ELITechGroup
EMPOWERING IVD

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NOTICE of CHANGE dated 29/09/2022

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HSV1 ELITe MGB® Kit»
Ref. RTS031PLD

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

- *Update for the use of the product for CSF matrix in association with «ELITe BeGenius®» instrument (REF INT040).*
- *Confirmed LoD and ULoQ/LLoQ value calculated on CSF matrix*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

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

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HSV1 ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS031PLD CE IVD -20°C

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INTENDED USE

The **«HSV1 ELITe MGB® Kit»** product is a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of type 1 Herpes Simplex human virus (HSV1)** in DNA samples extracted from cerebrospinal fluid (CSF), whole blood collected in EDTA, plasma collected in EDTA.

The product is intended for use in the diagnosis and monitoring of HSV1 infections, alongside clinical data of the patient and other laboratory tests outcomes.

ASSAY PRINCIPLES

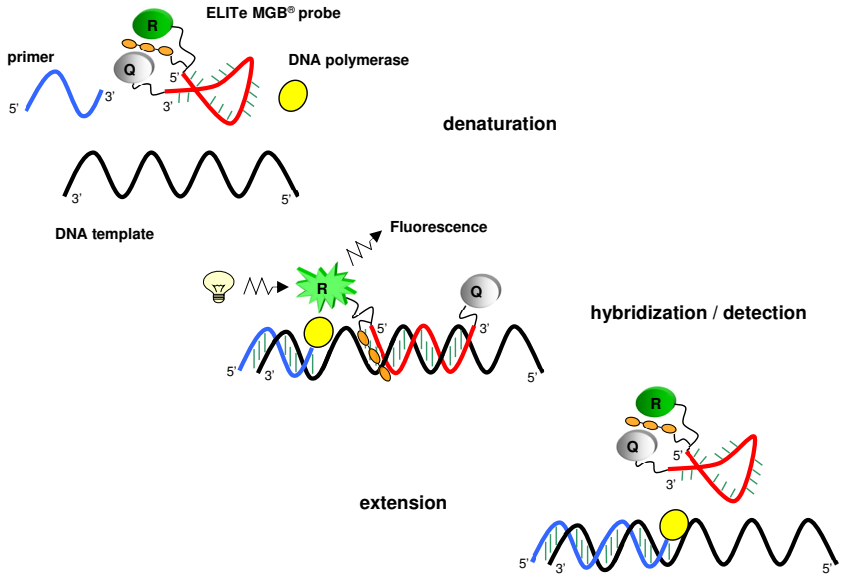
The assay consists of a real time amplification reaction with a programmable thermostat provided with a fluorescence detection optical system (real time amplification thermal cycler).

In each well, two amplification reactions are performed starting from DNA extracted from the samples being tested: a specific reaction for a region of the **glycoprotein D (gpD)** of HSV1 and a specific reaction for a region of the **human beta Globin gene** (Internal Control of inhibition). The HSV1 specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the HSV1 amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with AP525 fluorophore (analogous to VIC), is activated when hybridizes with the specific product of the amplification reaction for the Internal Control. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data allows detecting the presence and the titre of HSV1 DNA in the starting sample.

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

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

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



PRODUCT DESCRIPTION

The **«HSV1 ELITe MGB® Kit»** product supplies the **ready to use** complete mixture HSV1 Q - PCR Mix for real time amplification in a stabilising solution, **aliquoted into four disposable test tubes**. Each tube contains **540 µL** of solution, sufficient for **24 tests** (by processing at least 2 samples per session) in association with **«ELITe InGenius®»** and **«ELITe BeGenius®»** and **25 tests** in association with other systems.

The primers and the HSV1 specific probe (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non fluorescent molecule) are specific for a region of the **gpD** of HSV1 (region US6).

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

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

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

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

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
HSV1 Q - PCR Mix	Complete reaction mixture	**4 x 540 µL**	**-**

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.
- Programmable thermostat with optical fluorescence detection system 7300 Real Time PCR System or 7500 Fast Dx Real-Time PCR Instrument calibrated following manufacturer's instructions.
- Programmable thermostat with optical fluorescence detection system cobas z 480 analyzer, calibrated following manufacturer's instructions.

OTHER PRODUCTS REQUIRED

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

For manual DNA extraction from samples to be analyzed, it is validated the use of generic products **«EXTRAgen»** (ELITechGroup S.p.A., ref. EXTG01), kit for the extraction of DNA from non-cellular samples and **«EXTRAblood»** (ELITechGroup S.p.A., ref. EXTB01), kit for the extraction of DNA from cellular and non-cellular samples.

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

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

- for the calibrators **«HSV1 ELITe_STD»**,
- for the positive control of amplification **«HSV1 ELITe_PC»**,
- for negative control of amplification **«HSV1 ELITe_NC»**,
- for samples analysis **«HSV1 ELITe_WB_200_100»**, **«HSV1 ELITe_PL_200_100»** and **«HSV1 ELITe_CSF_200_100»**.

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

For automatic DNA extraction, amplification and interpretation of sample analysis, the instrument **«ELITe BeGenius®»** (ELITechGroup S.p.A., ref. INT040) and the following specific Assay protocols (ELITechGroup S.p.A.), are required:

for the calibrators **«HSV1 ELITe_Be_STD»**,

for the positive control of amplification **«HSV1 ELITe_Be_PC»**,

for negative control of amplification **«HSV1 ELITe_Be_NC»**,

for samples analysis **«HSV1 ELITe_Be_WB_200_100»**, **«HSV1 ELITe_Be_PL_200_100»** and **«HSV1 ELITe_Be_CSF_200_100»**.

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

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

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

For automatic DNA extraction from samples to be analyzed, the products **«QIAsymphony® DNA Mini Kit»** (QIAGEN GmbH, ref. 931236) and **«QIAsymphony® DSP Virus / Pathogen Midi kit»** (QIAGEN GmbH, ref. 937055), kits for extraction of nucleic acid from biological samples, with the instrument **«QIAsymphony® SP/AS»** (QIAGEN GmbH, ref. 9001297, 9001301) and related generic products are also validated.

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

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

When 7300 Real-Time PCR System is used, it is required the use of generic product: **«MicroAmp™ Optical 96-Well Reaction Plate»** (Life Technologies, ref. N8010560), microplates with 0.2 mL wells and adhesive sealing sheets for real time amplification.

When 7500 Fast Dx Real-Time PCR Instrument is used, it is required the use of generic product: **«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.

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

If detection of HSV1 DNA is required for qualitative analysis, use the product **«HSV1 - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR031PLD) or the product **«HSV1 - ELITe Positive Control RF»** (ELITechGroup S.p.A., ref. CTR031PLD-R), positive control of plasmid DNA.

If detection and quantification of HSV1 DNA is required for quantitative analysis, use the product **«HSV1 ELITe Standard»** (ELITechGroup S.p.A., ref. STD031PLD), four dilutions of known quantity plasmid DNA to obtain the standard curve.

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

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

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

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

Warnings and precautions for molecular biology

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

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

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

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

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

Amplification products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination. The pipettes used to handle amplification products must be exclusively used for this purpose.

Warnings and precautions specific for the components

The **HSV1 Q - PCR Mix** must be stored at -20°C in the dark.

The **HSV1 Q - PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

The **HSV1 Q - PCR Mix** can be used for 5 independent work sessions of 3 hours each ("Extraction+PCR" run mode) or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each ("Extraction+PCR" run mode).

ELITe InGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Whole blood collected in EDTA

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

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

Note: when the DNA extraction from whole blood is carried out with the **ELITe InGenius®** and **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocol **HSV1 ELITe WB_200_100**. This protocol processes 200 µL of sample, adds the **CPE** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information.

Plasma collected in EDTA

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

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

Note: when the DNA extraction from plasma is carried out with the **ELITe InGenius®** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocols **HSV1 ELITe_PL_200_100.** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information.

Cerebrospinal Fluid (CSF)

The CSF samples for nucleic acid extraction must be collected according to laboratory guidelines avoiding contamination by patient blood, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of four hours, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods. It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when the DNA extraction from whole blood is carried out with the **ELITe InGenius®** and with **ELITe InGenius** Software version 1.3 (or later equivalent versions), use the extraction protocol **HSV1 ELITe_CSF_200_100**. This protocol processes 200 µL of sample, adds the CPE Internal Control at 10 µL / extraction and elute the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: suspensions of leucocytes, suspensions of granulocytes and amniotic fluid.

Interfering substances

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

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

Amplification calibrators and amplification controls

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

as calibrator set, use the four concentration levels of the **HSV1 ELITe Standard**, in association with the protocol **«HSV1 ELITe_STD»**,

as amplification Positive Control use the **HSV1 - ELITe Positive Control**, in association with the protocol **«HSV1 ELITe_PC»**,

as amplification Negative Control, use molecular grade water (not provided with this kit) in association with the protocol **«HSV1 ELITe_NC»**.

Note: ELITe InGenius® system requires approved and valid results of calibration curve and amplification controls for each lot of amplification reagent stored in its database.
The calibration curves, approved and stored in the database, will expire after **60 days**. At expiration date it is necessary to re-run the Q-PCR Standards in association with the amplification reagent lot.
The amplification control results, approved and stored in the database, will expire after **15 days**. At the expiration date it is necessary to re-run the Positive and Negative Controls in association with the amplification reagent lot.
Furthermore, the calibrators and amplification controls must be re-run when:

- a new lot of reagents is started,
- the results of Quality control analysis (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius®** instrument.

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used. External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable.

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PROCEDURE

The procedure to use the **«HSV1 - ELITe MGB® Kit»** with the system ELITe InGenius consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and export of results.

System readiness verification

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

- switch on the **ELITe InGenius®** and select the login mode "**CLOSED**";
- - verify that the Calibrators (**HSV1 Q - PCR Standard**) have been run, approved and not expired (Status) in association with the amplification reagent lot to be used. This can be checked under the "Calibration" menu in the Home page. If there are not Calibrators approved or valid, run them as described in the following paragraphs,
- - verify that the amplification Controls (**HSV1 - Positive Control**, **HSV1 Negative Control**) have been run, approved and not expired (Status) in association with the amplification reagent lot to be used. This can be checked under the "Control" menu in the Home page. If there are not amplification Controls approved or valid, run them as described in the following paragraphs,
- choose the type of run and set up the run, following the instructions Graphical User Interface (GUI) for the session set up and using the Assay Protocols provided by ELITechGroup. These IVD protocols were specifically validated with ELITe MGB Kits, the ELITe InGenius instrument and the cited matrices.

The Assay protocols available for **«HSV1 ELITe MGB® Kit»** are described in the table below.

Assay protocols for HSV1 ELITe MGB Kit and ELITe InGenius®

Name	Matrix	Report unitage	Characteristics
HSV1 ELITe_WB_200_100	Whole Blood	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HSV1 ELITe_PL_200_100	Plasma	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HSV1 ELITe_CSF_200_100	CSF	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Protocols for qualitative analysis are available on request.

Setup of the session

The product **HSV1 ELITe MGB® Kit** can be used with the **ELITe InGenius®** system in order to perform:

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

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

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

The main steps for the setup of the three types of runs are described in the following paragraphs.

A. Integrated run

To setup an integrated run with sample extraction and amplification, carry out the following steps as per the GUI:

1. Thaw **HSV1 Q - PCR Mix** tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

Note: Thaw **HSV1 Q - PCR Mix** in the dark because this reagent is sensitive to the light.

2. Thaw the CPE tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home" screen.
4. Ensure that the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
5. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
6. Select the assay protocol to be used in the "Assay" column (e.g. HSV1 ELITe_PL_200_100).
7. Ensure that the "Protocol" displayed is: "Extract + PCR".
8. Select the sample loading position in the "Sample Position" column:
   if a primary tube is used select "Primary Tube",
   if a secondary tube is used select "Extraction Tube".
   Click "Next" to continue the setup.
9. Load CPE and HSV1 Q-PCR Mix on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of HSV1 Q - PCR Mix and CPE. Click "Next" button to continue the setup.
10. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
11. Load the "PCR Cassettes", the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the samples to be extracted, following the GUI instruction. Click "Next" to continue the setup.
12. Close the instrument door.
13. Press "Start" to start the run.

After process completion, the **ELITe InGenius®** allows users to view, approve, store the results and to 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 °C. Avoid spilling the Extracted Sample.

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

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

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B. Amplification run

To set up the amplification run starting from extracted DNA, carry out the following steps as per GUI:

1. Thaw a sufficient number of **HSV1 Q - PCR Mix** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

Note: Thaw **HSV1 Q - PCR Mix** in the dark because this reagent is sensitive to the light.

2. Select "Perform Run" from the "Home" screen.
3. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
4. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
5. Select the assay protocol to be used in the "Assay" column (e.g. HSV1 ELITe_PL_200_100).
6. Select "PCR Only" in the "Protocol" column.
7. Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Click "Next" to continue the setup.
8. Load HSV1 Q-PCR Mix on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of HSV1 Q - PCR Mix. Click "Next" button to continue the setup.
9. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load the "PCR Cassettes" and the extracted Nucleic Acid samples following the GUI instruction. Click "Next" to continue the setup.
11. Close the instrument door.
12. Press "Start" to start the run.

After process completion, the **ELITe InGenius®** system allows users to view, approve, store the results and to 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 and stored at -20 °C for one month. Avoid the spilling of the Extracted Sample.

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

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

C. Calibration run

To set up the Calibration run for Q-PCR Standards, carry out the following steps as per GUI:

1. Thaw **HSV1 Q - PCR Mix** tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

Note: Thaw **HSV1 Q - PCR Mix** in the dark because this reagent is sensitive to the light.

2. Thaw **HSV1 Q - PCR Standard** tubes (Cal1: HSV1 Q - PCR Standards 10^2, Cal2: HSV1 Q - PCR Standards 10^3, Cal3: HSV1 Q - PCR Standards 10^4, Cal4: HSV1 Q - PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home” screen.
4. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
5. In the Track of interest, select the Assay Protocol to be used in the “Assay” column.
6. Select the Assay Protocol “HSV1 ELITe_STD” in the “Assay” column and fill in the lot number and expiry date of HSV1 Q-PCR Standard.
7. Click “Next” to continue the setup.
8. Load HSV1 Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of HSV1 Q - PCR Mix. Click “Next” button to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the “PCR Cassettes” and the **HSV1 Q-PCR Standard** tubes following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

After process completion, the ELITe InGenius® system allows users to view, approve, store the results and to 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 and stored at -20 °C for one month. Avoid the spilling of the Extracted Sample.

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

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

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D. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, carry out the following steps as per GUI:

1. Thaw a sufficient number of **HSV1 Q - PCR Mix** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

Note: Thaw **HSV1 Q - PCR Mix** in the dark because this reagent is sensitive to the light**.**

2. Thaw **HSV1 - Positive Control** tubes at room temperature (~+25°C) for 30 minutes for Positive Control amplification session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water for the sessions to an Elution tube, provided with the ELITe InGenius SP Consumable Set.
4. Select “Perform Run” from the “Home” screen.
5. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 50 µL.
6. In the Track of interest, select the Assay Protocol to be used in the “Assay” column.
7. For the Positive Control, select the Assay Protocol “HSV1 ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of HSV1 Positive Control.
8. For the Negative Control, select the Assay Protocol “HSV1 ELITe_NC” and fill in the lot number and expiry date of the molecular biology grade water.
9. Click “Next” to continue the setup.
10. Load HSV1 Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of HSV1 Q - PCR Mix. Click “Next” button to continue the setup.
11. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
12. Load the “PCR Cassettes”, the HSV1 Positive Control tube and the Negative Control tube following the GUI instruction. Click “Next” to continue the setup.
13. Close the instrument door.
14. Press “Start” to start the run.

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

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

Note: At the end of the run the remaining Positive Control can be removed from the instrument, capped and stored at -20 °C. The remaining Negative Control must be disposed.

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

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

Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports ("Sample Report" or "Track Report"). Refer to the instrument user's manual for more details.

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

The **ELITe InGenius®** generates the results with the product **«HSV1 ELITe MGB® Kit»** through the following procedure:

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

A. Validation of Calibration curve

The fluorescence signals emitted by the specific HSV1 probe ("HSV1") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol "HSV1 ELITe_STD".

The Calibration curve, specific for the amplification reagent lot, are recorded in the database (Calibration). It can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

The Calibration curve, specific for the amplification reagent lot, will expire **after 60 days**.

Note: if the Calibration curve does not meet the acceptance criteria, the "Failed" message is shown on the "Calibration" screen and it is not possible to approve curve. The Calibrator amplification reactions have to be repeated.

Note: if the Calibration curve is run together with samples and its result is invalid, the samples are not quantified and cannot be approved. In this case, the amplification of all samples must be repeated too.

B. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the specific HSV1 probe ("HSV1") and by the specific Internal Control probe ("IC") in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocols "HSV1 ELITe_PC" and "HSV1 ELITe_NC".

The results of Positive Control and Negative Control amplification, specific for the lot of amplification reagent used, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

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

Before analysing any sample, it is absolutely mandatory to verify that Positive Control and Negative Control amplification were run with the lot of amplification reagent to be used and results are approved and valid. The availability of "Approved" (Status) results of Positive Control and Negative Control amplification is shown in the "Controls" window of the GUI. If the results of Positive Control and Negative Control amplification are missing, generate them as described above.

The results of Positive Control and Negative Control amplification runs are used by the instrument software to calculate the setup the "Control Charts". Four Positive Control and Negative Control results, from four different runs are requested to set up the "Control Chart". After that, the results of Positive Control and Negative Control are used for monitoring the amplification step performances. Refer to the user's manual of the instrument for more details.

Note: if the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the "Failed" message is shown on the "Controls" screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction has to be repeated.

Note: if the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the samples can be approved but the results are not validated. In this case, the amplification of all samples must be repeated too.

C. Validation of Samples results

The fluorescence signals emitted by the specific HSV1 probe (Channel "HSV1") and by the specific Internal Control probe (Channel "IC") in each sample amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol.

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

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

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

1) Calibration curve	**Status**
HSV1 Q - PCR Standard	APPROVED
2) Positive Control	**Status**
HSV1 - Positive Control	APPROVED
3) Negative Control	**Status**
HSV1 - Negative Control	APPROVED

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

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

Result of Sample run	Interpretation
HSV1: DNA Detected, quantity equal to XXX copies / mL	**HSV1 DNA detected** within the measurement range of the assay, quantity as shown.
HSV1: DNA Detected, quantity below LLoQ copies / mL	**HSV1 DNA detected** below the lower limit of quantification of the assay
HSV1: DNA Detected, quantity beyond ULoQ copies / mL	**HSV1 DNA detected** beyond the upper limit of quantification of the assay
HSV1: DNA Not Detected or below LoD copies / mL	**HSV1 DNA not detected or below the Limit of Detection** of the assay.
Invalid - Retest Sample	**Not valid assay result** due to Internal Control failure (Incorrect extraction or presence of inhibitor).

Samples reported as "Invalid - Retest Sample" by the **ELITe InGenius software** are not suitable for result interpretation. In this case, the Internal Control DNA was not efficiently detected due to problems in the amplification or extraction step (degradation or loss of DNA during the extraction or inhibitors carry-over in the eluate), which may cause incorrect results.

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

Samples reported as "HSV1: DNA Not Detected or below LoD" are suitable for analysis but was not possible to detect HSV1 DNA. In this case it cannot be excluded that the HSV1 DNA is present at a concentration below the limit of detection of the assay (see "Performance and Characteristic).

HSV1 positive samples at a concentration below the LoD, when are detected by the assay, are reported as "HSV1: DNA Detected, quantity below LLoQ" (see "Performance characteristics").

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

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

D. Samples result reporting

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

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

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

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

ELITe BeGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Whole blood collected in EDTA

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

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

Note: when the DNA extraction from whole blood is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius® Software** version **2.0.0** (or later equivalent versions), use the extraction protocol **HSV1 ELITe_Be_WB_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information.

Plasma collected in EDTA

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

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

Note: when the DNA extraction from 200 µL of plasma is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius® Software** version **2.0.0** (or later equivalent versions), use the extraction protocol **HSV1 ELITe_Be_PL_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information.

Cerebrospinal Fluid (CSF)

The CSF samples for nucleic acid extraction must be collected according to laboratory guidelines avoiding contamination by patient blood, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of four hours, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods. It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when the DNA extraction from whole blood is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius®** Software version **2.0.0** (or later equivalent versions), use the extraction protocol **HSV1 ELITe_Be_CSF_200_100**. This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elute the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: suspensions of leucocytes, suspensions of granulocytes and amniotic fluid.

Interfering substances

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

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

Amplification calibrators and amplification controls

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

as calibrator set, use the four concentration levels of the **HSV1 ELITe Standard**, in association with the protocol **« «HSV1 ELITe_Be_STD»**,
as amplification Positive Control use the **HSV1 - ELITe Positive Control**, in association with the protocol **«HSV1 ELITe_Be_PC»**,
as amplification Negative Control, use molecular grade water (not provided with this kit) in association with the protocol **«HSV1 ELITe_Be_NC»**.

Note: ELITe BeGenius® with **ELITe BeGenius® Software** allows generation of the calibration curve and the validation of amplification Controls for each lot of amplification reagent to be stored in its database.
Calibration curves, approved and stored in the database, will expire after **60 days**. At expiration date it is necessary to re-run the calibrator set.

Amplification validation Control results, approved and stored in the database, will expire after **15 days**. At expiration date it is necessary to re-run the Positive and Negative Controls.

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

Quality controls

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

PROCEDURE

The procedure to use the **«HSV1 ELITe MGB Kit»** with the system **ELITe BeGenius®** consists of three steps:

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

System readiness verification

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

- switch on the **ELITe BeGenius®** and select the mode “**CLOSED**”;
- verify that the Calibrators (**HSV1 Q-PCR Standard**) have been run, approved and not expired (status). This can be checked under the “Calibration” menu in the Home page;
- verify that the amplification Controls (**HSV1 - Positive Control**, **HSV1 Negative Control**) have been run, approved and not expired (status). This can be checked under the “Control” menu in the Home page;
- choose the type of run and set up the run, following the instructions Graphical User Interface (GUI) for the session set up and using the Assay Protocols provided by ELITechGroup. These IVD protocols were specifically validated with ELITe MGB Kits, matrices and ELITe BeGenius® instrument.

The Assay protocols available for **«HSV1 ELITe MGB® Kit»** are described in the table below.

Assay protocols for «HSV1 ELITe MGB® Kit» and ELITe BeGenius®			
Name	**Matrix**	**Report unitage**	**Characteristics**
HSV1 ELITe_Be_WB_200_100	Whole Blood	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HSV1 ELITe_Be_PL_200_100	Plasma	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HSV1 ELITe_Be_CSF_200_100	CSF	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Setup of the session

The **HSV1 ELITe MGB Kit** in association to the **ELITe BeGenius®** can be used in order to perform:

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

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

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

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

A. Sample run

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

1. Thaw a sufficient number of HSV1 Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw a sufficient number of CPE tubes for the session. Each new tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. Remove the Racks from the "Cooler Unit" and place them on the preparation table.
5. Select the "run mode": "Extract + PCR".
6. Load the samples into the Racks 5 and 4 (start always from Rack 5).
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.

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

8. Check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).

9. Select the assay protocol to be used in the "Assay" column (i.e. HSV1 ELITe_Be_WB_200_100). Click "Next" to continue the setup.
10. If used, repeat step 7 to 9 for Rack 4.
11. Load the Elution tubes into the Racks 3 and 2 (start always from Rack 3).

Note: Elution tubes can be labelled to improve traceability.

12. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
13. If used, repeat step 12 for Rack 2.
14. Load CPE and HSV1 Q-PCR Mix into the Rack 1.
15. Insert the Rack 1 into the "Cooler Unit". Click "Next" to continue the setup.
16. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
17. Load the Basket with "PCR Cassette" in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
18. Load the Basket with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables by following the GUI instruction. Click "Next" to continue the setup.
19. Close the instrument door.
20. Press "Start" to start the run.

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

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

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

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

B. Amplification run

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

1. Thaw a sufficient number of HSV1 Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw a sufficient number of CPE tubes for the session. Each new tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. Remove Racks 1, 2 and 3 from the "Cooler Unit" and place them on the preparation table.
5. Select the "run mode": "PCR Only".
6. Load the samples into the Racks 3 and 2 (start always from Rack 3).
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
8. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
9. Select the assay protocol to be used in the "Assay" column (e.g. HSV1 ELITe_Be_WB_200_100). Click "Next" to continue the setup.
10. Repeat step from 7 to 9 for Rack 2.
11. Load CPE and HSV1 Q-PCR Mix into the Rack 1.
12. Insert the Rack 1 into the "Cooler Unit". Click "Next" to continue the setup.

13. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
14. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
15. Close the instrument door.
16. Press “Start” to start the run.

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

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

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

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

C. Calibration run

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

1. Thaw a sufficient number of HSV1 Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the HSV1 Q - PCR Standard tubes (Cal1: HSV1 Q-PCR Standards 10^2, Cal2: HSV1 Q-PCR Standards 10^3, Cal3: HSV1 Q-PCR Standards 10^4, Cal4: HSV1 Q-PCR Standards 10^5). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home screen”.
4. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
5. Select the “run mode”: “PCR Only”.
6. Load the Calibrator tubes into the Racks 3.
7. Select the assay protocol to be used in the “Assay” column (HSV1 ELITe_Be_STD). Click “Next” button to continue the setup.
8. Load HSV1 Q-PCR Mix into the Rack 2.
9. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
11. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

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

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

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

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

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D. Positive Control and Negative Control run

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

1. Thaw a sufficient number of HSV1 Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the product HSV1 - ELITe Positive Control, for Positive Control amplification. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of the molecular biology grade water (as Negative Control) for the sessions in one Elution tube, provided with the ELITe InGenius SP Consumable Set.
4. Select “Perform Run” from the “Home screen”.
5. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
6. Select the “run mode”: “PCR Only”.
7. Load the Positive Control and Negative Control tubes into the Racks 3.
8. Select the assay protocol to be used in the “Assay” column (HSV1 ELITe_Be_PC and HSV1 ELITe_Be_NC). Click “Next” button to continue the setup.
9. Load HSV1 Q-PCR Mix into the Rack 2.
10. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
11. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
12. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
13. Close the instrument door.
14. Press “Start” to start the run.

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

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

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

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

Review and approval of results

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

The ELITe BeGenius® generates the results using the HSV1 ELITe MGB Kit through the following procedure:

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

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

PERFORMANCE CHARACTERISTICS ELITe InGenius® and ELITe BeGenius®

Analytical sensitivity: Limit of Detection

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

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

The results are reported in the following table.

Sample	N	Valid	Positive	Negative
10 copies plasmid DNA + 500 ng of human genomic DNA	24	24	24	0

The Limit of Detection (LoD) of HSV1 ELITe MGB® Kit was verified in association with **Whole Blood** collected in EDTA**, Plasma** collected in EDTA and **CSF** and **ELITe InGenius®** and **ELITe BeGenius®** systems (Extr + PCR mode).

For Whole Blood:

The LoD of this assay was verified by testing 20 replicates of Whole blood sample spiked at 211 copies / mL on ELITe InGenius® and ELITe BeGenius® systems in "Extract + PCR" mode. The samples were spiked using the reference material Herpes Simplex Virus Type 1 (HSV-1) Culture Fluid Heat Inactivated, ZeptoMetrix.

The LoD is confirmed if at least 18 out of 20 replicates give a positive result as per CLSI EP17-A guideline.

The results are reported in the following tables.

Limit of Detection for Whole Blood samples and ELITe InGenius®					
Sample	LoD	N	Valid	Positive	Negative
Whole blood collected in EDTA	211 copies / mL	20	20	19	1

Limit of Detection for Whole Blood samples and ELITe BeGenius®					
Sample	LoD	N	Valid	Positive	Negative
Whole blood collected in EDTA	211 copies / mL	20	20	20	0

The LoD value for HSV1 target was confirmed at 211 copies / mL for Whole Blood collected in EDTA.

For Plasma:

The LoD of this assay was verified by testing 20 replicates of Plasma sample spiked at 250 copies / mL on ELITe InGenius® and ELITe BeGenius® systems in "Extract + PCR" mode. The samples were spiked using the reference material Herpes Simplex Virus Type 1 (HSV-1) Culture Fluid Heat Inactivated, ZeptoMetrix.

The LoD is confirmed if at least 18 out of 20 replicates give a positive result as per CLSI EP17-A guideline.

The results are reported in the following tables.

Limit of Detection for Plasma samples and ELITe InGenius®					
Sample	LoD	N	Valid	Positive	Negative
Plasma collected in EDTA	250 copies / mL	20	20	20	0

Limit of Detection for Plasma samples and ELITe BeGenius®					
Sample	LoD	N	Valid	Positive	Negative
Plasma collected in EDTA	250 copies / mL	20	20	20	0

The LoD value for HSV1 target was confirmed at 250 copies / mL for Plasma collected in EDTA.

For Cerebrospinal Fluid (CSF):

The LoD of this assay was verified by testing 20 replicates of CSF sample spiked at 250 copies / mL on ELITe InGenius® and ELITe BeGenius® systems in "Extract + PCR" mode. The samples were spiked using the reference material Herpes Simplex Virus Type 1 (HSV-1) Culture Fluid Heat Inactivated, ZeptoMetrix.

The LoD is confirmed if at least 18 out of 20 replicates give a positive result as per CLSI EP17-A guideline.

The results are reported in the following tables.

Limit of Detection for CSF samples and ELITe InGenius®					
Sample	LoD	N	Valid	Positive	Negative
Cerebrospinal Fluid	250 copies / mL	20	20	20	0

Limit of Detection for CSF samples and ELITe BeGenius®					
Sample	LoD	N	Valid	Positive	Negative
Cerebrospinal Fluid	250 copies / mL	20	20	20	0

The LoD value for HSV1 target was confirmed at 250 copies / mL for Cerebrospinal Fluid.

Linear measuring range and Limits of quantification

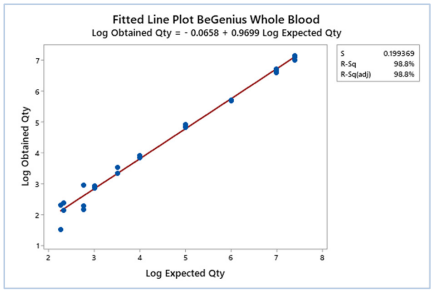
The linear measuring range of HSV1 ELITe MGB® Kit used in association with **Whole Blood** collected in EDTA**, Plasma** collected in EDTA and **CSF** and **ELITe InGenius® and ELITe BeGenius®** was verified with a panel of HSV1 dilutions. The panel was prepared by diluting the Herpes Simplex Virus Type 1 (HSV-1) Culture Fluid Heat Inactivated (ZeptoMetrix), in HSV1 DNA - negative matrices.

For Whole Blood:

The panel consisted of ten dilution points from about 2.5 x 10^7 copies / mL to about 178 copies / mL. Each sample of the panel was tested in 3 replicates.

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Whole Blood samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.997 for **ELITe InGenius®** and 0.988 for **ELITe BeGenius®**.

The results are reported in the following graphs.



The Lower Limit of Quantification (LLoQ) was set at the LoD concentration, that gives quantitative results precise (Standard Deviation equal to 0.253 Log copies / mL for **ELITe InGenius®** and 0.305 Log copies / mL for **ELITe BeGenius®**) and accurate (Bias equal to 0.133 Log copies / mL for **ELITe InGenius®** and 0.491 Log copies / mL for **ELITe BeGenius®**): 211 copies / mL.

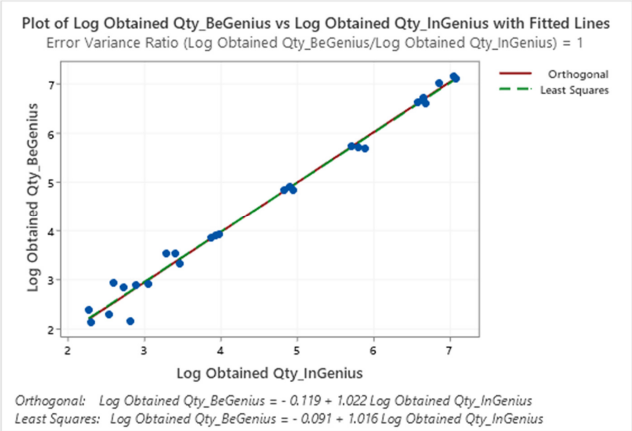
The Upper Limit of Quantification (ULoQ) was set at the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.117 Log copies / mL for **ELITe InGenius®** and 0.068 Log copies / mL for **ELITe BeGenius®**) and accurate (Bias equal to 0.393 Log copies / mL for **ELITe InGenius®** and 0.297 Log copies / mL for **ELITe BeGenius®**): 25,000,000 copies / mL.

The final results are summarized in the following table.

Linear measuring range for Whole Blood samples and ELITe InGenius® and ELITe BeGenius®		
Unit of measure	lower limit	upper limit
copies / mL	**211**	**25,000,000**

The results obtained by **ELITe InGenius** and **ELITe BeGenius** were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



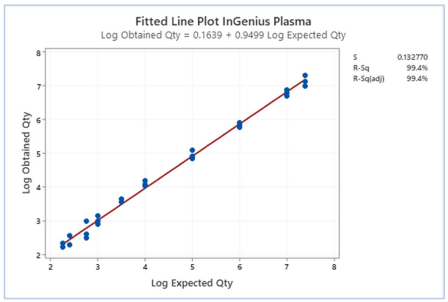
In this test, the orthogonal regression analysis generated a slope equal to 1.022 (95% CI: 0.977; 1.067) and an intercept equal -0.119 (95% CI: - 0.333; 0.094). The linear regression analysis generated a R2 of 0.988.

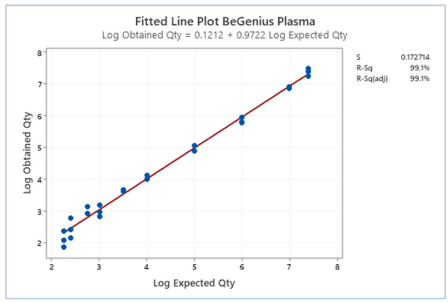
For Plasma:

The panel consisted of ten dilution points from about 2.5 x 10^7 copies / mL to about 178 copies / mL. Each sample of the panel was tested in 3 replicates.

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Whole Blood samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.994 for **ELITe InGenius®** and 0.991 for **ELITe BeGenius®**.

The results are reported in the following graphs.





The Lower Limit of Quantification (LLoQ) was set at the LoD concentration, that gives quantitative results precise (Standard Deviation equal to 0.192 Log copies / mL for **ELITe InGenius®** and 0.270 Log copies / mL for **ELITe BeGenius®**) and accurate (Bias equal to 0.197 Log copies / mL for **ELITe InGenius®** and 0.163 Log copies / mL for **ELITe BeGenius®**): 250 copies / mL.

HSV1 ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS031PLD

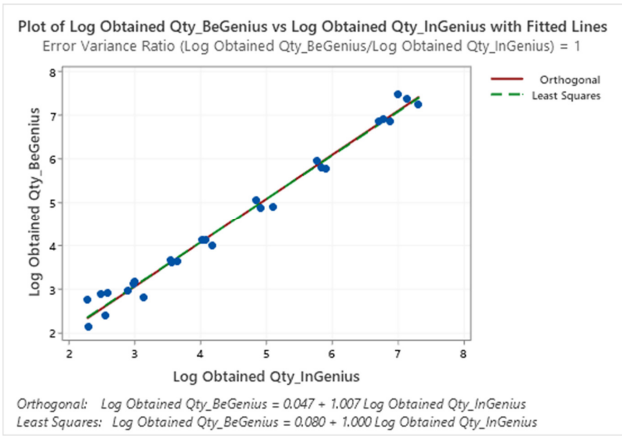
The Upper Limit of Quantification (ULoQ) was set at the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.117 Log copies / mL for **ELITe InGenius®** and 0.068 Log copies / mL for **ELITe BeGenius®**) and accurate (Bias equal to 0.393 Log copies / mL for **ELITe InGenius®** and 0.297 Log copies / mL for **ELITe BeGenius®**): 25,000,000 copies / mL.

The final results are summarized in the following table.

Linear measuring range for Plasma samples and ELITe InGenius® and ELITe BeGenius®		
Unit of measure	lower limit	upper limit
copies / mL	**250**	**25,000,000**

The results obtained by **ELITe InGenius®** and **ELITe BeGenius®** were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



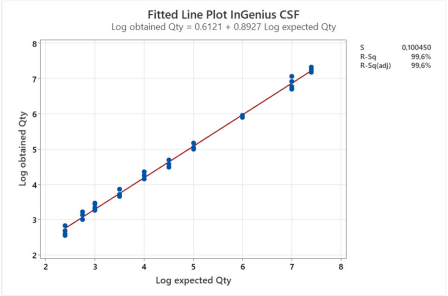
In this test, the orthogonal regression analysis generated a slope equal to 1.007 (95% CI: 0.960; 1.054) and an intercept equal to 0.047 (95% CI: - 0.180; 0.274). The linear regression analysis generated a R2 of 0.986.

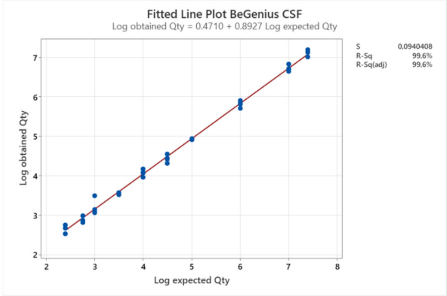
For Cerebrospinal Fluid CSF:

The panel consisted of ten dilution points from about 2.5 x 10^7 copies / mL to about 250 copies / mL. Each sample of the panel was tested in 4 replicates.

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

The results are reported in the following graphs.





The Lower Limit of Quantification (LLoQ) was set at the LoD concentration, that gives quantitative results precise (Standard Deviation equal to 0.1209 Log copies / mL for **ELITe InGenius®** and 0.0941 Log copies / mL for **ELITe BeGenius®**) and accurate (Bias equal to 0.2680 Log copies / mL for **ELITe InGenius®** and 0.2527 Log copies / mL for **ELITe BeGenius®**): 250 copies / mL.

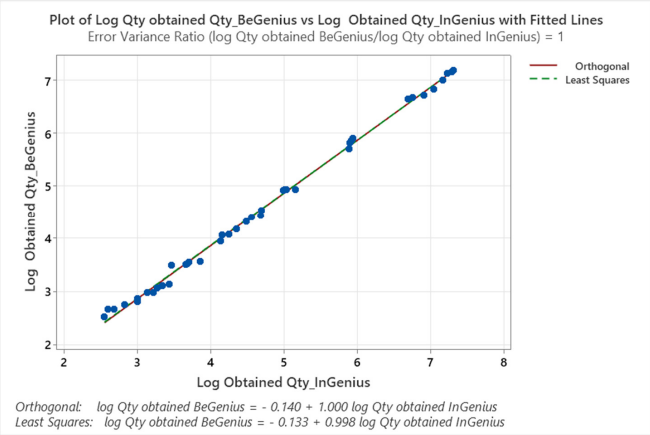
The Upper Limit of Quantification (ULoQ) was set at the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0661 Log copies / mL for **ELITe InGenius®** and 0.0811 Log copies / mL for **ELITe BeGenius®**) and accurate (Bias equal to 0.1434 Log copies / mL for **ELITe InGenius®** and 0.2804 Log copies / mL for **ELITe BeGenius®**): 25,000,000 copies / mL.

The final results are summarized in the following table.

Linear measuring range for CSF samples and ELITe InGenius® and ELITe BeGenius®		
Unit of measure	lower limit	upper limit
copies / mL	**250**	**25,000,000**

The results obtained by **ELITe InGenius®** and **ELITe BeGenius®** were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



In this test, the orthogonal regression analysis generated a slope equal to 1.000 (95% CI: 0.982; 1.016) and an intercept equal to 0.140 (95% CI: - 0.223; 0.056). The linear regression analysis generated a R2 of 0.997.

Repeatability

The Repeatability of results obtained by the product HSV1 ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Whole Blood samples collected in EDTA. The panel included one negative sample and two samples spiked by HSV1 certified reference material (Herpes Simplex Virus Type 1 (HSV-1) Culture Fluid Heat Inactivated, ZeptoMetrix) at concentration of 3 x LoD (about 633 copies / mL) and of 10 x LoD (about 2110 copies / mL).

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

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

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

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe InGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	-	-	-	24 / 24	23.59	0.41	1.72
3 x LoD	8 / 8	36.31	0.51	1.40				
10 x LoD	8 / 8	34.25	0.42	1.22				

Inter – Session Repeatability ELITe InGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 16	-	-	-	48 / 48	23.64	0.55	2.31
3 x LoD	16 / 16	36.15	0.52	1.44				
10 x LoD	16 / 16	34.24	0.42	1.21				

In the Repeatability test on **ELITe InGenius**, the assay detected the HSV1 target as expected and showed Ct values with %CV below 5% for HSV1 and for Internal Control.

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

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

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

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe BeGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	-	-	-	24 / 24	26.87	0.59	2.19
3 x LoD	8 / 8	37.82	0.65	1.73				
10 x LoD	8 / 8	35.82	0.47	1.32				

Inter – Session Repeatability ELITe BeGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 16	-	-	-	48 / 48	27.04	0.57	2.12
3 x LoD	16 / 16	37.53	0.64	1.71				
10 x LoD	16 / 16	35.55	0.53	1.50				

In the Repeatability test on **ELITe BeGenius®**, the assay detected the HSV1 target as expected and showed Ct values with %CV below 5% for HSV1 and for Internal Control

Reproducibility

The Reproducibility of results obtained by the product HSV1 ELITe MGB Kit in association with the **ELITe InGenius®** and **ELITe BeGenius®** systems was tested by analysing a panel of whole blood samples. The panel included one negative sample and two samples spiked by HSV1 certified reference material (Herpes Simplex Virus Type 1 (HSV-1) Culture Fluid Heat Inactivated, ZeptoMetrix) at concentration of 3 x LoD (about 633 copies / mL) and of 10 x LoD (about 2110 copies / mL).

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

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

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

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe InGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	-	-	-	24 / 24	23.55	0.57	2.40
3 x LoD	8 / 8	36.91	0.77	2.10				
10 x LoD	8 / 8	35.15	0.48	1.37				

Inter – Batch Reproducibility ELITe InGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	-	-	-	24 / 24	23.09	0.63	2.73
3 x LoD	8 / 8	36.99	0.61	1.64				
10 x LoD	8 / 8	34.71	0.53	1.51				

In the Reproducibility test on **ELITe InGenius®**, the assay detected the HSV1 target as expected and showed Ct values with %CV below 5% for HSV1 and for Internal Control.

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

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

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

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe BeGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	-	-	-	24 / 24	26.84	0.76	2.81
3 x LoD	8 / 8	37.26	0.59	1.58				
10 x LoD	8 / 8	36.12	0.79	2.18				

Inter – Batch Reproducibility ELITe BeGenius®								
Sample	**HSV1**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	-	-	-	24 / 24	26.55	0.85	3.21
3 x LoD	8 / 8	37.96	1.08	2.83				
10 x LoD	8 / 8	36.40	0.69	1.91				

In the Reproducibility test on **ELITe BeGenius®**, the assay detected the HSV1 target as expected and showed Ct values with %CV below 5% for HSV1 and for Internal Control.

HSV1 ELITe MGB® Kit
reagent for DNA Real Time amplification

REF **RTS031PLD**

Analytical sensitivity: reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of value of a calibrated reference material, was evaluated using as reference material the calibrated panel «HSV1 Molecular "Q" Panel» (Qnostics, Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **«ELITe InGenius®»** and ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius®»				
Sample	Nominal titre copies/mL	Nominal titre Log_{10}. copies/mL	Positive / Replicates	Mean results Log_{10} copies / mL
HSV1MQP01-High	10^5	5.000	2/2	4.890
HSV1MQP01-Medium	10^4	4.000	2/2	3.859
HSV1MQP01-Low	10^3	3.000	2/2	2.736
HSV1MQP01-Negative	negative	-	0/2	-

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

Further tests were carried out using as reference material QCMD 2014 Herpes Simplex virus EQA Panel (Qnostics Ltd, UK) a panel of HSV1 dilutions. Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, using **«ELITe InGenius»** and ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius®»				
Sample	Consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} copies / mL
HSVDNA14-01	HSV1, 3.657	0.563	2/2	3.716
HSVDNA14-02	Negative, N.A.	N.A.	0/2	Not detected
HSVDNA14-03	HSV1, 3.001	0.578	2/2	2.520
HSVDNA14-04	HSV1, 2.256	0.512	1/2	0.940
HSVDNA14-05	HSV1, 4.070	0.481	2/2	3.774
HSVDNA14-06	HSV2, 3.033	0.906	0/2	Not detected
HSVDNA14-07	HSV2, 2.394	0.618	0/2	Not detected
HSVDNA14-08	HSV2, 3.504	0.899	0/2	Not detected
HSVDNA14-09	HSV1, 2.481	0.477	2/2	1.976
HSVDNA14-10	VZV, N.A.	N.A.	0/2	Not detected

All negative samples were correctly detected as negative and all positive samples were detected as positive in agreement with qualitative results defined by EQA consensus. The sample HSVDNA14-04 gave only a positive result out of 2 replicates. This can be explained because the sample titer is below the detection limit. All samples above detection limit of the method were quantified within the range defined by the Real Time PCR Commercial Consensus ± 2 Standard Deviation.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing some positive clinical samples of whole blood collected in EDTA, plasma collected in EDTA and Cerebrospinal fluid in association with **ELITe InGenius®**. As **ELITe BeGenius®** showed equivalent analytical performances to **ELITe InGenius®**, it can be assumed that the results of Diagnostic sensitivity obtained in association with **ELITe InGenius®** are applicable also to **ELITe BeGenius®**.

The diagnostic sensitivity was evaluated using 50 samples of whole blood collected in EDTA negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV12-04, a sample of QCMD 2012 Herpes Simplex Virus DNA EQA Panel (Qnostics Ltd, UK) (N=30) and adding HSV1 ELITe-IQC High" (ELITech Group S.p.A.) (N=20) at a titre of 750 copies / mL, 30 samples of plasma collected in EDTA negative for HSV1 DNA, that were spiked for HSV1 DNA adding "HSV1 ELITe-IQC High" (ELITech Group S.p.A.) at a titre of 750 copies / mL and 20 samples of CSF negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV1 ELITe-IQC High" (ELITech Group S.p.A.) at a titre of 750 copies / mL.

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

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked for HSV1 DNA	50	49	1
Plasma collected in EDTA spiked for HSV1 DNA	30	30	0
Cerebrospinal fluid spiked for HSV1 DNA	20	20	0

49 out of 50 whole blood samples were confirmed as positives. One sample tested negative. This might be explained by the imprecision of the titre of the calibrated material used for spiking (HSV12-04 sd = 0. 517 Log). In the spiked samples this might result in viral load below the limit of detection and the samples may stochastically test negative.

The diagnostic sensitivity of the assay in association to whole blood in this test was equal to 98%.

All plasma and Cerebrospinal fluid samples were valid and positive.

The diagnostic sensitivity of the assay in association to plasma and Cerebrospinal fluid in this test was equal to 100%.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analyzing some clinical samples of whole blood collected in EDTA, plasma collected in EDTA and Cerebrospinal fluid, negative for HSV1 DNA in association with **ELITe InGenius®**. As **ELITe BeGenius®** showed equivalent analytical performances to **ELITe InGenius®**, it can be assumed that the results of Diagnostic specificity obtained in association with **ELITe InGenius®** are applicable also to **ELITe BeGenius®**.

The diagnostic specificity was evaluated using 34 whole blood samples collected in EDTA from healthy donors that were presumably negative for HSV1 DNA, 38 plasma samples collected in EDTA from healthy donors that were presumably negative for HSV1 DNA and 22 CSF samples from healthy donors that were presumably negative for HSV1 DNA.

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

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA negative for HSV1 DNA	34	0	34
Plasma collected in EDTA negative for HSV1 DNA	38	0	38
Cerebrospinal fluid negative for HSV1 DNA	22	0	22

All whole blood, plasma and Cerebrospinal fluid samples were valid and negative.

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

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

SAMPLES AND CONTROLS

Samples

This product must be used with **DNA extracted** from the following clinical samples: cerebrospinal fluid (CSF), whole blood collected in EDTA, plasma collected in EDTA.

Cerebrospinal fluid (CSF)

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

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

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

Note: when you carry out the DNA extraction from CSF using **«EXTRAgen»** kit, please, follow the instructions for use manual: start from **300 µL** of sample, add **5 µL** of **CPE** for the internal control at the beginning of the extraction. Dissolve the pellet of extracted nucleic acids in **60 µL** of ultrapure water.

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

Note: when you carry out the DNA extraction from cerebrospinal fluid with the **«ELITe GALAXY»** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses 300 µL of sample and elutes the extract in 200 µL (the elution takes actually place in 210 µL of which 200 µL are recovered). Samples in primary tubes can be directly loaded on **«ELITe GALAXY»**. A minimum volume 400-650 µL, dependent on the tube class used, is always required for each sample. Add **10 µL / sample** of **CPE.** The CPE must be added to **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from cerebrospinal fluid with the instrument **«NucliSENS® easyMAG®»**, please follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip and run the extraction. After the 10 minutes incubation, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica** and proceed with the extraction. Elute the nucleic acids in **100 µL** of elution buffer.

Whole blood collected in EDTA

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

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

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

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

Note: when you carry out the DNA extraction from whole blood with the **«ELITe GALAXY»** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses

300 µL of sample and elutes the extract in 200 µL. Samples in primary tubes can be directly loaded on **«ELITe GALAXY»**. A minimum volume 400-650 µL, dependent on the tube class used, is always required for each sample. Add **10 µL** / **sample** of **CPE.** The CPE must be added to **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from whole blood using **«EXTRAblood»** kit, please, follow the instructions for use manual: start from **200 µL** of sample (no more than 2 millions of leucocytes), elute the DNA in **100 µL** of elution buffer.

Plasma collected in EDTA

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

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

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

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

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

Note: When you carry out the DNA extraction from plasma with the instrument **«NucliSENS® easyMAG®»**, please follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip and run the extraction. After the 10 minutes incubation, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica** and proceed with the extraction. Elute the nucleic acids in **100 µL** of elution buffer.

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

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: suspensions of leucocytes, suspensions of granulocytes and amniotic fluid.

Interfering substances

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

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

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

Amplification controls

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

For the negative control, use molecular biology grade water (not provided with this product) added to

the reaction in place of the DNA extracted from the sample.

For the positive control, use the **«HSV1 - ELITe Positive Control»** product or the **«HSV1 ELITe Standard»** product.

PROCEDURE

Setting of the real time amplification session

(To perform in the amplification / detection of amplification products area)

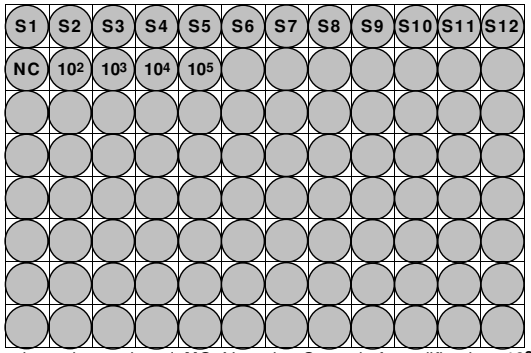
When **7300 Real-Time PCR System** instrument is used.

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

- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session;
- set (Detector Manager) the "detector" for the HSV1 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "HSV1";
- set (Detector Manager) the "detector" for the Internal Control probe with the "reporter" = "VIC" (AP525 is analogous to VIC) and the "quencher" = "none" (non fluorescent) and call it "IC";
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

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

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



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

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

Note: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60°C.
- modify timing as indicated in the table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;
- optional: add dissociation stage (Add Dissociation Stage) and set the temperature from **40°C** to **80°C**.

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

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

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session and set “Run mode: Fast 7500”;
- set (Detector Manager) the "detector" for the HSV1 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “HSV1”;
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "VIC" (AP525 is similar to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”;
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "CY5" (AP593 is used instead of CY5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

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

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

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

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

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

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

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

Before starting the session, it is important to do the following:
- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **HSV1 Q - PCR Mix** tubes required for the session, remembering that each tube is sufficient for preparing **25 reactions**. Mix gently, spin down the contents for 5 seconds and keep them on ice;
- take and thaw the **HSV1 - ELITe Positive Control** or the **HSV1 Q - PCR Standard** tubes. Mix them gently, centifuge them for 5 seconds spinning down the contents and keep them on ice;
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powder-free gloves and not to damage the wells.

1. Accurately pipet **20 µL** of **HSV1 Q - PCR Mix** on the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

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

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

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

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

   - When a **qualitative** result is required (detection of HSV1 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **HSV1 - ELITe Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the **HSV1 - ELITe Positive Control** three times into the reaction mixture. Avoid creating bubbles.

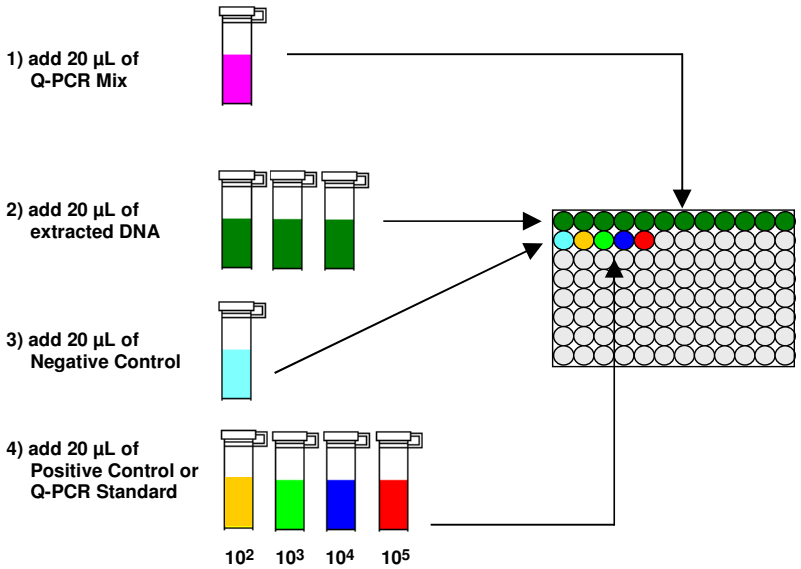
   - When a **quantitative** result is required (quantification of HSV1 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **HSV1 Q - PCR Standard 10^2** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **HSV1 Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **HSV1 Q - PCR Standards** (**10^3, 10^4, 10^5**).

5. Accurately seal the **Amplification microplate** with the **Amplification Sealing Sheet**.

6. Transfer the **Amplification microplate** into the real time thermal cycler (in the amplification / detection of amplification products area) and start the thermal cycle for the amplification saving the session setting with an univocal and recognizable file name (e.g. "year-month-day-HSV1-EGSpA").

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

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



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

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

HSV1 ELITe MGB® Kit
reagent for DNA Real Time amplification
REF RTS031PLD

Qualitative analysis of the results

The recorded values of the fluorescence emitted by the specific HSV1 probe (FAM detector "HSV1") and by the specific Internal Control probe (VIC detector "IC") in the amplification reactions must be analysed by the instrument software.

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

Note: In the case of a positive sample with a high titre of HSV1 DNA, the FAM fluorescence of the HSV1 specific probe may begin to increase before the cycle 15. In this case the calculation range for the **Baseline** must be adapted from cycle 6 to the cycle in which the FAM fluorescence of the sample begins to increase, as detected by the instrument software (Results > Component).

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

- set manually the **Threshold** for the FAM detector “HSV1” to **0.1**;
- set manually the **Threshold** for the VIC detector "IC" to **0.05**.

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

- set manually the **Threshold** for the FAM detector “HSV1” to **0.2**;
- set manually the **Threshold** for the VIC detector "IC" to **0.1**.

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

In the **Positive Control*** amplification reaction, the **Ct** value of HSV1 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Positive Control reaction detector FAM "HSV1"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for HSV1, the target DNA was not correctly detected. This means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mix or of the positive control, degradation of the reaction mix or of the positive control, incorrect setting of the position of the positive control, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

***Note:** When this product is used for the quantification of HSV1 DNA, the **Q - PCR Standard** reactions were set up instead of the **Positive Control** reaction. In this case, validate the amplification and the detection by referring to the amplification reaction of **Q - PCR Standard 10^5** (**Ct ≤ 25**).

In the **Negative control** amplification reaction, the **Ct** value of HSV1 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Negative control reaction detector FAM "HSV1"	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative control** amplification reaction is different from **Ct Undetermined** for HSV1, the target DNA was detected. This means that problems occurred during the amplification step (contamination), which may lead to incorrect results and false positives. The session is not valid and needs to be repeated starting from the amplification step.

In the amplification reaction of each **sample**, the **Ct** value of HSV1 is used to detect the target DNA while the **Ct** value of Internal Control is used to validate extraction, amplification and detection.

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

This product is able to detect a minimal quantity of about 10 copies of DNA of the gpD gene of HSV1 in the amplification reaction, corresponding to 10 genome Equivalents per reaction (limit of detection, see Performance Characteristics paragraph).

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

Sample reaction		Sample suitability	Assay result	HSV1 DNA
detector FAM "HSV1"	detector VIC "IC"			
Ct Undetermined	Ct > 35 or Ct Undetermined	unsuitable	invalid	-
	Ct ≤ 35	suitable	valid, negative	NOT DETECTED
Ct Determined	Ct > 35 or Ct Undetermined	suitable*	valid, positive	DETECTED
	Ct ≤ 35	suitable	valid, positive	DETECTED

If the result of the amplification reaction of a sample is **Ct Undetermined** for HSV1 and **Ct > 35** or **Ct Undetermined** for the Internal Control, it means that it is impossible to detect efficiently the DNA for the Internal Control. In this case problems have occurred during the amplification step (inefficient or absent amplification) or during the extraction step (loss of DNA during the extraction or presence of inhibitors) which may lead to incorrect results and false negatives. The sample is not suitable, the assay, is invalid and it needs to be repeated starting from the extraction of a new sample.

If the result of the amplification reaction of a sample is **Ct Undetermined** for HSV1 and **Ct ≤ 35** for the Internal Control, it means that the HSV1 DNA is not detected in the DNA extracted from the sample; but it cannot be excluded that the HSV1 DNA has a lower titre than the detection limit of the product (see the paragraph about Performance Characteristics). In this case the result could be a false negative.

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

***Note:** When in the amplification reaction of a sample the HSV1 DNA is detected, the Internal Control may result as Ct > 35 or Ct Undetermined. In fact, the low efficiency amplification reaction for the Internal Control may be displaced by competition with the high efficiency amplification reaction for HSV1 DNA. In this case the sample is nevertheless suitable and the positive result of the assay is valid.

Quantitative analysis of the results

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

In the amplification reactions of the four **Q - PCR standards**, the **Ct** values of HSV1 are used to calculate the **Standard Curve** (Results > Standard Curve) for the amplification session, to validate the amplification and the detection as described in the following table:

Standard Curve detector FAM "HSV1"	Acceptability range	Amplification / Detection
Correlation coefficient (R2)	0.990 ≤ R2 ≤ 1.000	CORRECT

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

The **Ct** values of HSV1 in the amplification reaction of each **sample** and the **Standard Curve** of the amplification session are used to calculate the **Quantity** of target DNA present in the amplification reactions of the samples.

This product is able to quantify from 1,000,000 to 10 copies of DNA of the gpD gene of HSV1 in the amplification reaction, corresponding to the genome Equivalents per reaction (linear measuring range, see Performance Characteristics paragraph), as described in the following table:

Sample result detector FAM "HSV1"	HSV1 genome Equivalents per reaction
Quantity > 1 x 10^6	MORE THAN 1,000,000
1 x 10^1 ≤ Quantity ≤ 1 x 10^6	= Quantity
Quantity < 1 x 10^1	LESS THAN 10

The results (**Quantity**) of each **samples** (Results > Report) are used to calculate the genome Equivalents **(gEq)** of HSV1 present in the extracted sample (**Nc**) according to this formula:

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

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

When «**EXTRAgen**» extraction kit is used with cerebrospinal fluid samples and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for cerebrospinal fluid and «EXTRAgen»
Nc (gEq / mL) = 12.5 x Quantity

When **«ELITe STAR»** extraction system is used with whole blood, plasma collected in EDTA or cerebrospinal fluid samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

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

When **«ELITe GALAXY»** extraction system is used with whole blood, plasma collected in EDTA or cerebrospinal fluid samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

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

When «**EXTRAblood**» extraction kit is used with whole blood samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for whole blood and «EXTRAblood»
Nc (gEq / mL) = 25 x Quantity

When «**NucliSENS® easyMAG®**» extraction system is used with plasma collected in EDTA or cerebrospinal fluid samples and the result **expressed in gEq / mL** is required, the formula becomes:

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

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

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

Calculation of the linear measuring range limits

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

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

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

When «**EXTRAgen**» extraction kit is used with cerebrospinal fluid samples, the formula becomes:

Linear measuring range limits (gEq / mL) «EXTRAgen»
Lower limit (gEq / mL) = 12.5 x 10 gEq
Upper limit (gEq / mL) = 12.5 x 1,000,000 gEq
from 125 to 12,500,000 gEq / mL

When «**EXTRAblood**» extraction kit is used with whole blood samples collected in EDTA, the formula becomes:

Measuring range limits (gEq / mL) with «EXTRAblood»
Lower limit (gEq / mL) = 25 x 10 gEq
Upper limit (gEq / mL) = 25 x 1,000,000 gEq
from 250 to 25,000,000 gEq / mL

When «**ELITe STAR**» is used with whole blood, plasma collected in EDTA or cerebrospinal fluid samples, the formula becomes:

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

When «**ELITe GALAXY**» extraction system is used with whole blood, plasma collected in EDTA or cerebrospinal fluid samples, the formula becomes:

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

HSV1 ELITe MGB® Kit
reagent for DNA Real Time amplification
REF RTS031PLD

When «**NucliSENS® easyMAG®**» extraction system is used with plasma samples collected in EDTA or cerebrospinal fluid samples, the formula becomes:

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

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

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

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: limit of detection

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

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

The final results are summed up in the following table.

Samples	**No.**	**positive**	**negative**
10 copies plasmid DNA + 500 ng of human genomic DNA	50	50	0

The analytical sensitivity of this assay used in association to whole blood samples and **«ELITe GALAXY»** was verified with a panel of HSV1 dilutions within the limiting concentration. The panel was prepared by diluting the HSV08-01 sample of the "QCMD 2008 Herpes Simplex Virus EQA Panel (Qnostics, Ltd, UK)" in HSV1 DNA - negative EDTA whole blood. The viral concentrations ranged from 10 gEq / mL to 560 gEq / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for whole blood samples and «ELITe GALAXY» (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**211 gEq / mL**	135 gEq / mL	498 gEq / mL

The analytical sensitivity of this assay used in association to plasma samples and **«ELITe GALAXY»** was verified with a panel of HSV1 dilutions within the limiting concentration. The panel was prepared by diluting the HSV08-01 sample of the "QCMD 2008 Herpes Simplex Virus EQA Panel" (Qnostics, Ltd, UK) in HSV1 DNA - negative EDTA plasma. The viral concentrations ranged from 10 gEq / mL to 560 gEq / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for plasma samples and «ELITe GALAXY» (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**95 gEq / mL**	55 gEq / mL	554 gEq / mL

Analytical sensitivity: linear measuring range

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

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

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

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

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

The final results are summed up in the following table:

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

The linear measuring range limits as gEq / mL referred to the used extraction kit are calculated at page 25.

Analytical sensitivity: Precision and Accuracy

The precision of the assay, as the variability of results obtained with several replicates of the same sample tested within the same session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 22.7% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The accuracy of the assay, as the difference between the mean of results obtained with several replicates of a sample within the same session and the theoretical concentration of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 10.1% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

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

Analytical sensitivity: reproducibility with calibrated reference material

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

The tests were carried out using as calibrated reference material a panel of dilutions of HSV1 within the concentration limit (QCMD 2007 Herpes simplex virus EQA Panel, Qnostics Ltd, UK). Each sample was used in duplicates carrying out the whole analysis, extraction with **«EXTRAgen»** and amplification by ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «EXTRAgen»				
Sample	Commercial assay consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
HSV07-01	HSV2, 4.243	0.730	0 / 2	Not detected
HSV07-02	HSV1, 4.282	0.363	2 / 2	4.292
HSV07-03	Negative, NA	NA	0 / 2	Not detected
HSV07-04	HSV1, 2.593	0.536	2 / 2	3.040
HSV07-05	HSV2, 2.695	1.301	0 / 2	Not detected
HSV07-06	Negative, NA	NA	0 / 2	Not detected
HSV07-07	HSV1, 7.292	0.387	2 / 2	7.396
HSV07-08	HSV1, 4.204	0.339	2 / 2	4.336
HSV07-09	HSV2, 1.890	0.313	0 / 2	Not detected
HSV07-10	HSV1, 5.275	0.292	2 / 2	5.351
HSV07-11	HSV2, 6.134	0.897	0 / 2	Not detected
HSV07-12	VZV, NA	NA	0 / 2	Not detected

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

Further tests were carried out using as calibrated reference material a panel of dilutions of HSV1 within the concentration limit (QCMD 2012 Herpes Simplex Virus DNA EQA Panel, Qnostics Ltd, UK). Each sample was tested carrying out the whole analysis procedure: extraction with **«ELITe STAR»** and amplification with ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «ELITe STAR»				
Sample	Commercial assay consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
HSV12-01	Negative, NA	-	0 / 2	-
HSV12-02	HSV1, 3.910	0.582	2 / 2	4.047
HSV12-03	HSV2, 1.948	0.305	0 / 2	-
HSV12-04	HSV1, 3.680	0.547	2 / 2	4.070
HSV12-05	HSV2, 1.352	0.629	0 / 2	-
HSV12-06	HSV1, 2.318	0.441	2 / 2	2.353
HSV12-07	HSV1, 2.014	0.296	0 / 2	-
HSV12-08	HSV2, 3.424	1.098	0 / 2	-
HSV12-09	Negative, NA	-	0 / 2	-
HSV12-10	HSV2, 3.417	1.042	0 / 2	-

All negative samples were correctly reported. The positive samples within the theoretical LoD of the system (280 copies/mL) were correctly detected within the range of the mean "Consensus" value of the commercial assay ± 1 Standard Deviation. One sample below the theoretical limit of detection of the system (103 copies/mL) was reported negative. Samples with titer below the limit of detection can be stochastically reported as positive or negative.

Further tests were carried out using as calibrated reference material a panel of dilutions of HSV1 within the concentration limit (QCMD 2012 Herpes Simplex Virus DNA EQA Panel, Qnostics Ltd, UK). Each sample was tested carrying out the whole analysis procedure: extraction with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «ELITe GALAXY»				
Sample	Commercial assay consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
HSV12-01	Negative, NA	-	0/2	-
HSV12-02	HSV1, 3.910	0.582	2/2	3.895
HSV12-03	HSV2, 1.948	0.305	0/2	-
HSV12-04	HSV1, 3.680	0.547	2/2	3.867
HSV12-05	HSV2, 1.352	0.629	0/2	-
HSV12-06	HSV1, 2.318	0.441	2/2	2.215
HSV12-07	HSV1, 2.014	0.296	1/2	1.962
HSV12-08	HSV2, 3.424	1.098	0/2	-
HSV12-09	Negative, NA	-	0/2	-
HSV12-10	HSV2, 3.417	1.042	0/2	-

All negative samples were correctly reported. The positive samples were correctly detected within the range of the mean "Consensus" value of the commercial assay ± 1 Standard Deviation. One out of two replicates of sample HSV12-07 was not detected. The discrepant result can be explained by the low sample titre (103.28 gEq/mL) being around the limit of detection of the method. The sample was anyway reported as positive.

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

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

The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probe in the alignment of the sequences available in the database for the gpD gene of HSV1 showed conservation and absence of significant mutations.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested using some clinical samples of cerebrospinal fluid and whole blood collected in EDTA, tested positives for HSV1 DNA.

The diagnostic sensitivity was evaluated using as reference material 21 negative cerebrospinal fluid samples, that were spiked to lower titre for HSV1 DNA adding HSV07-02, HSV07-08, HSV07-10 samples from QCMD 2007 Herpes simplex virus Proficiency Panel (Qnostics Ltd, UK) and 20 samples of whole blood collected in EDTA from normal donors presumably negative for HSV1 DNA (Biological Sample Library Europe S.A.S., Lyon, France), spiked to a low titre for HSV1 DNA by adding HSV08-03 sample from QCMD 2008 Herpes simplex virus EQA Panel (Qnostics Ltd, UK). Each sample of Cerebrospinal fluid was used to carrying out the whole analysis, extraction with **«EXTRAgen»** and amplification by ELITechGroup S.p.A. products. Each sample of Whole blood was used to carrying out the whole analysis, extraction with **«EXTRAblood»** and amplification by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid spiked for HSV1 DNA	21	21	0
Whole blood collected in EDTA spiked for HSV1 DNA	20	20	0

All spiked samples were correctly detected as positive for HSV1 DNA.
The diagnostic sensitivity of the assay in this test was equal to 100%.

The diagnostic sensitivity was evaluated using 22 samples of cerebrospinal fluid negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV08-07 sample, from QCMD 2008 Herpes Simplex Virus EQA Panel (Qnostics Ltd, UK), 30 samples of plasma negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV08-03 sample, from QCMD 2008 Herpes Simplex Virus EQA Panel (Qnostics Ltd, UK) and 30 whole blood samples negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV08-03, from QCMD 2008 Herpes Simplex Virus EQA Panel (Qnostics Ltd, UK). Each sample was used to carry out the whole analysis procedure: extraction with «**ELITe STAR**» and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid spiked for HSV1 DNA	22	22	0
Plasma collected in EDTA spiked for HSV1 DNA	30	30	0
Whole blood collected in EDTA spiked for HSV1 DNA	30	27	1

Two HSV1 positive samples resulted invalid.
One sample reported a negative result. The diagnostic sensitivity of the assay in this test was equal to 98.7%.

The diagnostic sensitivity was evaluated using 20 samples of cerebrospinal fluid negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV08-07 sample, from QCMD 2008 Herpes Simplex Virus EQA Panel (Qnostics Ltd, UK), 30 samples of plasma negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV08-07 sample, from QCMD 2008 Herpes Simplex Virus DNA EQA Panel, (Qnostics Ltd, UK) and 30 whole blood samples negative for HSV1 DNA, that were spiked for HSV1 DNA adding HSV08-07 sample, from QCMD 2008 Herpes Simplex Virus DNA EQA Panel, (Qnostics Ltd, UK) and HSV10-07 sample, from QCMD 2010 Herpes Simplex Human virus DNA EQA Panel, (Qnostics Ltd, UK). Each sample was used to carry out the whole analysis procedure: extraction with «**ELITe GALAXY**» and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid spiked for HSV1 DNA	20	20	0
Plasma collected in EDTA spiked for HSV1 DNA	30	30	0
Whole blood collected in EDTA spiked for HSV1 DNA	30	30	0

All spiked samples were correctly detected as positive for HSV1 DNA.
The diagnostic sensitivity of the assay in this test was equal to 100 %.

Analytical specificity: absence of cross-reactivity potential interfering markers

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

The analysis of the alignment of the sequences of the primers and of the fluorescent probe with the sequences available in databases for organisms other than HSV1, including the HSV2 and the VZV complete genomes, the human herpetic virus that is most similar to HSV1, showed their specificity and the absence of significant homology.

The analytical specificity of the assay, as absence of cross-reactivity with other potential interference markers, was checked by testing a proficiency panel.

The analytical specificity was evaluated using as calibrated reference material a panel including HSV2 positive and VZV positive samples (QCMD 2007 Herpes simplex virus EQA Panel, Qnostics Ltd, UK). Each sample was tested in duplicates carrying out the whole analysis procedure, extraction and amplification by ELITechGroup S.p.A. products.

The results are reported in the paragraph “Analytical sensitivity: reproducibility with calibrated reference material”.

No cross-reactivity was detected with HSV2 and VZV positive samples.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was tested using some HSV1 DNA negative clinical samples of cerebrospinal fluid and whole blood collected in EDTA, tested negatives for HSV1 DNA.

The diagnostic specificity was evaluated using as reference material 28 cerebrospinal fluid samples that were negative for HSV1 DNA (tested with a real time amplification CE IVD product) and 24 sample of whole blood collected in EDTA from presumably negative donors for HSV1 DNA (Biological Sample Library Europe S.A.S., Lyon, France). Each sample of Cerebrospinal fluid was used to carrying out the whole analysis, extraction with **«EXTRAgen»** and amplification by ELITechGroup S.p.A. products. Each sample of Whole blood was used to carrying out the whole analysis, extraction with **«EXTRAblood»** and amplification by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid negative for HSV1 DNA	28	0	27
Whole blood collected in EDTA negative for HSV1 DNA	24	1	23

One sample of cerebrospinal fluid gave an invalid result, possibly for the presence of an inhibitor.

One whole blood sample gave a discordant result with a very low viral titre (lesser than 1 gEq / reaction). This sample, resulted negative but valid in an independent amplification session, is lower than the detection limit of the product that gives randomly either negative or positive results in different sessions.

This discordant result may be explained considering whole blood sample is just presumably negative for HSV1 DNA, a virus largely widespread in the population in a latent way. The diagnostic specificity of the assay in this test was equal to 98.0%.

The diagnostic specificity was evaluated using 24 cerebrospinal fluid samples that were negative for HSV1 DNA, 30 plasma samples collected in EDTA that were negative for HSV1 DNA and 30 whole blood samples collected in EDTA that were negative for HSV1 DNA (tested with a real time amplification CE IVD product). Each sample was used to carry out the whole analysis procedure: extraction with **«ELITe STAR»** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid negative for HSV1 DNA	24	0	24
Plasma collected in EDTA negative for HSV1 DNA	30	0	30
Whole blood collected in EDTA negative for HSV1 DNA	30	1	28

A HSV1 negative sample resulted invalid.

One sample reported a positive result with a viral titre equal to 65 gEq/mL. Due to the low viral titre, the samples couldn't have been detected during the analysis with the reference method. The diagnostic specificity of the assay in this test was equal to 98.8 %

The diagnostic specificity was evaluated using 22 cerebrospinal fluid samples that were negative for HSV1 DNA, 34 plasma samples collected in EDTA that were presumably negative for HSV1 DNA and 36 whole blood samples collected in EDTA that were presumably negative for HSV1 DNA. Each sample was used to carrying out the whole analysis procedure: extraction with **«ELITe GALAXY»** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid negative for HSV1 DNA	22	0	22
Plasma collected in EDTA negative for HSV1 DNA	34	0	34
Whole blood collected in EDTA negative for HSV1 DNA	36	0	36

All samples were correctly detected as negative for HSV1 DNA.

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

Roche cobas z 480 analyzer

SAMPLES AND CONTROLS

Samples

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

Whole blood collected in EDTA

The whole blood samples for DNA extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of three days, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods. It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

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

Plasma collected in EDTA

The plasma samples for nucleic acid extraction must be collected in EDTA according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of three days, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods. It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

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

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: cerebrospinal fluid, suspensions of leucocytes, suspensions of granulocytes and amniotic fluid.

Interfering substances

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

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

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

Amplification controls

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

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

For the positive control, use the **«HSV1 - ELITe Positive Control»** product or alternatively **«HSV1 - ELITe Positive Control RF»** product or the **«HSV1 ELITe Standard»** product.

Quality controls

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

PROCEDURE

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

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

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

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

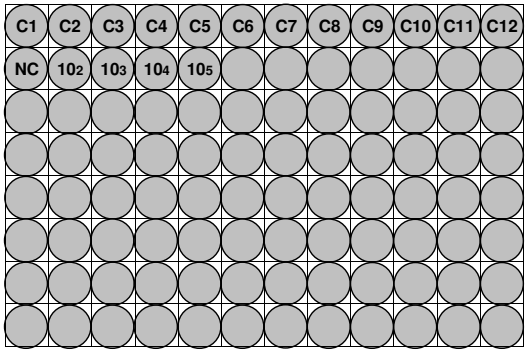
Note: fluorescence acquisition occurs individually, set Ramp Rate (°C/sec) to 4.4 °C/sec.

- select the signal detection channels: "detector" for the HSV1 sensor with "channel FAM 465-510" and "detector" for the IC internal control sensor with "channel VIC 540-580";

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

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

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



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

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

Before starting the session, it is necessary to:
- retrieve and thaw the test tubes containing the samples to be analyzed. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve and thaw the test tubes containing **HSV1 Q - PCR Mix** required for the session, remembering that the contents of each tube is enough to perform **25 reactions**. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve and thaw the test tubes containing **HSV1 – Positive Control** or alternatively **HSV1 - ELITe Positive Control RF** or **HSV1 Q - PCR Standard**. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve the **AD-plate** to be used in the session, making sure you handle it wearing dust-free gloves and do not damage the wells.

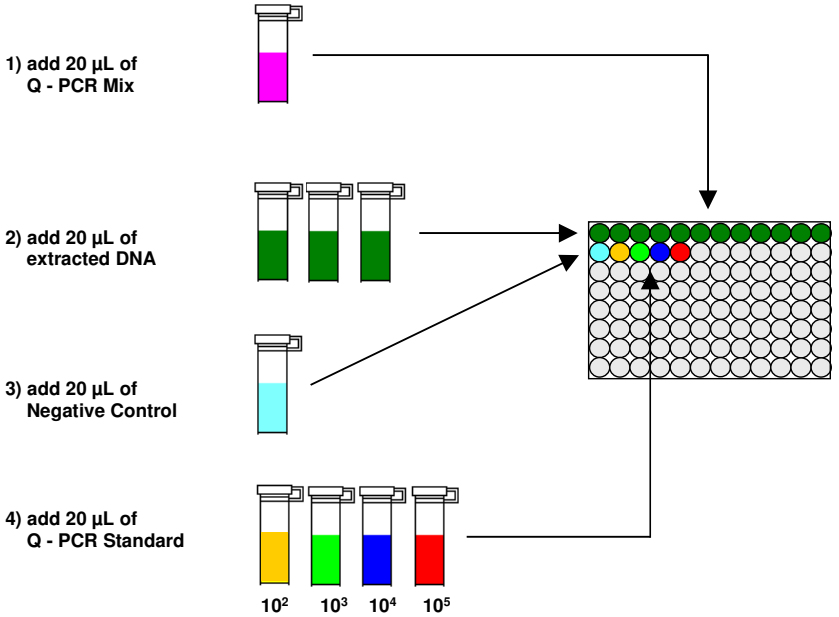
1. Without creating any bubbles and depositing it precisely on the bottom, transfer **20 µL** of reaction mixture **HSV1 Q - PCR Mix** into the wells on the **AD-plate** as previously established in the **Work Plan**.

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

2. Depositing it precisely into the reaction mixture, transfer **20 µL** of **extracted DNA** from the first sample in the corresponding well on the **AD-plate** as previously established in the **Work Plan**. Mix the sample well by pipetting the **extracted DNA** three times into the reaction mixture. Be sure not to create any bubbles. Proceed in the same manner with all the other **extracted DNA**.
3. Depositing it precisely into the reaction mixture, transfer **20 µL** of **ultra-pure molecular biology grade water** (not supplied with the product) into the well on the **AD-plate** containing the negative amplification control as previously established in the **Work Plan**. Mix the negative control well by pipetting the **ultra-pure molecular biology grade water** three times into the reaction mixture. Be sure not to create any bubbles.
4. On the basis of the result required (qualitative or quantitative), one of these two options must be followed:
   - When a **qualitative** result is required (detection of HSV1 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **HSV1 - Positive Control** or alternatively **«HSV1 - ELITe Positive Control RF»** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the **HSV1 - Positive Control** three times into the reaction mixture. Avoid creating bubbles.
   - When a **quantitative** result is required (quantification of HSV2 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **HSV1 Q - PCR Standard 10^2** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **HSV1 Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **HSV1 Q - PCR Standards** (**10^3, 10^4, 10^5**)..
5. Carefully seal the **AD-plate** using the **Sealing Film.**
6. Transfer the **AD-plate** into the real-time Thermal Cycler in the amplification/detection of amplification products area and start the amplification thermal cycle, saving the session settings under a unique and recognizable identifier (e.g. "year-month-day-HSV1-EGSpA").

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

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



Qualitative results analysis

The emitted fluorescence values recorded by the HSV1 detector and Internal Control (IC) detector during the amplification reactions must be analyzed by the instrument's software.

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

Select the group of samples to be analyzed

In accordance with the instrument's documentation, before starting the analysis you must:
- manually enter the calculation range (Background button) for the **Background Fluorescence Level** from cycle 2 to cycle 6.

For **Plasma** samples

- manually set the **Threshold** and **Noiseband** for the FAM "HSV1" detector to **0.55**;
- manually set the **Threshold** and **Noiseband** for the VIC "IC" detector to **1.2**

For **Whole blood** samples

- manually set the **Threshold** and **Noiseband** for the FAM "HSV1" detector to **0.80**;
- manually set the **Threshold** and **Noiseband** for the VIC "IC" detector to **1.5**

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

The **Ct** values for HSV1 in the amplification reactions of the four **Q - PCR Standard** are used to calculate the **Standard Curve** (Results > Standard Curve) of that amplification session and to validate the amplification and detection as shown in the following table:

Reaction Q - PCR Standard 10^5 "HSV1" detector	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for HSV1, the target DNA was not correctly detected. This means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mix or of the positive control, degradation of the reaction mix or of the positive control, incorrect setting of the position of the positive control, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

*** Note:** When this product is used for the quantification of HSV1 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**).

During the **Negative Control** amplification reaction, the value of **Ct** for HSV1 (Analysis window) is used to validate amplification and detection as shown in the following table:

Negative Control Reaction "HSV1" detector	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative Control** amplification reaction is other than **Ct Undetermined** for HSV1, the presence of the DNA target has been detected. Problems occurred during the amplification stage (contamination) which can lead to incorrect results and false positives. The session is invalid and must be repeated from the amplification stage.

During the amplification reactions for each **sample**, the value of **Ct** for HSV1 is used to detect the presence of the DNA target, whilst the value of **Ct** for the Internal Control is used to validate the extraction, amplification and detection.

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

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

Sample reaction		Sample suitability	Assay result	HSV1 DNA
"HSV1" detector	"IC" detector			
Ct Undetermined	Ct > 35 or Ct Undetermined	not suitable	invalid	-
	Ct ≤ 35	suitable	valid, negative	NOT DETECTED
Ct Determined	Ct > 35 or Ct Undetermined	suitable	valid, positive	DETECTED
	Ct ≤ 35	suitable	valid, positive	DETECTED

If the result of a sample's amplification reaction is **Ct Undetermined** for HSV1 and **Ct > 35** or **Ct Undetermined** for the Internal Control, it was not possible to detect the Internal Control DNA efficiently. In this case, problems occurred during the amplification stage (inefficient or null amplification) or in the extraction stage (degraded sample DNA, sample with insufficient number of cells, loss of DNA during extraction or presence of inhibitors in the extracted DNA) which can cause incorrect results and false negatives. The sample is not suitable, the assay is not valid and must be repeated starting from the extraction of a new sample.

If the result of a sample's amplification reaction is **Ct Undetermined** for HSV1 and **Ct ≤ 35** for the Internal Control, the HSV1 DNA was not detected in the DNA extracted from the sample but it cannot be excluded that the HSV1 DNA is present at a concentration lower than the product's limit of detection (see Performance Characteristics). In this case, the result would constitute a false negative.

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

Note: When HSV1 DNA is detected during the amplification reaction of a sample, amplification of the Internal Control can produce a result of Ct > 35 or Ct Undetermined. In fact, the low-efficiency Internal Control amplification reaction can be eliminated from the competition with the high-efficiency HSV1 reaction. In this case, the sample is then suitable and the positive assay result is valid.

Quantitative results analysis

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

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

The **Ct** values for HSV1 in the amplification reactions of each **sample** and the **Standard Curve** (**Standard Curve** button) from the amplification session are used to calculate the **Quantity** of DNA target present in the amplification reactions relating to the samples.

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

Sample result FAM "HSV1" detector	HSV1 copies per reaction
Quantity > 1×10^6	**GREATER THAN 1,000,000**
$1.0 \times 10^1 \leq$ Quantity $\leq 1 \times 10^6$	**= Quantity**
Quantity < 1.0×10^1	**LESS THAN 10**

The results (**Quantity**) relating to each **sample** (Analysis window) are used to calculate the **copies** of HSV1 present in the source sample (**Nc**) according to this formula:

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

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

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

Simplified formula for whole blood and MagNA Pure 24
Nc (copies / mL) = 25 x Quantity

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PERFORMANCE CHARACTERISTICS

Analytical sensitivity: limit of detection

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

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

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

Analytical sensitivity: linear measuring range

The analytical sensitivity of this assay, as linear measuring range, allows for the quantification from around 1,000,000 to 10 copies in 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of this assay was evaluated using a panel of dilutions (1 Log_{10} between one dilution and the next) of plasmid DNA containing the amplification product, whose initial concentration was measured using a spectrophotometer. The points of the panel from 10^7 molecules per reaction to 10^1 molecules per reaction were used in 9 replicates to carry out amplification using ELITechGroup S.p.A. products. Analysis of the obtained data, performed using linear regression, showed that the assay has a linear response for all panel points (linear correlation coefficient greater than 0.99).

The lower limit of the linear measuring range was set at around 10 copies / reaction within one logarithm from the lowest concentration of Q - PCR Standard amplification standard (10^2 copies / 20 µL).

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

The results are shown in the following table.

Linear measuring range using MagNA Pure 24		
	Lower limit	Upper limit
copies / mL	**25**	**25,000,000**
copies / reaction	**10**	**10,000,000**

Conversions from copies / mL to copies / reaction and vice versa were calculated as shown on page 39.

Analytical sensitivity: Precision and Accuracy

The precision of this assay, in terms of the variability of the results obtained in the same amplification session using different replicates of a sample, allowed to obtain a mean Variation Coefficient percentage (VC%) of the values of Ct lower than 2% in the range from 10^6 molecules to 10^1 molecules in 20 µL of DNA added to the amplification reaction.

The precision of this assay, in terms of the variability of the results obtained in the same amplification session using different replicates of a sample, allowed to obtain a mean Variation Coefficient percentage (VC%) of the measured quantities of around 11% in the range from 10^6 molecules to 10^1 molecules in 20 µL of DNA added to the amplification reaction.

The accuracy of this assay, in terms of the difference between the mean of the results obtained in the same amplification session using different replicates of a sample and the sample's theoretical concentration value, allowed to obtain a mean Inaccuracy percentage of the measured Log quantity of around 3% in the range from 10^6 molecules to 10^1 molecules in 20 µL of DNA added to the amplification reaction.

Precision and accuracy were determined using the data obtained during the experiments assessing the linear measuring range.

Analytical sensitivity: reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of value of a calibrated reference material, was evaluated using as reference material the «HSV1 Molecular 'Q' Panel» (Qnostics Ltd, UK). Each sample of the panel was tested in 4 replicates carrying out the whole analysis procedure: extraction using the **MagNA Pure 24** automatic extraction system and amplification using ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «MagNA Pure 24»	
Sample	**Positives / Replicates**
HSV1MQP01-High	2/2
HSV1MQP01-Medium	2/2
HSV1MQP01-Low	2/2
HSV1MQP01-Negative	0/2

All samples were correctly detected.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity was evaluated using as reference material 30 samples of whole blood collected in EDTA, negative for HSV1 DNA, which were spiked for HSV1 DNA adding HSV1MQP01-High sample (Qnostics Ltd, UK), and 29 samples of plasma collected in EDTA, negative for HSV1 DNA, which were spiked for HSV1 DNA adding HSV1MQP01-High sample (Qnostics Ltd, UK).

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

Samples	N	positives	negatives
Whole blood collected in EDTA spiked for HSV1 DNA	30	30	0
Plasma collected in EDTA spiked for HSV1 DNA	29	29	0

All samples were valid at first test and confirmed positive for HSV1 DNA.

The total diagnostic sensitivity of the assay was 100%.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity was evaluated using as reference material 40 samples of whole blood collected in EDTA presumably negative for HSV1 DNA and 34 samples of plasma collected in EDTA presumably negative for HSV1 DNA.

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

Samples	N	positives	negatives
Whole blood collected in EDTA presumably negative for HSV1 DNA	40	1	39
Plasma collected in EDTA presumably negative for HSV1 DNA	34	0	34

All whole blood samples were valid at first test and thirty-nine (39) out of forty (40) whole blood samples were confirmed negative for HSV1 DNA, while one sample showed a positive discrepant result.

The diagnostic specificity of the assay associated to whole blood samples was 98%.

All plasma samples were valid at first test and confirmed negative for HSV1 DNA.

The diagnostic specificity of the assay associated to plasma samples was 100%.

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Robustness: invalid results using clinical samples

The robustness of this assay, in terms of the evaluation of invalid results using clinical samples in first analysis, was verified by analyzing clinical samples.

The number of invalid samples was verified using the results of clinical samples which were negative and positive for HSV1 DNA having been analyzed using the **MagNA Pure 24** automatic extraction system and through amplification using ELITechGroup S.p.A. products. The results are shown in the following table.

Samples	N	Invalid	%
Whole blood collected in EDTA	70	0	0
Plasma collected in EDTA	63	0	0

Note: The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instruments are recorded in Section 7 of the Product Technical File for the " HSV1 ELITe MGB®Kit", FTP RTS031PLD.

REFERENCES

E. Aurelius et al. (1993) *J. Med. Virology* 39: 179 - 186
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30

PROCEDURE LIMITATIONS

Use this product only with DNA extracted from the following clinical samples: cerebrospinal fluid (CSF), whole blood collected in EDTA, plasma collected in EDTA.

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.

Use the different platforms only with the clinical samples indicated in the section "Samples and Controls".

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

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

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the HSV1 positive clinical samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations. However, the cross-contaminations can be avoided only by good laboratory practices and following carefully these instructions for use.

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

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

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

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

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

Due to inherent differences between technologies, it is recommended that users perform method

correlation studies to estimate technology differences prior to switching to a new technology.

A negative result obtained with this product means that the HSV1 DNA is not detected in the DNA extracted from the sample; but it cannot be excluded that the HSV1 DNA has a lower titre than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

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

Possible polymorphisms within the region of the viral genome covered by the product primers and probes may impair detection and quantification of HSV1 DNA.

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

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

TROUBLESHOOTING

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

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

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

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

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but different from that of the other samples and of the standards or positive control.	Check for detector FAM Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target DNA with a possible mutation. The target DNA of the sample should be sequenced to confirm mutation.
With ELITe InGenius and ELITe BeGenius®: Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - repeat the amplification of eluted sample in molecular biology grade water, in a "PCR only" session or - repeat the extraction with a dilution of the primary sample in molecular biology grade water, in a "Extract + PCR" session.

SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch code.


Use by (last day of month).

IVD *in vitro* diagnostic medical device.

CE Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.


Contains sufficient for "N" tests.


Attention, consult instructions for use.

CONT Contents.


Keep away from sunlight.


Manufacturer.

HSV1 ELITe MGB® Kit
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NOTICE TO PURCHASER: LIMITED LICENSE

This product content LTC licensed reagents.

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

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

HSV1 ELITe MGB® kit used with Genius series platforms

Ref: RTS031PLD

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

A. Intended use

The «**HSV1 ELITe MGB® Kit**» product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of type 1 Herpes Simplex human virus (HSV1)** in DNA samples extracted from cerebrospinal fluid (CSF), whole blood collected in EDTA, plasma collected in EDTA.
The product is intended for use in the diagnosis and monitoring of HSV1 infections alongside clinical data of the patient and other laboratory tests outcomes. The assay is CE-IVD validated in combination with the instruments **ELITe InGenius®** and **ELITe BeGenius®**.

B. Amplified sequence

Target	Gene	Fluorophore
HSV1	Glicoprotein D (gpD)	FAM
Internal Control	Human beta globin gene	AP525

C. Validated matrix

› **Whole Blood** EDTA, **Plasma EDTA, CSF**

D. Kit content

HSV1 Q-PCR Mix
 X 4
Ready-to-use PCR Master Mix 4 tubes of 540 µL 96 reactions per kit 5 freeze-thaw cycles per tube

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

E. Material required not provided in the kit

› **ELITe InGenius®** instrument: INT030
› **ELITe BeGenius® instrument:** INT040
› **ELITe InGenius SP200** extraction cartridge: INT032SP200
› **ELITe InGenius PCR Cassette** amplification cartridge: INT035PCR
› **ELITe InGenius SP200 Consumable Set** consumable for extraction: INT032CS
› **HSV1 - ELITe Standard:** STD031PLD
› **HSV1 - ELITe Positive Control:** CTR031PLD
› **CPE – Internal Control:** CTRCPE
› **ELITe InGenius Waste Box:** F2102-000
› **300 µL Filter Tips Axygen:** TF-350-L-R-S
› **1000 µL Filter Tips Tecan :** 30180118

F. Protocol

› Sample volume	200 µL
› CPE Internal Control volume	10 µL
› Total eluate volume	100 µL
› PCR eluate input volume	20 µL
› Q-PCR Mix volume	20 µL
› Unit of quantitative result	Copies/mL
› Frequency of controls	15 days
› Frequency of calibration	60 days

G. Performance ELITe InGenius® and ELITe BeGenius®

Matrix	Limit of Detection	Linearity Range	Diagnostic Sensitivity	Diagnostic Specificity
Whole Blood	**211 cp / mL**	**211 – 25,000,000**	**98%** 49/50*	**100%** 34/34*
Plasma	**250 cp /mL**	**250 – 25,000,000**	**100%** 30/30*	**100%** 38/38*
CSF	**250 cp / mL**	**250 – 25,000,000**	**100%** 20/20*	**100%** 22/22*

**confirmed samples/ tested samples*

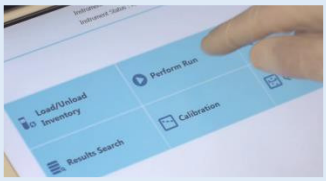
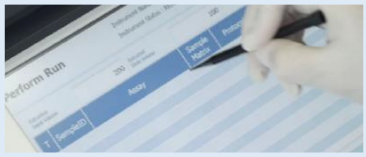
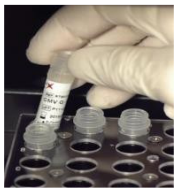
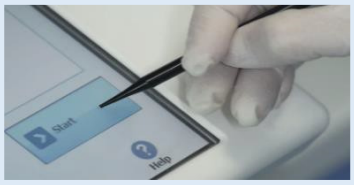
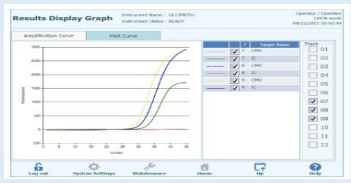
H. Procedures ELITe InGenius®

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

Before analysis

1. Switch on ELITe InGenius Identification with username and password Select the mode "Closed"	**2.** Verify calibrators: HSV1 Q-PCR Standard in the "Calibration menu" Verify controls: HSV1 positve and negative controls in the "Control menu" *N.B:* Both have been run, approved and not expired	**3.** Thaw the Q- PCR-Mix and the Internal Control tubes Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

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

Procedure 2 - PCR only

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

Procedure 3 - Extraction only

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

L. Procedures ELITe BeGenius®

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

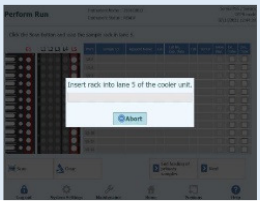
Before analysis

1. Switch on ELITe BeGenius Identification with username and password Select the mode "Closed"	**2.** Verify calibrators: HSV1 Q-PCR standard in the "Calibration menu" Verify controls: HSV1 pos. and neg. controls in the "Control menu" *NB:* Both have been run, approved and not expired	**3.** Thaw the HSV1 Q- PCR-Mix and the CPE Internal Control tubes Vortex gently Spin down 5 sec

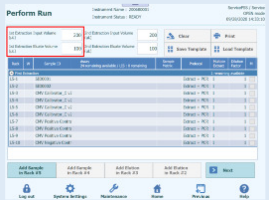
Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen and then click on the run mode «Extraction and PCR»

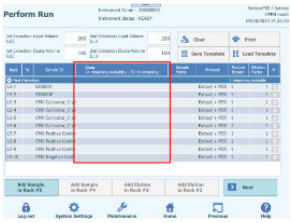
2. Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active



3. Verify the extraction volumes: Input: "200 µL", Eluate: "100 µL"

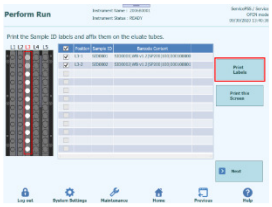


4. Select the "Assay protocol" of interest



Note: if a second extraction is performed repeat steps from 2 to 4

5. Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area



6. Load the Q-PCR-Mix and the CPE Internal Control in Reagent Rack and insert it in the cooling area

7. Load: Filter Tips, Extraction rack, and PCR rack

8. Close the door. Start the run

9. View, approve and store the results

Procedure 2 - PCR only

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

Procedure 3 - Extraction only

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

HSV1 ELITe MGB® Kit used with ABI PCR instrument

Ref: RTS031PLD

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

A. Intended use

The «**HSV1 ELITe MGB® Kit**» product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of type 1 Herpes Simplex human virus (HSV1)** in DNA samples extracted from cerebrospinal fluid (CSF), whole blood collected in EDTA, plasma collected in EDTA.
The product is intended for use in the diagnosis and monitoring of HSV1 infections alongside clinical data of the patient and other laboratory tests outcomes. The assay is CE-IVD validated in combination with **ABI PCR thermal cyclers** (Thermo-Fisher) and the following extraction systems: **ELITe STAR** (ELITechGroup), **ELITe GALAXY** (ELITechGroup), **easyMAG** (BioMérieux) or **QIAsymphony** (Qiagen).

B. Amplified sequence

Target	Gene	Fluorophore
HSV1	Glicoprotein D (gpD)	FAM
Internal Control	Human beta globin gene	AP525

C. Validated matrix

› **Whole blood** EDTA › **Plasma** EDTA › **Cerebrospinal fluid**

D. Kit content

HSV1 Q-PCR Mix
 X 4
Ready-to-use PCR Master Mix 4 tubes of 540 µL 100 reactions per kit 5 freeze-thaw cycles per tube

› Maximum shelf-life: 24 months
› Storage Temperature: - 20°C

E. Material required not provided in the kit

› **7500 Fast Dx and 7300 PCR Instrument**
› **ELITe STAR:** INT010
› **ELITe STAR 200 extraction kit:** INT011EX
› **ELITe GALAXY:** INT020
› **ELITe GALAXY 300 extraction kit:** INT021EX
›
› **HSV1 ELITe Standard:** STD031PLD
› **HSV1 - ELITe Positive Control:** CTR031PLD
› **CPE - Internal Control:** CTRCPE
› **easyMAG** - Generic protocol 2.0.1
› **QIAsymphony** - DNA Mini kit or DSP Virus/Pathogen Midi kit
› **Molecular biology grade water**

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
ELITe STAR - ABI	Whole blood	**10 gEq/reaction**	**96%** (27/28)*	**97%** (28/29)*
	Plasma	**10 gEq/reaction**	**100%** (30/30)*	**100%** (30/30)
	CSF	**10 gEq/reaction**	**100%** (22/22)*	**100%** (24/24)*
ELITe GALAXY - ABI	Whole blood	**211 gEq/mL**	**100%** (30/30)*	**100%** (36/36)*
	Plasma	**95 gEq/mL**	**100%** (30/30)*	**100%** (34/34)*
	CSF	**10 gEq/reaction**	**100%** (20/20)*	**100%** (22/22)*

**confirmed samples/tested samples*

G. Procedure

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

Extraction - Validated systems

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

Amplification - Settings of 7500 Fast Dx and 7300 PCR instruments

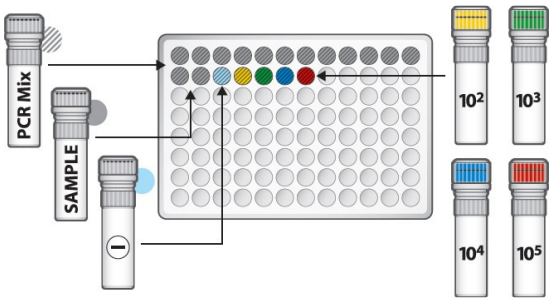
1. Switch on the thermal-cycler
2. Set "HSV1" detector with "FAM" and quencher "none"
3. Set "Internal Control" detector with "VIC" and quencher "none"
4. Set passive fluorescence as "Cy5" with 7500 Fast Dx and as "ROX" with 7300 instrument
5. Set up the thermal profile as indicated. Fluorescence acquisition must be set during hybridation step at 60°C

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

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

Amplification - PCR Set -up

1. Thaw HSV1 Q-PCR Mix and Q-PCR standard tubes
2. Mix gently and spin-down
3. Pipet **20 µL** of Q-PCR-Mix in all microplate wells in use
4. Add, **20 µL** of extracted DNA in sample wells, **20 µL** of molecular grade water in Negative Control well, and **20 µL** of the 4 Q-PCR standards in standard curve wells
   Each one has to be mixed by pipetting 3 times into the reaction mixture
5. Seal the microplate with the amplification sealing sheet
6. Transfer the microplate in the thermocycler and start



Amplification – Baseline and Threshold for qualitative analysis

Instrument	Baseline	HSV1 FAM	Internal Control VIC
7500 Fast Dx Real Time PCR	6 - 15	0.2	0.1
7300 Real Time PCR	6 - 15	0.1	0.05

Interpretation - Qualitative results

HSV1 Ct value	Internal Control Ct value	Interpretation
Determined	_	Positive
Undetermined	Ct ≤ 35	Negative
	Ct >35 or Undetermined	Invalid*

**Repeat the assay starting from the extraction*

Interpretation - Quantitative results

The HSV1 Ct value obtained for each sample and the standard curve generated are used to calculate the quantity of target DNA in the reaction.
The sample quantification ranges from approximately 10 to 10^6 cp/reaction or approximately from 100 to 10^7 cp/mL.

HSV-1 ELITe MGB® Kit used with Cobas-Z 480 PCR instruments

Ref.: RTS031PLD

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

A. Intended use

The «**HSV1 ELITe MGB® Kit**» product is part of a qualitative and quantitative nucleic acids amplification assay for **the detection and quantification of the DNA of type 1 Herpes Simplex human virus (HSV1)** in DNA samples extracted from cerebrospinal fluid (CSF), whole blood collected in EDTA, plasma collected in EDTA.

The product is intended for use in the diagnosis and monitoring of HSV1 infections alongside clinical data of the patient and other laboratory tests outcomes. The assay is CE-IVD validated in combination with **Cobas – Z 480 analyzer (Roche)** and the following extraction systems: **MagNA Pure 24 System**.

B. Amplified sequence

Target	Gene	Fluorophore
HSV1	**Glicoprotein D (gpD)**	**FAM**
Internal Control	**human beta globin gene**	**AP525**

C. Validated matrix

› **Whole blood** EDTA › **Plasma** EDTA

D. Kit content

HSV1 Q-PCR Mix
 X 4
Ready-to-use PCR Master Mix 4 tubes of 540 µL 100 reactions per kit 5 freeze-thaw cycles per tube

› Maximum shelf-life: 24 months
› Storage Temperature: - 20°C

E. Material required not provided in the kit

› **Cobas – Z 480 analyzer PCR Instrument**
› **MagNA Pure 24 System,** software 1.0
› **HSV1 - ELITe Positive Control:** CTR031PLD
› **HSV1 ELITe Standard:** STD031PLD
› **CPE Internal Control:** CTRCPE

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
MagNA Pure 24	Whole blood	**10 cp/reaction**	**100%** (29/29)*	**97.5%** (39/40)*
	Plasma	**10 cp/reaction**	**100%** (30/30)*	**100%** (34/34)*

**confirmed samples/tested samples*

G. Procedure

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

Extraction - Validated systems

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

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

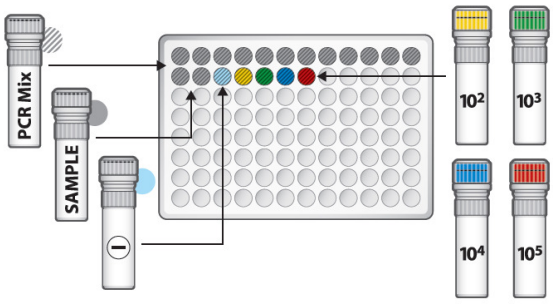
1. Switch on the thermal-cycler
2. Set "HSV1" detector with "FAM" and quencher "465 - 510"
3. Set "Internal Control" detector with "VIC" and quencher "540 -580"

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

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

Amplification - PCR Set-up

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



Amplification – Background and Threshold for qualitative analysis*

Instrument	Matrix	Background	HSV1 FAM	Internal Control VIC
Cobas-Z 480 PCR instruments	Plasma	2 - 6	0.55	1.2
Cobas-Z 480 PCR instruments	WB	2 - 6	0.8	1.5

**manually set the Threshold and Noiseband*

Interpretation - Qualitative results

HSV-1 Ct value	Internal Control Ct value	Interpretation
Determined	-	Positive
Undetermined	Ct ≤ 35	Negative
	Ct >35 or Undetermined	Invalid*

**Repeat the assay starting from the extraction*

Interpretation - Quantitative results

The HSV1 Ct value obtained for each sample and the standard curve generated are used to calculate the quantity of target DNA in the reaction.The sample quantification ranges from approximately 10 to 10^6 copies/reaction or approximately from 100 to 10^7 copies/mL.

WORK SHEET

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												