeGenius.

11.12 Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with **ELITe InGenius** analysing clinical samples of whole blood collected in EDTA, plasma collected in EDTA and CSF, certified positive for the target or spiked with reference material. As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Table 28 Diagnostic Sensitivity

Samples	N	Positive	Negative	% Diagnostic Sensitivity
Whole blood collected in EDTA and spiked for HSV2 DNA	53	53	0	100
Plasma collected in EDTA and spiked for HSV2 DNA	52	51		
Cerebrospinal fluid positive for HSV2 DNA	1	1	0	100
Cerebrospinal fluid spiked for HSV2	55	55	0	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 for the "HSV2 ELITe MGB® Kit", FTP032PLD.

12 SPECIMENS AND CONTROLS FOR ABI 7500 Fast Dx Real-Time PCR Instrument

12.1 Specimens

The following specimens and nucleic acid extraction methods are validated for use with the **HSV2 ELITe MGB Kit** using the ABI 7500 Fast Dx Real-Time PCR Instrument.

Table 29

Specimen type	Kit/Method	Protocol	Input volume (µL)	Elution volume (µL)	Primary tube minimum volume (µL)	Special instruction
Whole blood	ELITe GALAXY	xNA Extraction (Universal)	300	200	400-650	Add 10 µL/ sample of CPE to the IC + Carrier solution

12.2 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.

Do not use samples collected in heparin, which is a known reverse transcription and PCR inhibitor

12.3 Amplification controls

It is 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 kit) added to the reaction in place of the DNA extracted from the sample.

For the Positive Control, use the **HSV2 - ELITe Positive Control** product or the **HSV2 - ELITe Standard** product.

12.4 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.

13 ABI 7500 Fast Dx Real-Time PCR Instrument PROCEDURE

13.1 Setting of the real time amplification session

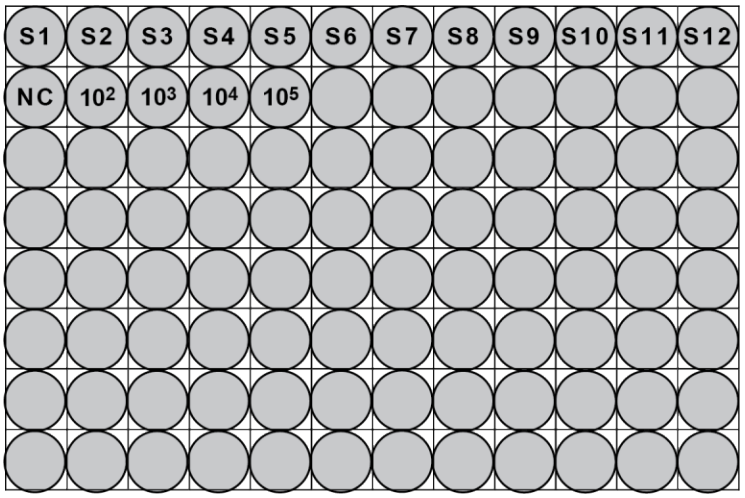
Before starting the session, refer to the instrument documentation to:

- switch on the instrument, switch on the computer, open the dedicated software and an "absolute quantification" session and set “Run mode: Fast 7500”;
- set (Detector Manager) the "detector" for the HSV2 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and label it “HSV2”;
- 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 label it “IC”;
- for each well in use in the microplate, set (Well Inspector) the "detector" (fluorescence to be measured), the "passive reference" = "Cy5" (AP593 is used instead of Cy5 for normalisation of fluorescence levels), and the type of reaction (sample, negative control, positive control or known quantity standard).

NOTE

To quantify the DNA in the starting sample, include a series of reactions using the **Q-PCR Standards** (10^5 copies / rxn, 10^4 copies / rxn, 10^3 copies / rxn, 10^2 copies / rxn) to obtain the **Standard curve**.

See below, by way of example, how to set up the quantitative analysis of 12 samples.



Legend: S1 -S12: Samples to be analysed; **NC**: Negative Control of amplification;

10 ²: 10^2 standard copies; **10³**: 10^3 standard copies; **10 ⁴**: 10^4 standard copies; **10 ⁵**: 10^5 standard copies.

Refer to the instrument documentation to set the **thermal cycling** parameters (Instrument > Thermal Cycler Protocol > Thermal Profile):

- add a **20 second extension at 72 °C** (Add Step);

NOTE

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

- modify thermal cycling temperatures and times as indicated in the table **"Thermal cycle"**;
- set the number cycles at **45**
- set the sample volume at **30 µL**
- optional: add a dissociation stage (Add Dissociation Stage), set the starting temperature at **40 ° C** and the ending temperature at **80 °C**.

Table 30 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.

Table 30 Thermal cycle (continued)

Stage	Temperatures	Timing
Dissociation (optional)	95 °C	15 sec.
	40 °C	1 min.
	80 °C	15 sec.
	60 °C	15 sec.

13.2 Real-time PCR session set-up

(Performed by the **ELITe GALAXY** instrument)

To perform the PCR session set up:

- thaw the **Q-PCR Mix** tubes required for the session (each tube is sufficient for **25 reactions**)
- thaw the **Positive Control** (qualitative analysis: detection of extracted DNA) or the **Q - PCR Standard** (quantitative analysis: quantification of extracted DNA) tubes
- mix gently the reagents and spin down the contents for 5 seconds
- prepare the **Negative Control** (not provided) as per the instruction of use of the instrument
- prepare a **Q-PCR microplate**. Handle it with powderless gloves and do not damage the wells

NOTE

To prepare the PCR on the **ELITe GALAXY**, load the elution microplate containing the extracted DNA samples, the reagents and the **Q-PCR microplate** as indicated in the instrument user manual and follow the steps on the GUI.

The instrument automatically performs the PCR set-up dispensing in each well of the **Q-PCR microplate**:

- **20 µL** of **Q-PCR Mix**
- **20 µL** of **extracted DNA** / **Q-PCR Standard** / **Controls**

NOTE

If not all the Q—PCR Mix is used, store the remaining volume in the dark at -20°C for no longer than one month. Freeze and thaw the Q—PCR Mix for a maximum of **5 TIMES**.

After the PCR set-up performed by the instrument:

- seal the **Q-PCR microplate** with an optical seal
- transfer the **Q-PCR microplate** onto the **7500 Fast Dx Real-Time PCR Instrument** and start the PCR. Save the run file with a unique and recognizable name (e.g. "year-month-day-TARGET-EGSpA")

NOTE

At the end of the PCR the **Q-PCR microplate** must be discarded following all governmental and environmental regulations. In order to avoid spilling the PCR products, the **optical seal must not be removed from the Q-PCR microplate**.

13.3 General settings for analysis of results

Before starting the analysis, refer to the instrument documentation to:

- manually adjust the calculation range for the **Baseline** (fluorescence background level) from cycle 6 to cycle 15 (Results > Amplification plot > delta Rn vs Cycle);

NOTE

The FAM fluorescence of the HSV2 probe in a sample with a high concentration of HSV2 DNA may begin to increase before cycle 15. In this case, lower the **Baseline** calculation range to the cycle at which the FAM fluorescence of the sample begins to increase (Results > Component).

- manually set the thresholds for the detectors:

set the FAM detector “HSV2” **threshold** at **0.2**;

set the VIC detector "IC" **threshold** at **0.1**.

The PCR cycle at which a sample’s fluorescence level reaches the **threshold** value determines the **threshold cycle (Ct)** for that sample.

The instrument software automatically analyses the fluorescence levels in the controls, standards and sample reactions and calculates Ct values.

13.4 Qualitative analysis of results

The HSV2 **Ct** value of the **Positive Control** is used to validate the PCR. The PCR run is valid when results are as described in the following table:

Table 31

Positive Control reaction Detector FAM " HSV2 "	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive Control** is **Ct > 25** or **Ct Undetermined** for Detector FAM “ HSV2”**,** the session is not valid and must be repeated starting from the PCR step. This may indicate an issue during the PCR setup, the PCR or the detection step (e. g., incorrect dispensation or degradation of the Q-PCR Mix or positive control, incorrect placement of the positive control, incorrect thermal cycle settings), which may lead to incorrect results.

NOTE

When the product is used for the quantification of HSV2 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**).

The HSV2 Ct value of the **Negative Control** is used to validate the PCR. The PCR run is valid when results are as described in the following table:

Table 32

Negative control reaction Detector FAM " HSV2 "	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative control** amplification reaction is different from **Ct Undetermined** for Detector FAM “ HSV2”, the session is not valid and must be repeated starting from the PCR step. This may indicate issues occurred during the amplification step (contamination) which may lead to incorrect results and false positive results.

The **Ct** value of HSV2 in each sample is used to detect the target DNA while the **Ct** value of the internal control is used to validate the extraction, PCR, and detection.

NOTE

Verify by the amplification plot (Results > Amplification plot > delta Rn vs Cycle) that the **Ct** of each sample was determined by a fast and regular increase in fluorescence and not by peaks or an increase in background signal (irregular or high background).

Possible sample results (Results > Report) are described in the following table:

Table 33

Sample reaction		Sample suitability	Assay sample result	HSV2 DNA
Detector FAM " HSV2"	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

A sample result of **Ct Undetermined** for HSV2 and **Ct > 35** or **Ct Undetermined** for the internal control is invalid and indicates an issue during nucleic acid extraction or PCR (e. g., degradation of sample DNA, loss of DNA during extraction, presence of inhibitors in the DNA, inefficient or absent amplification), which may lead to incorrect results. The sample is not suitable for the analysis and the assay needs to be repeated starting from nucleic acid extraction of a new sample.

A sample result of **Ct Undetermined** for HSV2 and **Ct ≤ 35** for the internal control is a valid result and indicates that HSV2 DNA was not detected in the sample. The sample may contain no HSV2 DNA or it contains HSV2 DNA at a concentration lower than the detection limit of the product (see 14 Performance Characteristics page 37). A sample result of **Ct Determined (Ct ≤ 45)** for HSV2 and **Ct** > **35**, **Ct Undetermined**, or **Ct ≤ 35** for the IC is a valid result and indicates that HSV2 DNA was detected in the sample

NOTE

In case of Ct Determined for HSV2 and Ct > 35 or Undetermined for the IC, the PCR efficiency of the IC may have been impacted by competition with the high PCR efficiency of the HSV2 DNA. In this case the sample is suitable, and the positive result is valid.

NOTE

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

13.5 Quantitative analysis of the results

In the amplification reactions of the four **Q - PCR standards**, the HSV2 **Ct** values 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:

Table 34

Standard Curve Detector FAM " HSV2 "	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, the session is not valid and must be repeated starting from the PCR step. This may indicate an issue during the PCR or the detection step (e. g., incorrect dispensation or degradation of the Q-PCR Mix or of the standards, incorrect placement of the standards, incorrect thermal cycle settings or cross-contamination), which may lead to incorrect results.

Table 35

Sample result for detector FAM "HSV2"	HSV2 copies per reaction
Quantity > 1 x 10 ^{6}	**MORE THAN 1 x 10 ^{6}**
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 copies of HSV2 present in the specimen used in the extraction (**Nc**) according to this formula:

Table 36

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

where:

Ve is the total volume **in µL** of the extracted DNA sample (elution volume)

Quantity is the **copies/reaction** of the sample calculated by the instrument software (PCR result)

Vc is the volume of the specimen used for nucleic acid extraction (input volume) expressed in the required unit of measurement

Va is the volume in **µL** of the extracted DNA sample (eluate) used in the PCR

Ep is the efficiency of the procedure (extraction and PCR) **expressed as a decimal**

To convert the sample quantity from copies/mL to IU/mL, multiply the copies/mL value by the **conversion factor (Fc)**. The Fc was calculated using calibrated certified reference material (1st WHO International Standard for HSV2 DNA, NIBSC ref.17/122, United Kingdom) (See 14 Performance Characteristics page 37).

For convenience, the following are simplified formulas in which Ve/(Vc x Va x Ep) and its conversion to IU/mL have been calculated.

Table 37

Matrix	Nucleic acid extraction method	Ve/ (Vc x Va x Ep)	Formula to quantify Nc (copies/mL)	Fc (IU/copy)	Formula to quantify Nc (IU/mL)
Whole blood	ELITe GALAXY	35	35 x Quantity	0.2	7 x Quantity

14 PERFORMANCE CHARACTERISTICS WITH ABI 7500 Fast Dx Real-Time PCR Instrument

14.1 Limit of detection (LoD)

The Limit of Detection (LoD) of the assay in association to whole blood collected in EDTA was verified on the ELITe GALAXY and ABI 7500 Fast Dx Real-Time PCR instruments, by testing a panel of HSV2 negative matrix spiked with certified reference material (QCMD 2008 Herpes Simplex Virus EQA Panel's sample HSV08-12, Qnostics, Ltd). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

Table 38 Limit of Detection with ELITe GALAXY (IU / mL)

Matrix	95% positivity	95% confidence range	
		lower limit	upper limit
whole blood	34	22	101

The LoD as copies / mL for each matrix is calculated by applying the specific conversion factor reported at paragraph 14.6 Conversion to International Units page 39.

The analytical sensitivity as copies / mL is reported below.

Table 39 Limit of Detection with ELITe GALAXY (copies / mL)

Matrix	95% positivity	95% confidence range	
		lower limit	upper limit
whole blood	171	112	505

14.2 Linear measuring range

The linear measuring range of the assay was determined in association with whole blood collected in EDTA on **ELITe GALAXY** and **ABI 7500 Fast Dx Real-Time PCR** instruments using a panel of dilution of a plasmid DNA containing the amplification product.

The linear measuring range as copies / mL is calculated by applying the specific conversion factor reported at paragraph 14.6 Conversion to International Units page 39

The final results are summarized in the following table.

Table 40 Linear measuring range for whole blood samples

Unit of Measure	lower limit	upper limit
IU / mL	2	200,000
copies / reaction	10	1,000,000

14.3 Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

The inclusivity of the assay, as efficiency of detection for different genotypes of *Herpes Simplex Virus Type 2* was evaluated by *in silico* analysis of the sequences available in nucleotide databases. The analysis showed sequence conservation and absence of significant mutations. So, efficient detection for the different strains or isolates is expected.

14.4 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, protozoa and fungi). Therefore, no cross-reactivity should be expected.

14.5 Reproducibility

The Reproducibility of the assay was evaluated on ABI 7500 Fast Dx Real-Time PCR instrument by analysis of a panel of samples spiked by a plasmid DNA containing the amplification product HSV2 and Internal Control at different concentrations.

A summary of Inter-batch Reproducibility (on ten lots of HSV2 ELITe MGB Kit) is shown in the table below:

Table 41 Inter-batch Reproducibility on ABI 7500 Fast Dx Real-Time PCR

Sample copies / reaction	N	Ct Mean HSV2	Ct Mean IC	SD HSV2	SD IC	%CV HSV2	%CV IC	Out-come
100,000 target	30	23.19	-	0.64	-	2.76	-	Passed
50,000 target + 150,000 IC	30	24.00	22.26	0.56	0.40	2.34	1.79	Passed
5,000 target + 150,000 IC	30	27.44	22.59	0.58	0.37	2.12	1.63	Passed
500 target + 150,000 IC	30	30.76	22.56	0.71	0.37	2.30	1.62	Passed
10 target + 150,000 IC	90	36.71	22.61	0.99	0.38	2.70	1.70	Passed
150,000 IC	30	-	22.49	-	0.40	-	1.77	Passed
6,000 IC	90	-	27.99	-	0.51	-	1.83	Passed

In the Reproducibility test, the HSV2 ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

14.6 Conversion to International Units

The conversion factor to report the quantitative results in International Units / mL starting from copies / mL, was calculated, for whole blood collected in EDTA, using the certified calibrated reference material (1st WHO International Standard for HSV2 DNA, NIBSC ref.17/122, United Kingdom).

The results are summarized in the following table.

Table 42 Conversion factor to International Units with ABI 7500 Fast Dx Real-Time PCRand Whole blood

Instrument	Fc (IU / copies)
ELITe GALAXY	0.2

14.7 Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated analyzing, in association with **ELITe GALAXY** and ABI 7500 Fast Dx Real-Time PCR Instrument, samples certified negative for the target.

The results are summed up in the following table.

Table 43 Diagnostic specificity

Samples	N	positive	negative	% Diagnostic Specificity
Whole blood collected in EDTA and negative for HSV2 DNA	57	0	57	100

14.8 Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated analyzing, in association with **ELITe GALAXY** and ABI 7500 Fast Dx Real-Time PCR Instruments, samples spiked with HSV2 reference material of the target.

The results are summed up in the following table

Table 44 Diagnostic sensitivity

Samples	N	positive	negative	% Diagnostic Sensitivity
Whole blood collected in EDTA and spiked for HSV2 DNA	54	54	0	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 "HSV2 ELITe MGB Kit", FTP032PLD.

15 REFERENCES

E. T. E. Fenner et al. (1991) J Clin Microbiology 29: 2621 - 2622

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

16 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: whole blood collected in EDTA (all the instruments), plasma collected in EDTA and CSF (ELITe InGenius and ELITe BeGenius).

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 inhibition caused by antiviral, antibiotic, chemotherapeutic, or immunosuppressant drugs.

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 (see 11 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 22). 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.

17 TROUBLESHOOTING

ELITe InGenius and ELITE BeGenius

Table 45

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix, Q-PCR Standards and Positive Control. Check the volumes of Q-PCR Mix, Q-PCR Standards and Positive Control.
PCR Mix degradation.	Do not use the Q-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 Q-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 Q-PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of Q-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 46

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix and Negative Control. Check the volumes of Q-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 Q-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 47

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix, Internal Control, and sample. Check the volumes of Q-PCR Mix, Internal Control and sample.
PCR Mix degradation.	Do not use the Q-PCR Mix for more than 5 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the Q-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 Q-PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of Q-PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification of eluted sample with a 1:2 dilution in molecular biology grade water in a “PCR Only” session. Repeat the extraction of the sample with a 1:2 dilution in molecular biology grade water in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 48

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm 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 49

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 50

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 Q-PCR Mix and / or Internal Control

Open Platform

Table 51

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Check the volumes of PCR Mix, Q-PCR Standards and Positive Control dispensed in the Q-PCR microplate.
Q-PCR Mix degradation.	Do not freeze and thaw the PCR mix more than 5 times. Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of Q-PCR Mix.
Q-PCR Standards or Positive Control degradation.	Do not freeze and thaw the Q-PCR standard more than 4 times. Use new aliquots of Q-PCR Standards or Positive Control.
Instrument setting error.	Check the position of PCR Mix, Q-PCR Standards and Positive Control on the instrument. Check the thermal cycle settings on the instrument.

Table 52

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix and Negative Control. Check the volumes of Q-PCR Mix and Negative Control.
Microplate badly sealed.	Take care when sealing the Q-PCR microplate with the optical seal.
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 Q-PCR Mix.
Contamination of the preparation area, racks and micropipette.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

Table 53

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix, Internal Control and sample. Check the volumes of Q-PCR Mix, Internal Control and sample.
PCR Mix degradation.	Do not freeze and thaw the PCR mix more than five times. Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of Q-PCR Mix
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification of eluted sample with a 1:2 dilution in molecular biology grade water. Repeat the extraction of the sample with a 1:2 dilution in molecular biology grade water.

Table 54

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Check the volumes of reagents and samples dispensed in the Q-PCR microplate.
Baseline setting error.	If the calculation range for the Baseline set from cycle 6 to cycle 15 is not proper to normalize the background, set the calculation range within cycles where the background fluorescence has already stabilized (check Results > Component) and the target fluorescence has not yet started to increase.

Table 55

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but 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.

18 SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch code.


Use by (last day of month).

IVD *in vitro* diagnostic medical device.

CE 0123 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.

UDI Unique Device Identification


Contains sufficient for "N" tests.


Consult instructions for use.

CONT Contents.


Keep away from sunlight.


Manufacturer.

19 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.

20 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 HSV2 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 **HSV2 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 Herpes Simplex virus type 2 (HSV2)** 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 whole blood collected in EDTA, plasma collected in EDTA and cerebrospinal fluid (CSF).

The assay is also validated in association with the **ELITe GALAXY**, automatic extraction and PCR set-up system and **7500 Fast Dx Real-Time PCR Instrument**, Real-Time PCR platform, using human specimens of whole blood collected in EDTA.

The product is intended for use as an aid in the diagnosis and monitoring of HSV2 infections in patients suspected of having or undergoing monitoring of HSV2 infections.

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

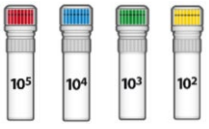
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	Glicoprotein G (gpG)	FAM	HSV2
Internal Control	Human beta globin gene	AP525	IC

Validated matrix

- Whole blood collected in EDTA
- Plasma collected in EDTA
- CSF

Kit content and related products

HSV2 ELITe MGB Kit	HSV2 ELITe Standard	HSV2 - ELITe Positive Control
 X 4	 X 2	 X 2
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, 10^4, 10^3, 10^2 2 sets of 4 tubes of 200 µL 4 freeze-thaw cycles	Ready-to-use PC 2 tubes of 160 µL 8 reactions per kit 4 freeze-thaw cycles

Maximum shelf-life: **24 months**

Storage Temperature: **-20 °C**

Other products required not provided in the kit

• ELITe InGenius instrument: INT030. • ELITe BeGenius instrument: INT040. • ELITe InGenius SP 200: INT032SP200. • ELITe InGenius SP1000: INT033SP1000 • ELITe InGenius SP 200 Consumable Set: INT032CS.	• 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.

ELITe InGenius and ELITe BeGenius protocol

› Sample volume › CPE volume › Total elution volume	200 µL(InGenius and BeGenius) or 1000 µL (InGenius only) 10 µL 100 µL	› Eluate PCR input volume › Q—PCR Mix volume › Frequency of controls	20 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection		Diagnostic Sensitivity	Diagnostic Specificity
	IU/mL	copies/mL		
whole bood	33	165	100	100
Plasma	12	119	98	100
CSF	24	119	100	100

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.

Sample type	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 ° C	-70 ± 15 ° C
Whole blood	EDTA	≤ 1 d	≤ 3d	≤ 30 d	≤ 30 d
Plasma	EDTA	≤ 1 d	≤ 3d	≤ 30 d	≤ 30 d
CSF	-	≤ 4 hours	≤ 4 hours	≤ 30 d	≤ 30 d

C EDTA, Ethylenediaminetetraacetic acid; d, day.

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.

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: HSV2 ELITe_WB_200_100 or HSV2 ELITe_PL_200_100 or HSV2 ELITe_CSF_200_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: HSV2 ELITe_PC and HSV2 ELITe_NC, or HSV2 ELITe_STD or HSV2 ELITe_WB_200_100 or HSV2 ELITe_PL_200_100 or HSV2 ELITe_CSF_200_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 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.

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 HSV2 ELITe_Be_WB_200_100 or HSV2 ELITe_Be_PL_200_100 or HSV2 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, 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: HSV2 ELITe_PC and HSV2 ELITe_NC, or HSV2 ELITe_STD or HSV2 ELITe_Be_WB_200_100 or HSV2 ELITe_Be_PL_200_100 or HSV2 ELITe_Be_CSF_200_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 the Elution tube rack with the extracted nucleic acid	**8.** Close the door. Start the run	**9.** View, approve and store the results

Appendix B HSV2 ELITe MGB Kit used in association with ABI 7500 Fast Dx Real-Time PCR Instrument

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 **HSV2 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 Herpes Simplex virus type 2 (HSV2)** 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 whole blood collected in EDTA, plasma collected in EDTA and cerebrospinal fluid (CSF).

The assay is also validated in association with the **ELITe GALAXY**, automatic extraction and PCR set-up system and **7500 Fast Dx Real-Time PCR Instrument**, Real-Time PCR platform, using human specimens of whole blood collected in EDTA.

The product is intended for use as an aid in the diagnosis and monitoring of HSV2 infections in patients suspected of having or undergoing monitoring of HSV2 infections.

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

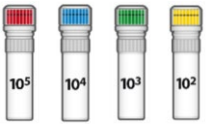
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	Glicoprotein G (gpG)	FAM	HSV2
Internal Control	Human beta globin gene	AP525	IC

Validated matrix

- Whole blood collected in EDTA

Kit content and related products

HSV2 ELITe MGB Kit	HSV2 ELITe Standard	HSV2 - ELITe Positive Control
 X 4	 X 2	 X 2
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, 10^4, 10^3, 10^2 2 sets of 4 tubes of 200 µL 8 freeze-thaw cycles	Ready-to-use PC 2 tubes of 160 µL 8 reactions per kit 8 freeze-thaw cycles

Maximum shelf-life: **24 months**

Storage Temperature: **-20 °C**

Other products required not provided in the kit

• **ELITe GALAXY:** INT020 • **ELITe GALAXY 300 extraction kit:** INT021EX • **ABI 7500 Fast Dx Real—Time PCR Instrument**	• CPE - Internal Control: CTRCPE • Molecular biology grade water

7500 Real-Time PCR Instrument Performances

Matrix	Limit of Detection	Diagnostic Specificity	Diagnostic Sensitivity	Linearity (IU/mL)	Formula to quantity (copies / mL)	Conversion factor copies/mL to IU/ mL
whole blood	171	100	100	2.0 - 2.0 x 105	35 x Quantity	0.2

7500 Real-Time PCR Instrument Procedures

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 Galaxy	WB	300 µL	400 µL	200 µL	10 µL

Amplification - Settings of 7500 Fast Dx

1. Switch on the thermal-cycler
2. Set “HSV2” detector with “FAM” and quencher “none”
3. Set “Internal Control” detector with “VIC” and quencher “none”
4. Set passive fluorescence as “Cy5”
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
Initial Denaturation	94°C	2 min
Amplification 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 (performed by ELITe GALAXY)

To perform the PCR session set up:

1. thaw the Q PCR-Mix and Positive Control / Q-PCR standard tubes
2. mix gently and spin-down
3. prepare the **Negative Control** (not provided)

4. prepare a **Q-PCR microplate**
5. the instrument automatically performs the PCR set-up dispensing in each well of the **Q-PCR microplate 20 µL** of **PCR Mix** and **20 µL** of **extracted DNA** / **Q-PCR Standard** / **Controls**.

After the PCR set-up performed by the instrument:

1. seal the **Q-PCR microplate** with an optical seal
2. transfer the **Q-PCR microplate** onto the **7500 Fast Dx Real-Time PCR Instrument** and start the PCR. Save the run file with a unique and recognizable name (e.g. "year-month-day-TARGET-EGSpA").

Amplification - Threshold for qualitative analysis

Instrument	HSV2 FAM	Internal Control VIC
7500 Fast Dx Real Time PCR	0.2	0.1

Interpretation

Qualitative results		
HSV2 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*

Quantitative results
The HSV2 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.

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