ELITechGroup
EMPOWERING IVD

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NOTICE of CHANGE dated 19/01/2022

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HHV8 ELITe MGB® Kit»
Ref. RTS038PLD

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

- *Update for the use of the product in association with «ELITe BeGenius®» instrument (REF INT040).*

- *Update of PERFORMANCE CHARACTERISTICS (pag.20):*

  - *Change in Limit of Detection (LoD)*
  - *Addition of Linear measuring range*
  - *Addition of Repeatability*
  - *Addition of Reproducibility*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

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

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HHV8 ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS038PLD

CE IVD

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INTENDED USE

The **«HHV8 ELITe MGB® Kit»** product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of Herpes human virus 8 (HHV8)** 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 HHV8 infections alongside clinical data of the patient and other laboratory test outcomes.

ASSAY PRINCIPLES

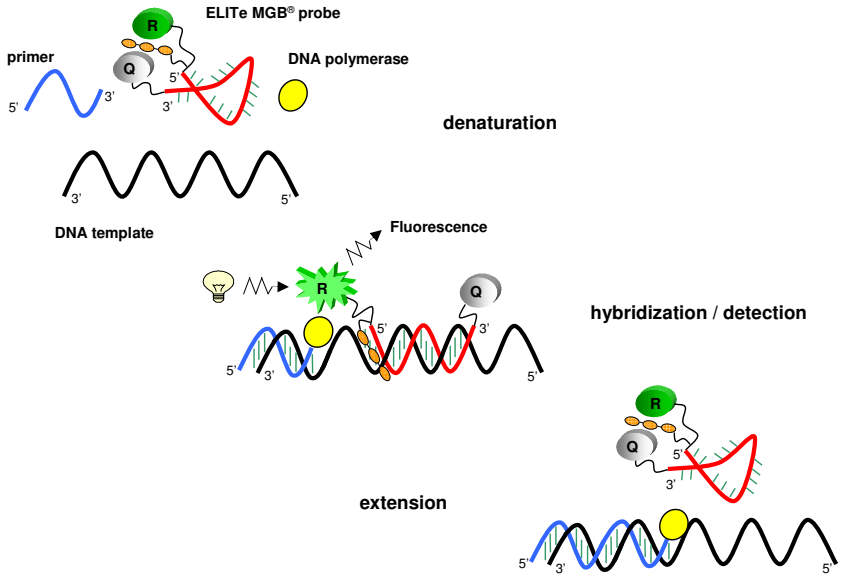
The assay consists of a real time amplification reaction with a programmable thermostat provided with a fluorescence detection optical system.

In each well, two amplification reactions are performed starting from DNA extracted from the samples being tested: a specific reaction for the region of the **minor capsid protein** gene (ORF26) of HHV8 and a specific reaction for a region of the human **beta Globin** gene (Internal Control of inhibition). The HHV8 specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the HHV8 amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with AP525 fluorophore (analogous to VIC), is activated when hybridizes with the specific product of the Internal Control amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data allows detecting the presence and the titre of HHV8 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 **«HHV8 ELITe MGB® Kit»** product supplies the **ready to use** complete mixture HHV8 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** in association with **«ELITe InGenius®»** and **«ELITe BeGenius®»** and **25 tests** in association with other systems.

The primers and the HHV8 specific probe (stabilized by MGB® group, labelled with FAM fluorophore and quenched by a non fluorescent molecule) are specific for the region of the **minor capsid protein** gene of HHV8.

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

The reaction mixture provides buffer, magnesium chloride, triphosphate nucleotides, AP593 fluorophore (used instead of ROX or Cy5) as passive reference for fluorescence normalisation, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product, the "hot start" DNA polymerase enzyme.

The product is sufficient for **96 tests in association with «ELITe InGenius®»** and **«ELITe BeGenius®»** 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
HHV8 Q - PCR Mix	complete reaction mixture	**4 x 540 µL**	**-**

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

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

OTHER PRODUCTS REQUIRED

The reagents for the extraction of DNA from the samples, the positive control of extraction, the positive control of the amplification and 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 product **«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 **«Filter tips 300»** (Axygen BioScience Inc., CA, USA, ref. 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 **«HHV8 ELITe_STD»**,
- for the positive control of amplification **«HHV8 ELITe_PC»**,
- for negative control of amplification **«HHV8 ELITe_NC»**,
- for samples analysis **«HHV8 ELITe_WB_200_100»** and **«HHV8 ELITe_PL_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 **«HHV8 ELITe_Be_STD»**,
for the positive control of amplification **«HHV8 ELITe_Be_PC»**,
for negative control of amplification **«HHV8 ELITe_Be_NC»**,
for samples analysis **«HHV8 ELITe_Be_WB_200_100» and «HHV8 ELITe_Be_PL_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 nucleic acid from biological 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 generic products **«NucliSENS® easyMAG® Reagents»** (bioMérieux SA, ref.s 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, it is also validated the use of the product **«QIAsymphony® DNA Mini Kit»** (QIAGEN GmbH, ref. 931236), kit for extraction of nucleic acid from biological samples, with the instrument **«QIAsymphony® SP/AS»** (QIAGEN GmbH, ref.s 9001297, 9001301) and related generic products.

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

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

If detection of HHV8 DNA is required (qualitative analysis), it is required the use of the product **«HHV8 - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR038PLD), positive control of plasmid DNA.

If detection and quantification of HHV8 DNA is required (quantitative analysis), it is required the use of product **«HHV8 ELITe Standard»** (ELITechGroup S.p.A., ref. STD038PLD), 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 in the product before running the assay.
While running the assay, follow the instructions provided in the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided in the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

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

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

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

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

The 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 **HHV8 Q - PCR Mix** must be stored at -20° C in the dark.

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The **HHV8 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 **HHV8 Q - 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.

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 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.

N.B.: 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 protocols **HHV8 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 on how to set up and perform the extraction procedure.

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.

N.B.: 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 **HHV8 ELITe_PL_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 on how to set up and perform the extraction procedure.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: cerebrospinal fluid and cutaneous biopsies.

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 **HHV8 ELITe Standard**, in association with the protocol **«HHV8 ELITe_STD»** for **ELITe InGenius**
as amplification Positive Control use the **HHV8 - ELITe Positive Control**, in association with the protocol **«HHV8 ELITe_PC»** for **ELITe InGenius**,
as amplification Negative Control, use molecular grade water (not provided with this kit) in association with the protocol **«HHV8 ELITe_NC»** for **ELITe InGenius.**

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 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.

ELITe InGenius PROCEDURE

The procedure to use the **«HHV8 - 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 (**HHV8 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 (**HHV8 - Positive Control**, **HHV8 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 and the ELITe InGenius instrument and the cited matrices.

The Assay protocols available for **«HHV8 ELITe MGB® Kit»**are described in the table below.

Assay protocols for «HHV8 ELITe MGB® Kit»			
Name	**Matrix**	**Report unitage**	**Characteristics**
HHV8 ELITE_WB_200_100	Whole Blood	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HHV8 ELITE_PL_200_100	Plasma	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO 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 **HHV8 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 **HHV8 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 **HHV8 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. HHV8 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 HHV8 Q-PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" button to continue the setup.
10. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
11. Load the "PCR 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.

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 HHV8 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 **HHV8 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. HHV8 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 **HHV8 Q-PCR Mix** on the "Inventory Block" selected by following the GUI instruction. Click "Next" to continue the setup.
9. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load the "PCR Cassettes" and the extracted Nucleic Acid samples following the GUI instruction. Click "Next" to continue the setup.

11. Close the instrument door.
12. Press "Start" to start the run.

After process completion, the **ELITe InGenius** 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 **HHV8 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 HHV8 Q - PCR Mix in the dark because this reagent is sensitive to the light.

2. Thaw **HHV8 Q - PCR Standard** tubes (Cal1: HHV8 Q - PCR Standards 10^2, Cal2: HHV8 Q - PCR Standards 10^3, Cal3: HHV8 Q - PCR Standards 10^4, Cal4: HHV8 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 "HHV8 ELITe_STD" in the "Assay" column and fill in the lot number and expiry date of HBV Q-PCR Standard.
7. Click "Next" to continue the setup.
8. Load the **HHV8 Q-PCR Mix** on the Inventory Block selected by following the GUI instruction. Click "Next" to continue the setup.
9. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load the "PCR Cassettes" and the **HHV8 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.

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 **HHV8 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 **HHV8 Q - PCR Mix** in the dark because this reagent is sensitive to the light**.**

2. Thaw **HHV8 - 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 “HHV8 ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of HHV8 Positive Control.
8. For the Negative Control, select the Assay Protocol “HHV8 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 **HHV8 Q-PCR Mix** on the “Inventory Block” selected by following the GUI instruction. Click “Next” 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 HHV8 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 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.

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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 **«HHV8 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 HHV8 probe ("HHV8") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “HHV8 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

T The fluorescence signals emitted by the specific HHV8 probe ("HHV8") 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 “HHV8 ELITe_PC” and “HHV8 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 HHV8 probe ("HHV8") and by the specific Internal Control probe ("IC") in each sample amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol.

N.B.: Before analysing any sample, it is absolutely mandatory to generate and to approve the Calibration curve and the result of 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**
HHV8 Q-PCR Standard	APPROVED
2) Positive Control	**Status**
HHV8 Positive Control	APPROVED
3) Negative Control	**Status**
HHV8 Negative Control	APPROVED

For each sample, the assay result is automatically interpreted by the system as established by the **ELITe InGenius software** algorithm and the Assay protocol parameters.

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

Result of Sample run	Interpretation
HHV8: DNA Detected, quantity equal to XXX copies / mL	**HHV8 DNA detected** within the measurement range of the assay, quantity as shown.
HHV8: DNA Detected, quantity below LLoQ copies / mL	**HHV8 DNA detected** below the lower limit of quantification of the assay
HHV8: DNA Detected, quantity beyond ULoQ copies / mL	**HHV8 DNA detected** beyond the upper limit of quantification of the assay
HHV8: DNA Not Detected or below LoD copies / mL	**HHV8 DNA not detected or below the Limit of Detection** of the assay.
Invalid - Retest Sample	**Not valid assay result** due to Internal Control failure (Incorrect extraction or inhibitor carry-over).

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

When the eluate volume is sufficient, the extracted sample can be retested via 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 suitable for analysis but in which it was not possible to detect HHV8 DNA are reported as: “DNA Not Detected or below LoD". In this case it cannot be excluded that the HHV8 DNA is present at a concentration below the limit of detection of the assay (see “Performance characteristics).

N.B.: 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 viewed as “Sample Report” and “Track Report”.

The “Sample Report” shows the details of a sample run sorted by Sample ID, i.e. by patient.

The “Track Report” shows the details of a sample run track by 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 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 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 **HHV8 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 on how to set up and perform the extraction procedure.

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 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 **HHV8 ELITe_Be_PL_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

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

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: cerebrospinal fluid and cutaneous biopsies.

Interfering substances

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

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

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

Amplification calibrators and amplification controls

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

as calibrator set, use the four concentration levels of the **HHV8 ELITe Standard**, in association with the protocol **«HHV8 ELITe_Be_STD»** for **ELITe BeGenius**,
as amplification Positive Control use the **HHV8 - ELITe Positive Control**, in association with the protocol **«HHV8 ELITe_Be_PC»** for **ELITe BeGenius**,
as amplification Negative Control, use molecular grade water (not provided with this kit) in association with the protocol **«HHV8 ELITe_Be_NC»** for **ELITe BeGenius**,

Note: ELITe BeGenius 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 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.

ELITe BeGenius PROCEDURE

The procedure to use the **«HHV8 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 (**HHV8 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 (**HHV8 - Positive Control**, **HHV8 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 **«HHV8 ELITe MGB® Kit»** are described in the table below.

Assay protocols for «HHV8 ELITe MGB Kit» and ELITe BeGenius

Name	Matrix	Report unitage	Characteristics
HHV8 ELITe_Be_WB_200_100	Whole Blood	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HHV8 ELITe_Be_PL_200_100	Plasma	copies/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL 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 **HHV8 ELITe MGB Kit** in association to the **ELITe BeGenius** can be used in order to perform:

A. Sample run,
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 HHV8 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. HHV8 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 HHV8 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 HHV8 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).

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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. HHV8 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 HHV8 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 HHV8 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 HHV8 Q - PCR Standard tubes (Cal1: HHV8 Q-PCR Standards 10^2, Cal2: HHV8 Q-PCR Standards 10^3, Cal3: HHV8 Q-PCR Standards 10^4, Cal4: HHV8 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 (HHV8 ELITe_Be_STD). Click “Next” button to continue the setup.
8. Load HHV8 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.

D. Positive Control and Negative Control run

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

1. Thaw a sufficient number of HHV8 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 HHV8 - 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 ofthe 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 (HHV8 ELITe_Be_PC and HHV8 ELITe_Be_NC). Click “Next” button to continue the setup.
9. Load HHV8 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 HHV8 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

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

The LoD of this assay was tested using plasmid DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmid DNA was diluted to a titre of about 10 copies / 20 µL in presence of plasmid DNA containing the internal control with a titre of 150,000 copies / 20 µL. This sample was tested in 18 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.

Samples	N	positive	negative
10 copies plasmid DNA HHV8+ 150,000 copies of internal control	18	18	0

The Limit of Detection (LoD) of HHV8 ELITe MGB® Kit was verified in association with **Plasma** and **Whole Blood** samples collected in EDTA 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 117 copies / mL on **ELITe InGenius** and **ELITe BeGenius** systems in “Extract + PCR” mode. The samples were spiked using the reference Material Human Herpes Virus Type 8 (HHV-8) Infectious Culture Fluid (ZeptoMetrix Corporation).

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	117 copies / mL	20	20	20	0

Limit of Detection for Whole Blood samples and ELITe BeGenius					
Sample	LoD	N	Valid	Positive	Negative
Whole blood collected in EDTA	117 copies / mL	20	20	20	0

The LoD value for HHV8 target was confirmed at 117 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 98 copies / mL on **ELITe InGenius** and **ELITe BeGenius** systems in "Extract + PCR" mode. The samples were spiked using the reference Material Human Herpes Virus Type 8 (HHV-8) Infectious Culture Fluid (ZeptoMetrix Corporation).

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	98 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	98 copies / mL	20	20	20	0

The LoD value for HHV8 target was confirmed at 98 copies / mL for Plasma collected in EDTA.

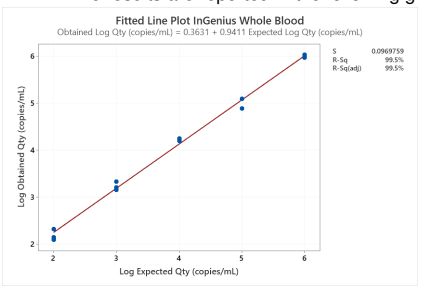
Linear measuring range and Limits of quantification

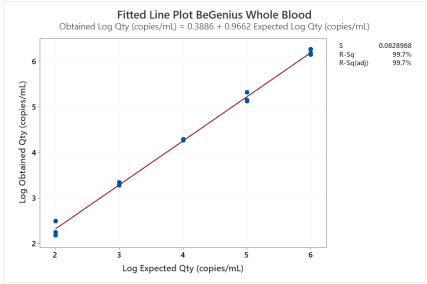
The linear measuring range of HHV8 ELITe MGB® Kit used in association with **Whole Blood** and **Plasma** collected in EDTA and **ELITe InGenius** and **ELITe BeGenius** was verified with a panel of HHV8 dilutions. The panel was prepared by diluting Human Herpes Virus Type 8 (HHV-8) Infectious Culture Fluid (ZeptoMetrix Corporation) in HHV8 DNA - negative matrices. The panel consisted of five dilution points from 1 x 10^6 copies / mL to about 2 x 10^2 copies / mL. Each sample of the panel was tested in 3 replicates.

For Whole Blood:

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 0.995 for **ELITe InGenius** and 0.997 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 0.1839 Log copies / mL for **ELITe InGenius** and 0.3488 Log copies / mL for **ELITe BeGenius**) and accurate (Bias equal to 0.0014 Log copies / mL for **ELITe InGenius** and 0.1329 Log copies / mL for **ELITe BeGenius**): 117 copies / mL.

The Upper Limit of Quantification (ULoQ) was set at, the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0302 Log copies / mL for **ELITe InGenius** and 0.06107 Log copies / mL for **ELITe BeGenius**) and accurate (Bias equal to 0.0078 Log copies / mL for **ELITe InGenius** and -0.1914 Log copies / mL for **ELITe BeGenius**): 1,000,000 copies / mL.

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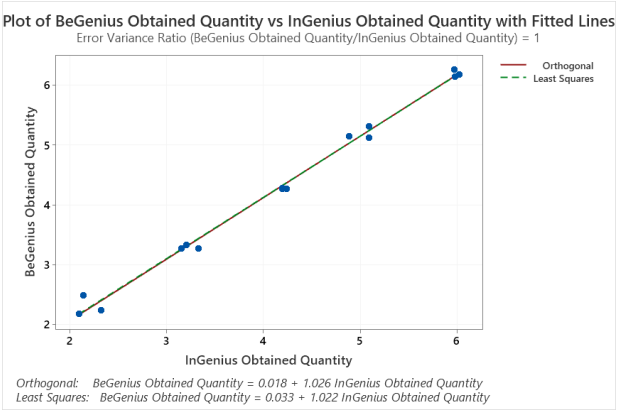
The final results are summarized in the following table.

Linear measuring range for Whole Blood samples and ELITe InGenius® and ELITe BeGenius®		
Unit of measure	lower limit	upper limit
copies / mL	**117**	**1,000,000**

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.

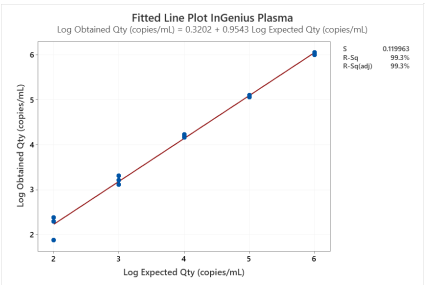


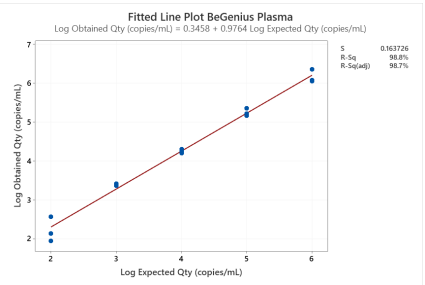
In this test, the orthogonal regression analysis generated a slope equal to 1.026 (95% CI: 0.980; 1.072) and an intercept equal 0.018 (95% CI: - 0.183; 0.219). The linear regression analysis generated a R2 of 0.993.

For Plasma:

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Plasma samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.993 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 0.1971 Log copies / mL for **ELITe InGenius** and 0.090 Log copies / mL for **ELITe BeGenius**) and accurate (Bias equal to 0.1537 Log copies / mL for **ELITe InGenius** and 0.2693 Log copies / mL for **ELITe BeGenius**): 98 copies / mL.

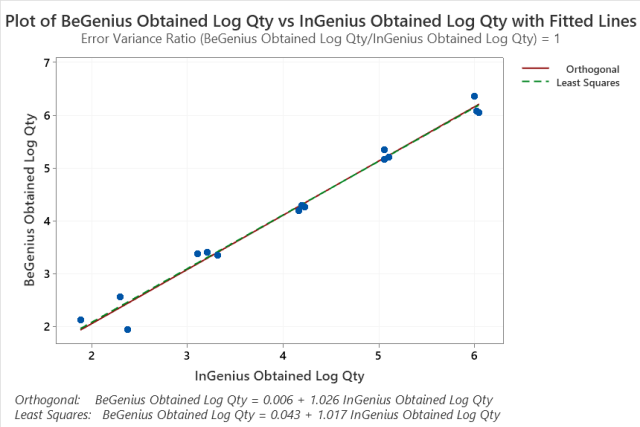
The Upper Limit of Quantification (ULoQ) was set at, the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0245 Log copies / mL for **ELITe InGenius** and 0.1731 Log copies / mL for **ELITe BeGenius**) and accurate (Bias equal to -0.0249 Log copies / mL for **ELITe InGenius** and -0.1647 Log copies / mL for **ELITe BeGenius**): 1,000,000 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	**98**	**1,000,000**

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 1.026 (95% CI: 0.953; 1.099) and an intercept equal to 0.006 (95% CI: - 0.312; 0.324). The linear regression analysis generated a R2 of 0.983.

Repeatability

The Repeatability of results obtained by the product HHV8 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 HHV8 certified reference material (Human Herpes Virus Type 8 (HHV-8) Infectious Culture Fluid ZeptoMetrix) at concentration of 3 x LoD (about 351 copies / mL) and of 10 x LoD (about 1170 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	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A:	N.A.	24 / 24	25.39	0.44	1.75
3 x LoD	8 / 8	35.66	0.39	1.10				
10 x LoD	8 / 8	33.95	0.28	0.84				

Inter – Session Repeatability ELITe InGenius								
Sample	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	25.22	0.93	3.69
3 x LoD	16 / 16	35.58	0.51	1.44				
10 x LoD	16 / 16	33.93	0.56	1.65				

In the Repeatability test on **ELITe InGenius**, the assay detected the HHV8 target as expected and showed low %CV of Ct values that did not exceed 1.65% for HHV8 and 3.69% 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	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24/24	28.70	0.95	3.29
3 x LoD	8 / 8	36.36	0.42	1.16				
10 x LoD	8 / 8	34.43	0.11	0.31				

Inter – Session Repeatability ELITe BeGenius								
Sample	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	28.54	1.16	4.05
3 x LoD	16 / 16	36.10	0.52	1.44				
10 x LoD	16 / 16	34.25	0.32	0.93				

In the Repeatability test on **ELITe BeGenius**, the assay detected the HHV8 target as expected and showed low %CV of Ct values that did not exceed 1.44% for HHV8 and 4.05% for Internal Control.

Reproducibility

The Reproducibility of results obtained by the product HHV8 ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HHV8 certified reference material (Human Herpes Virus Type 8 (HHV-8) Infectious Culture Fluid ZeptoMetrix) at concentration of 3 x LoD (about 351 copies / mL) and of 10 x LoD (about 1170 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, using the same lot and 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, using two different lots and the same instrument by the same operator. 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	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	24.40	1.49	6.11
3 x LoD	8 / 8	35.27	0.28	0.78				
10 x LoD	8 / 8	33.87	0.37	1.09				

Inter – Batch Repeatability ELITe InGenius								
Sample	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	25.69	1.03	3.99
3 x LoD	8 / 8	35.47	0.49	1.38				
10 x LoD	8 / 8	33.84	0.31	0.92				

In the Reproducibility test on **ELITe InGenius**, the assay detected the HHV8 target as expected and showed low %CV of Ct values that did not exceed 1.38% for HHV8 and 6.11%for Internal Control.

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

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

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

A summary of results is shown in the table below.

Inter – Instrument Repeatability ELITe BeGenius								
Sample	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	28.39	1.37	4.82
3 x LoD	8 / 8	36.19	0.61	1.70				
10 x LoD	8 / 8	24.24	0.42	1.22				

Inter – Batch Repeatability ELITe BeGenius								
Sample	**HHV8**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	28.83	1.02	3.55
3 x LoD	8 / 8	35.79	0.58	1.73				
10 x LoD	8 / 8	34.10	0.40	1.17				

In the Reproducibility test on **ELITe BeGenius**, the assay detected the HHV8 target as expected and showed low %CV of Ct values that did not exceed 1.7% for HHV8 and 4.8 %for Internal Control.

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Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analysing some clinical samples of Whole Blood and Plasma collected in EDTA positive for HHV8 DNA in association with **ELITe InGenius**. As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the diagnostic sensitivity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius**.

The diagnostic sensitivity was evaluated using 30 whole blood samples collected in EDTA that were negative for HHV8 DNA and that were spiked for HHV8 DNA adding HUMAN HERPES VIRUS TYPE 8 (HHV-8) Infectious Culture Fluid (ZeptoMetrix Corporation) with a titre of 750 copies /mL and 30 plasma samples collected in EDTA that were negative for HHV8 DNA and that were spiked for HHV8 DNA adding HHV8 Infectious Culture Fluid (ZeptoMetrix Corporation) with 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 with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked with HHV8 DNA	30	30	0
Plasma collected in EDTA spiked with HHV8 DNA	30	30	0

All samples were confirmed as positives.

The diagnostic sensitivity of the assay 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 analysing some clinical samples of Whole Blood and Plasma collected in EDTA negative for HHV8 DNA in association with **ELITe InGenius**. As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the diagnostic specificity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius**.

The diagnostic specificity was evaluated using 32 whole blood samples collected in EDTA from healthy donors that were presumably negative for HHV8 DNA and 32 plasma samples collected in EDTA from healthy donors that were presumably negative for HHV8 DNA.

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 negative for HHV8 DNA	32	0	32
Plasma collected in EDTA negative for HHV8 DNA	32	0	32

All samples were correctly detected.

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

OTHER SYSTEMS

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.

N.B.: when you carry out the DNA extraction from cerebrospinal fluid samples 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 minute 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 nucleic acids extraction must be collected in EDTA according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

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

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

N.B.: 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 million of leucocytes), elute the DNA in **100 µL** of elution buffer.

N.B.: 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_E100_S200_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.

N.B.: when you carry out the DNA extraction from whole blood with «**ELITe GALAXY**» and 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 of 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.

N.B.: when you carry out the DNA extraction from whole blood with the instrument **«NucliSENS® easyMAG®»**, please follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **100 µL** of sample in the 8 well strip, load the strip on the instrument and run the extraction without lysis incubation. After the instrument added **EasyMAG® Lysis Buffer**, without removing the strip, mix three times the strip content by the supplied multichannel pipet using the program number 3. Incubate for 10 minutes, then add the **NucliSENS® easyMAG® Magnetic Silica** to the strip content by the multichannel pipet using the program number 3 and proceed with the extraction. Elute the nucleic acids in **50 µL** of elution buffer.

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N.B.: when you carry out the DNA extraction from whole blood with the instrument **«QIAsymphony® SP/AS»** and the kit **«QIAsymphony® DNA Mini Kit»** with **software version 3.5**, use the extraction protocol **Virus Blood_200_V4_default IC** and follow these directions: the instrument is able to use a primary tube, sample volume required for the extraction is **200 µL**, it's always requested a minimum dead volume of 100 µL. Load on the instrument, in the "internal control" slot, the tubes containing buffer ATE, 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 **60 µL** (elution takes actually place in 90 µL, of which 60 µL are recovered). For details on the extraction procedure follow indications in the instruction for use manual of the kit.

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.

N.B.: 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_E100_S200_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.

N.B.: when you carry out the DNA extraction from plasma with **«ELITe GALAXY»** and 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 of 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.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: cutaneous biopsies.

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, use sterile bidistilled 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 **«HHV8 - ELITe Positive Control»** product or the **«HHV8 ELITe Standard»** product.

Quality controls

It is recommended to validate the whole analysis procedure of each extraction and amplification session by processing a negative tested sample and a positive tested sample or a calibrated reference material.

PROCEDURE

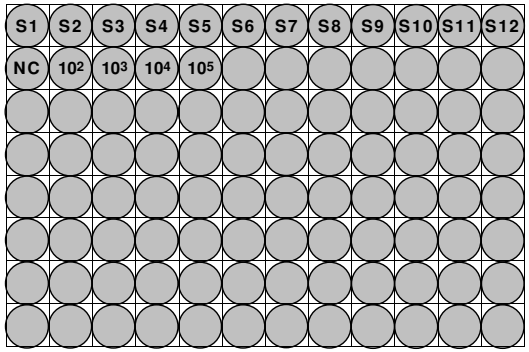
Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

When **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 HHV8 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “HHV8”;
- 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.

N.B.: 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**;

N.B.: 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 HHV8 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “HHV8”;
- 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.

N.B.: 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**;

N.B.: 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.

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

Before starting the session, it is necessary to:
- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **HHV8 Q - PCR Mix** tubes required for the session, remembering that each tube is sufficient for preparing **25 reactions**. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **HHV8 - Positive Control** or the **HHV8 Q - PCR Standard** tubes. Mix them gently, spin down the content for 5 seconds and keep them on ice;
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powder-free gloves and not to damage the wells.

1. Accurately pipet **20 µL** of **HHV8 Q - PCR Mix** on the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

N.B.: 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 **DNA extract** 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:

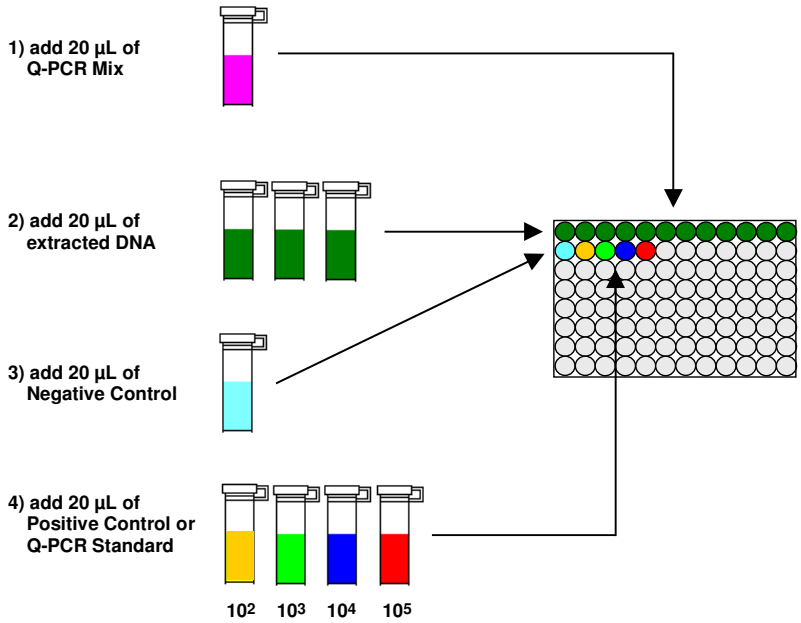
   - When a **qualitative** result is required (detection of HHV8 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **HHV8 - Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the volume of 20 µL three times into the reaction mixture. Avoid creating bubbles.

   - When a **quantitative** result is required (quantification of HHV8 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **HHV8 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 volume of 20 µL three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the **HHV8 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-HHV8-EGSpA").

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

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



N.B.: if the preparation of the amplification reaction is performed with the **«ELITe GALAXY»** instrument, load the elution microplate, the Q-PCR Mix and the amplification microplate as indicated in the instrument user manual and following the steps required by the GUI.

N.B.: 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.

Qualitative analysis of the results

The recorded values of the fluorescence emitted by the specific HHV8 probe (FAM detector "HHV8") 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;

N.B.: In the case of a positive sample with a high titre of HHV8 DNA, the FAM fluorescence of the HHV8 specific probe may begin to increase before the 15th cycle. 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 “HHV8” 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 “HHV8” 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 HHV8 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Positive Control reaction detector FAM "HHV8"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for HHV8, the target DNA has not been correctly detected. This means that problems have 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.

***N.B.:** When this product is used for the quantification of HHV8 DNA, the **Q - PCR Standard** reactions are 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 HHV8 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Negative control reaction detector FAM "HHV8"	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative control** amplification reaction is different from **Ct Undetermined** for HHV8, 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 HHV8 is used to detect the target DNA, while the **Ct** value of Internal Control is used to validate extraction, amplification and detection.

N.B: 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).

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This product is able to detect a minimal quantity of about 10 copies of DNA of the minor capsid protein gene of HHV8 in the amplification reaction, corresponding to the genome Equivalents per reaction (detection limit for the product, see Performance Characteristics paragraph).

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

Sample reaction		Sample suitability	Assay result	HHV8 DNA
detector FAM "HHV8"	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 HHV8 and **Ct > 35** or **Ct Undetermined** for the Internal Control, it means that it was impossible to detect efficiently the DNA for the Internal Control. In this case problems occurred during the amplification step (inefficient or absent amplification) or during the extraction step (loss of DNA during the extraction or presence of inhibitors) which may lead to incorrect results and false negatives. The sample is not suitable, the assay is invalid and it needs to be repeated starting from the extraction of a new sample.

If the result of the amplification reaction of a sample is **Ct Undetermined** for HHV8 and **Ct ≤ 35** for the Internal Control, it means that the HHV8 DNA is not detected in the DNA extracted from the sample; but it can not be excluded that the HHV8 DNA has a lower titre than the detection limit of the product (see the paragraph about Performance Characteristics). In this case the result could be a false negative.

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

N.B.: When in the amplification reaction of a sample the HHV8 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 HHV8 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 HHV8 are used to calculate the **Standard Curve** (Results > Standard Curve) for the amplification session and to validate the amplification and the detection as described in the following table:

Standard Curve detector FAM "HHV8"	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 HHV8 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 minor capsid protein gene of HHV8 in the amplification reaction, corresponding to the genome Equivalents per reaction (linear measuring range of the product, see Performance Characteristics,), as described in the following table:

Sample result detector FAM "HHV8"	HHV8 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 the amplification reactions for the **samples** (Results > Report) are used to calculate the genome Equivalents **(gEq)** of HHV8 present in the extracted sample (**Nc**) according to this formula:

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

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 **«ELITe STAR»** is used with whole blood, plasma collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

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

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

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

When «**NucliSENS® easyMAG®**» extraction system 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 «NucliSENS® easyMAG®»
Nc (gEq / mL) = 50 x Quantity

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

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

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

Simplified formula for whole blood and «QIAsymphony® SP/AS»
Nc (gEq / mL) = 23 x Quantity

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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

Calculation of the linear measuring range limits

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

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

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

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

Measuring range limits (gEq / mL) with «ELITe STAR System»
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**» is used with whole blood, plasma collected in EDTA, the formula becomes:

Measuring range limits (gEq / mL) with «ELITe GALAXY System»
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

When «**NucliSENS® easyMAG®**» extraction system is used with cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «NucliSENS® easyMAG®»
Lower limit (gEq / mL) = 50 x 10 gEq Upper limit (gEq / mL) = 50 x 1,000,000 gEq
from 500 to 50,000,000 gEq / mL

When «**NucliSENS® easyMAG®**» extraction system is used with non-cellular 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 cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «QIAsymphony® SP/AS»
Lower limit (gEq / mL) = 23 x 10 gEq **Upper limit (gEq / mL) = 23 x 1,000,000 gEq**
from 230 to 23,000,000 gEq / mL

When «**EXTRAblood**» extraction kit is used with cellular samples, the formula becomes:

Linear 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

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: detection limit

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

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

The final results are summed up in the following table.

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

The analytical sensitivity of this assay used in association to whole blood samples and **ELITe GALAXY** was verified with a panel of HHV8 dilutions within the limiting concentration. The panel was prepared by diluting the HHV8 Culture Fluid (ZeptoMetrix Corporation) in HHV8 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	**117 gEq / mL**	72 gEq / mL	326 gEq / mL

The analytical sensitivity of this assay used in association to plasma samples and **ELITe GALAXY** was verified with a panel of HHV8 dilutions within the limiting concentration. The panel was prepared by diluting the HHV8 Culture Fluid (ZeptoMetrix Corporation) in HHV8 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	**98 gEq / mL**	58 gEq / mL	336 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 plasmidic DNA containing the amplification product whose initial concentration was measured by a spectrophotometer. The dilutions from 10^7 molecules per reaction to 10^1 molecules per reaction were tested in 9 replicates carrying out the amplification by ELITechGroup S.p.A. products.

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

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

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

The final results are summed up in the following table.

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

The linear measuring range limits as gEq / mL referring to the used extraction kit are calculated at page 23.

Analytical sensitivity: Precision and Accuracy

The precision of the assay, as the variability of results obtained with several replicates of a sample tested within the same amplification session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 24.5% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The accuracy of the assay, as the difference between the mean of results obtained with several replicates of a sample within the same amplification session and the theoretical concentration value of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 8.8% 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.

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

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

The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probe in the alignment of the sequences available in the database for the minor capsid protein gene of HHV8 showed their conservation and absence of significant mutations.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested using some clinical samples of whole blood collected in EDTA positive for HHV8 DNA.

The diagnostic sensitivity was evaluated using as reference material 19 whole blood samples collected in EDTA, all positive for HHV8 DNA (tested with a CE IVD real time amplification product). Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Whole blood collected in EDTA positive for HHV8 DNA	19	18	1

One sample reported a negative result with ELITechGroup S.p.A. products. This discordance may be explained with a HHV8 titre very low and lower than the detection limit of method used (250 gEq/mL).

The diagnostic sensitivity of the assay in this test was 94.7%.

The diagnostic sensitivity was evaluated using 30 samples of plasma collected in EDTA negative for HHV8 DNA, that were spiked for HHV8 DNA adding HHV8 Culture fluid sample (ZeptoMetrix, USA) and 30 whole blood collected in EDTA samples negative for HHV8 DNA, that were spiked for HHV8 DNA adding HHV8 Culture fluid sample (ZeptoMetrix, USA). 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.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked for HHV8 DNA	30	30	0
Plasma collected in EDTA spiked for HHV8 DNA	30	30	0

All spiked samples were correctly detected as positive for HHV8 DNA.

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

The diagnostic sensitivity was evaluated using 31 samples of plasma negative for HHV8 DNA, that were spiked for HHV8 DNA adding HHV-8 Culture fluid sample (ZeptoMetrix, USA) and 30 whole blood samples negative for HHV8 DNA, that were spiked for HHV8 DNA adding HHV-8 Culture fluid sample (ZeptoMetrix, USA). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked for HHV8 DNA	30	30	0
Plasma collected in EDTA spiked for HHV8 DNA	31	30	0

One sample of plasma was excluded from the study because resulted invalid in amplification. This result was confirmed with a second amplification and is probably due to a presence of an inhibitor.

30 plasma samples resulted valid for analysis and were all confirmed positive. All spiked whole blood samples were correctly detected as positive for HHV8 DNA.

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

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

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

The analysis of the alignment of the sequences of the primers and of the fluorescent probe with the sequences available in databases for organisms other than HHV8, including EBV complete genome, the Herpes human virus that is most similar to HHV8, showed their specificity and the absence of significant homology.

The analytical specificity of the assay, as absence of cross-reactivity with other potential interfering markers, was checked using some clinical samples negative for HHV8 DNA and positive for DNA of other pathogens.

The analytical specificity was checked using as reference material 20 whole blood samples collected in EDTA, that were negative for HHV8 DNA but positive for DNA of BKV, EBV, HHV8 (tested with CE IVD amplification products). Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Whole blood samples collected in EDTA positive for BKV DNA	4	0	4
Whole blood samples collected in EDTA positive for EBV DNA	7	0	7
Whole blood samples collected in EDTA positive for HHV8 DNA	9	0	9

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

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was tested using some clinical samples of whole blood collected in EDTA negative for HHV8 DNA.

The diagnostic specificity was evaluated using as reference material 20 whole blood samples collected in EDTA, all negative for HHV8 DNA (tested with a CE IVD real time amplification product). Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Whole blood collected in EDTA negative for HHV8 DNA	20	0	20

The diagnostic specificity of the assay in this test was higher than 95%.

The diagnostic specificity was evaluated using 30 plasma samples collected in EDTA that were presumably negative for HHV8 DNA and 30 whole blood samples collected in EDTA that were presumably negative for HHV8 DNA (tested with a real time amplification method). 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.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA negative for HHV8 DNA	30	0	27
Plasma collected in EDTA negative for HHV8 DNA	30	0	30

Three samples resulted invalid.

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

The diagnostic specificity was evaluated using 30 plasma samples collected in EDTA that were presumably negative for HHV8 DNA and 30 whole blood samples collected in EDTA that were presumably negative for HHV8 DNA (tested with a real time amplification method). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **ELITe GALAXY System** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA negative for HHV8 DNA	30	0	30
Whole blood collected in EDTA negative for HHV8 DNA	30	0	30

All samples were correctly detected as negative for HHV8 DNA.
The diagnostic specificity of the assay in this test was equal to 100 %.

N. B.: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File "HHV8 ELITe MGB® Kit", FTP RTS038PLD.

REFERENCES

B. Bigoni et al (1996) *J Inf Dis* 173: 542 - 549
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: whole blood collected in EDTA, plasma collected in EDTA and cerebrospinal fluid (CSF).

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

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

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

There are no data available concerning product performances with DNA extracted from the following clinical samples: cutaneous biopsies.

Use this product only with the validated instruments and associated 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 acids extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the HHV8 positive clinical samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations; however, the cross-contaminations can be avoided only by good laboratory practices and following carefully these instructions for use manual.

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

HHV8 ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS038PLD

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.

When amplification session is manually setup, 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 HHV8 DNA was not detected in the DNA extracted from the sample; but it cannot be excluded that the HHV8 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 HHV8 DNA.

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

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

TROUBLESHOOTING

Target DNA not detected in the Positive Control or Q - PCR Standard reactions or invalid correlation coefficient of the Standard curve	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Take care when dispensing reactions into the microplate wells and comply with the work sheet. Check the volumes of reaction mixture dispensed. Check the volumes of positive control or standard dispensed.
Incorrect session setup on ELITe InGenius 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 or 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, positive control or standards. Check the volumes of reaction mixture, positive control or standards.
Error while setting the instrument.	Check the position settings of the samples, negative controls, positive controls or standards on the instrument.
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of the molecular grade biology 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.

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.

HHV8 ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS038PLD

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.

SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch ref.


Use by (last day of month).

IVD *in vitro* diagnostic medical device.


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.

NOTICE TO PURCHASER: LIMITED LICENSE

This product content LTC licensed reagents.

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

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

HHV8 ELITe MGB® kit used with Genius series platforms

Ref: RTS038PLD

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 HHV8 ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification of Herpes human virus 8 (HHV8)**. The assay is CE-IVD validated in combination with the instruments **ELITe InGenius®** and **ELITe BeGenius®**.

B. Amplified sequence

Target	Gene	Fluorophore
HHV8	minor capsid protein gene (ORF26)	FAM
Internal Control	Human beta globin gene	AP525 (VIC)

C. Validated matrix

› **Whole blood** EDTA

› **Plasma** EDTA

D. Kit content

HHV8 Q-PCR Mix
4 tubes of 540 µL

X 4

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

E. Material required not provided in the kit

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

› **HHV8 ELITe Standard :** STD038PLD
› **HHV8 - ELITe Positive Control :** CTR038PLD
› **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
› HHV8 Q-PCR Mix volume	20 µL

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

G. Performance

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Whole Blood	**117 cp/mL**	**100%** 30/30*	**100%** 32/32*
Plasma	**98 cp/mL**	**100%** 30/30*	**100%** 32/32*

**confirmed samples/ tested samples*

Matrix	Linearity (copies/mL)
Whole Blood	**117 – 1,000,000**
Plasma	**98 – 1,000,000**

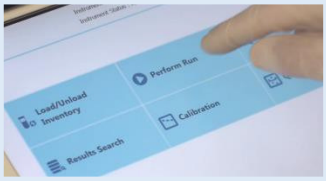
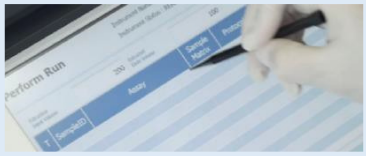
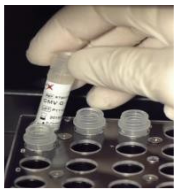
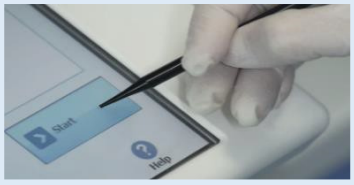
H. Procedures ELITe InGenius

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: HHV8 Q-PCR standard in the “Calibration menu” Verify controls: HHV8 pos. and neg. controls in the “Control menu” *NB:* Both have been run, approved and not expired	**3.** Thaw the HHV8 Q- PCR-Mix and the CPE Internal Control tubes Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

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

Procedure 2 - PCR only

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

Procedure 3 - Extraction only

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

I. 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.

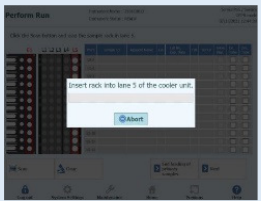
Before analysis

1. Switch on ELITe BeGenius Identification with username and password Select the mode "Closed"	**2.** Verify calibrators: HHV8 Q-PCR standard in the "Calibration menu" Verify controls: HHV8 pos. and neg. controls in the "Control menu" *NB:* Both have been run, approved and not expired	**3.** Thaw the HHV8 Q- PCR-Mix and the CPE Internal Control tubes Vortex gently Spin down 5 sec

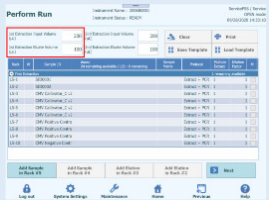
Procedure 1 - Complete run: Extraction + PCR

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

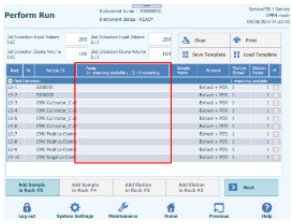
2. Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active



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

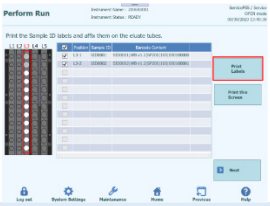


4. Select the "Assay protocol" of interest



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

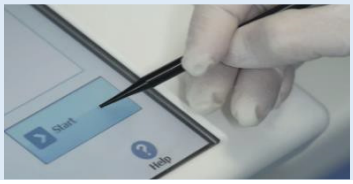
5. Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area



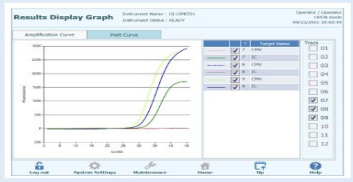
6. Load the Q-PCR-Mix and the CPE Internal Control in Reagent Rack and insert it in the cooling area

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

HHV8 ELITe MGB® Kit used with ABI PCR instrument

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 HHV8 ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **Herpes human virus 8 (HHV8)**.

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
HHV8	minor capsid protein gene (ORF26)	FAM
Internal Control	Human beta globin gene	AP525 (VIC)

C. Validated matrix

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

D. Kit content

HHV8 Q-PCR Mix
4 tubes of 540 µL

X 4

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

E. Material required not provided in the kit

› **7500 Fast Dx and 7300 PCR Instrument**
› **ELITe STAR:** INT010
› **ELITe STAR 200 extraction kit:** INT011EX
› **ELITe GALAXY:** INT020
› **ELITe GALAXY 300 extraction kit:** INT021EX

› **HHV8 ELITe Positive Control:** CTR038PLD
› **HHV8 ELITe Standard:** STD038PLD
› **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	–	**100%** (30/30)*	**100%** (27/30)*
	Plasma	–	**100%** (30/30)*	**100%** (30/30)*
ELITe GALAXY - ABI	Whole Blood	**117 gEq/mL**	**100%** (30/30)*	**100%** (30/30)*
	Plasma	**98 gEq/mL**	**100%** (30/30)*	**100%** (30/30)*

System	Linearity	Conversion factor cp/reaction to cp/mL
ELITe STAR - ABI	280 →28 x 10^6 (WB, PL)	28 (WB, PL)
ELITe GALAXY - ABI	350 →35 x 10^6 (WB, PL)	35 (WB, PL)
easyMAG - ABI	500 →50 x 10^6 (WB)	50 (WB)
QIAsymphony - ABI	230 →23 x 10^6 (WB) 120 →12 x 10^6 (PL)	23 (WB) 12 (PL)
EXTRAblood - ABI	250 →25 x 10^6 (WB)	25 (WB)

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	Whole Blood, Plasma	200 µL	700 µL	100 µL	200µL
ELITe Galaxy	Whole Blood, Plasma	300 µL	400 µL	200 µL	10 µL
EasyMAG	CSF	500 µL	-	100 µL	5 µL
EasyMAG	Whole Blood	100	-	50	
QIAsymphony	Whole Blood,	200 µL	400 µL	95 µL	10 µL

Amplification - Settings of 7500 Fast Dx and 7300 PCR instruments

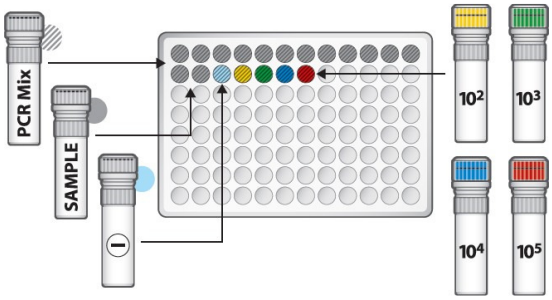
1. Switch on the thermal-cycler
2. Set "HHV8" detector with "FAM" and quencher "none"
3. Set "Internal Control" detector with "VIC" and quencher "none"
4. Set passive fluorescence as "Cy5" with 7500 Fast Dx and as "ROX" with 7300 instrument
5. Set up the thermal profil as indicated. Fluorescence acquisition must be set during hybridation step at 60°C

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

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

Amplification - PCR Set -up

1. Thaw HHV8 Q PCR-Mix and Q-PCR standard tubes
2. Mix gently and spin-down
3. Pipet **20 µL** of Q-PCR-Mix in all microplate wells in use
4. Add, **20 µL** of extracted DNA in sample wells, **20 µL** of molecular grade water in Negative Control well, and **20µL** of the 4 Q-PCR standards in standard curve wells, if quantitative, **20 µL** of the Positive Control, if qualitative. Each one has to be mixed by pipetting 3 times into the reaction mixture
5. Seal the microplate with the amplification sealing sheet
6. Transfer the microplate in the thermocycler and start



Amplification - Threshold for qualitative analysis

Instrument	HHV8 FAM	Internal Control VIC
7500 Fast Dx Real Time PCR	0.2	0.1
7300 Real Time PCR	0.1	0.05

Interpretation - Qualitative results

HHV8 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 HHV8 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 gEq/reaction.