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EMPOWERING IVD

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NOTICE of CHANGE dated 23/01/2024

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HHV7 ELITe MGB® Kit»
Ref. RTS037PLD

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

- *Extension of the use of the product in association with «ELITe BeGenius®» instrument (REF INT040) and whole blood and plasma matrices.*

- *Update of PERFORMANCE CHARACTERISTICS:*

  - *LoD, LLoD and ULoD values confirmed on matrix*
  - *Repeatability and Reproducibility calculated on matrix*
  - *Internal Cut-off value changed from 36 to 35*

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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HHV7 ELITe MGB® Kit

reagents for DNA Real-Time PCR

REF **RTS037PLD** **CE** **IVD** **-20 °C**

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INTENDED USE

The **HHV7 ELITe MGB® Kit** product is a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of Herpes human virus 7 (HHV7)** in DNA samples extracted from whole blood collected in EDTA and plasma collected in EDTA and cerebrospinal fluid (CSF).

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of whole blood and plasma collected in EDTA.

The assay is validated in association with the **7300 Real-Time PCR System and 7500 Real-Time PCR System**, using human specimens of whole blood, plasma collected in EDTA and cerebrospinal fluid.

The product is intended for use in the diagnosis and monitoring of HHV7 infections, alongside patient clinical data and other laboratory test outcomes.

ASSAY PRINCIPLE

The assay is a quantitative Real-Time PCR detecting HHV7 DNA, isolated from specimens and amplified using the assay reagent **HHV7 Q PCR Mix** that contains primers and probes with ELITe MGB and TaqMan™ MGB® technology.

The ELITe MGB and TaqMan MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycle (Ct) and the melting temperatures (Tm). The HHV7 DNA quantity is calculated based on a stored calibration curve.

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

PRODUCT DESCRIPTION

The **HHV7 ELITe MGB Kit** provides the assay reagent **HHV7 Q - PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- HHV7, **capsid protein gene (U57)** region, detected in Channel **HHV7;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye.
- Internal Control (IC), specific for the artificial DNA sequence IC2, detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled with AquaPhluor® 525 (AP525)dye.

The **HHV7 Q - PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, AP593 fluorophore (analogue of ROX or Cy5) as passive reference for fluorescence normalisation, the enzyme Uracil-N-glycosidase (UNG) to inactivate contamination by the amplification product, the "hot start" DNA Polymerase.

The product **HHV7 ELITe MGB Kit** contains sufficient reagents for **96 tests** on **ELITe InGenius** and **ELITe BeGenius, with 20** µLused per reaction.

The product **HHV7 ELITe MGB Kit** contains sufficient reagents for **100 tests on other systems**, **with 20** µLused per reaction.

The **HHV7 ELITe MGB Kit** can be also used in association with other equivalent instruments.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
HHV7 Q - PCR Mix ref. RTS037PLD	Mixture of reagents for Real-Time PCR in tube with **transparent cap**	**4 x 540 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~3,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Thermomixer.
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).

- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.
- 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 sample DNA, the extraction and inhibition internal control, the amplification positive and negative controls, the DNA standards and the consumables **are not** provided with this product.

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

Instruments and Software	Products and Reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030) **ELITe InGenius Software** version 1.3.0.17 (or later) **HHV7 ELITe_STD**, Assay Protocol with parameters for Calibrators analysis **HHV7 ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **HHV7 ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **HHV7 ELITe_WB_200_100**, Assay Protocol with parameters for whole blood specimen analysis **HHV7 ELITe_PL_200_100**, Assay Protocol with parameters for plasma specimen analysis	**ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS) **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR), **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000) **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only **CPE – Internal Control** (EG SpA, ref. CTRCPE) **HHV7 ELITe Standard** (EG SpA, ref. STD037PLD) **HHV7 – ELITe Positive Control** (EG SpA, ref. CTR037PLD)
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.1.0. (or later) **HHV7 ELITe_Be_STD**, Assay Protocol with parameters for Calibrators analysis **HHV7 ELITe_ Be_PC**, Assay Protocol with parameters for Positive Control analysis **HHV7 ELITe_ Be_NC**, Assay Protocol with parameters for Negative Control analysis **HHV7 ELITe_ Be_WB_200_100**, Assay Protocol with parameters for whole blood specimen analysis **HHV7 ELITe_ Be_PL_200_100**, Assay Protocol with parameters for plasma specimen analysis	
7300 Real-Time PCR System (ThermoFisher Scientific, ref. 4351101) **QIAsymphony® SP/AS** (QIAGEN GmbH, Ref. 9001297, 9001301) **NucliSENS® easyMAG®** (bioMérieux SA, Ref. 200111)	**MicroAmp™ Optical 96-Well Reaction Plate** (LifeTechnologies, ref. N8010560) **CPE – Internal Control** (EG SpA ,ref. CTRCPE) **HHV7 ELITe Standard** (EG SpA, ref. STD037PLD) **HHV7 – ELITe Positive Control** (EG SpA, ref. CTR037PLD) **QIAsymphony® Midi kit** (QIAGEN GmbH, Ref. 931236) **NucliSENS® easyMAG® Reagents** (bioMérieux SA, Ref. 280130, 280131, 280132, 280133, 280134, 280135)
7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific, ref. 4406985) **QIAsymphony SP/AS** (QIAGEN GmbH, Ref. 9001297, 9001301) **NucliSENS easyMAGe** (bioMérieux SA, Ref. 200111)	**MicroAmp Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL** (Life Technologies, ref. 4346906) **CPE – Internal Control** (EG SpA, ref. CTRCPE) **HHV7 ELITe Standard** (EG SpA, ref. STD037PLD) **HHV7 – ELITe Positive Control** (EG SpA, ref. CTR037PLD) **QIAsymphony Midi kit** (QIAGEN GmbH, Ref. 931236) **NucliSENS easyMAG Reagents** (bioMérieux SA, Ref. 280130, 280131, 280132, 280133, 280134, 280135)

HHV7 ELITe MGB® Kit
reagents for DNA Real-Time PCR

REF RTS037PLD

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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

Warnings and precautions for molecular biology

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

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.

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

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination. The PCR Cassette must be handled carefully and never opened to avoid PCR product diffusion into the environment and sample and reagent contamination.

Warnings and precautions specific for the components

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
HHV7 Q - PCR Mix	-20 °C or below (protected from light)	one month	up to five	up to five separate* sessions of three hours each or up to 7 consecutive hours (2 sessions of 3 hours each and the time needed to start a third session)

* with intermediate freezing

SPECIMENS AND CONTROLS for ELITe InGenius and ELITe BeGenius

Specimens

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

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Whole Blood	EDTA	≤ 24 hours	≤ 72 hours	≤ 1 month	> 1 month
Plasma	EDTA	≤ 24 hours	≤ 72 hours	≤ 1 month	> 1 month

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

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

Assay Protocols for HHV7 ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
Whole blood	**ELITe InGenius**	**HHV7 ELITe_WB_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	**HHV7 ELITe_Be_WB_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
Plasma	**ELITe InGenius**	**HHV7 ELITe_PL_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	**HHV7 ELITe_Be_PL_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL

For all protocols, transfer of the sample to the Extraction tube for (ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius) is optional.

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

Note: Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the "Warnings and Precautions" section.

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

Refer to "Potentially Interfering Substances" in the Performance Characteristics section to check data concerning interfering substances.

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

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.

PCR calibrators and controls

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

- For the calibration curve, use the four levels of the product **HHV7 ELITe Standard** (not provided with this kit) with the **HHV7 ELITe_STD** or **HHV7 ELITe_Be_STD** Assay Protocols.

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

- For the Positive Control, use the product **HHV7 - ELITe Positive Control** (not provided with this kit) with the **HHV7 ELITe_PC** or **HHV7 ELITe_Be_PC** Assay Protocols,
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **HHV7 ELITe_NC** or **HHV7 ELITe_Be_NC** Assay Protocols.

Note: The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the calibration curve and PCR control validation for each lot of PCR reagent.
Calibration curves expire after **60 days**, at which time it is necessary to re-run the calibration.
PCR control results expire after **15 days**, at which time it is necessary to re-run the Positive and Negative Controls.
The Calibrators and PCR controls must be re-run if any of the following events occur:

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

Quality controls

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

ELITe InGenius PROCEDURE

The procedure to use the **HHV7 - ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

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

STEP 1 – Verification of the system readiness

Before starting the session:
- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Calibration" menu on the Home page, verify the Calibrators (**HHV7 Q - PCR Standard**) are approved and valid (Status) for the PCR Mix lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- in the "Controls" menu on the Home page, verify the PCR Controls (**HHV7 - Positive Control**, **HHV7 Negative Control**) are approved and valid (Status) for the PCR Mix lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "Specimens and Controls").

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

Protocols for qualitative analysis are available on request.

STEP 2 – Session Setup

The **HHV7 ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),
B. Eluted sample run, (PCR Only),
C. Calibration run (PCR Only),
D. Positive Control and Negative Control run (PCR Only).

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

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

Before to setup a run:

Thaw the needed **HHV7 Q PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session). Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

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

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

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

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

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

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

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

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

Note: The **HHV7 Q-PCR Standard** can be used for 4 separate sessions of 2 hours each.

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

Note: The **HHV7 Positive Control** can be used for 4 separate sessions of 3 hours each.

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

HHV7 ELITe MGB® Kit
reagents for DNA Real-Time PCR

REF RTS037PLD

STEP 3 - Review and approval of results

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

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

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

The **ELITe InGenius** generates results with the **HHV7 ELITe MGB Kit** through the following procedure:

A. Validation of Calibration curve,

B. Validation of Positive Control and Negative Control results,

C. Validation of sample results,

D. Sample result reporting.

A. Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the target of the Calibrator reactions with the **HHV7 ELITe STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

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

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

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

B. Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the target of the Positive Control and Negative Control reactions with the **HHV7 ELITe_PC** and **HHV7 ELITe_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and used to verify the system (reagents lot and instrument).

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

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

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

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

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

C. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the target (Channel **HHV7**) and the Internal Control (Channel **IC**) with the **HHV7 ELITe_WB_200_100** and **HHV7 ELITe_PL_200_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Results Display" screen.

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

1) Calibration curve	**Status**
HHV7 Q-PCR Standard	APPROVED
2) Positive Control	**Status**
HHV7 Positive Control	APPROVED
3) Negative Control	**Status**
HHV7 Negative Control	APPROVED

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

The possible result messages are listed in the table below.

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

Result of sample run	**Interpretation**
HHV7:DNA Detected, quantity equal to "XXX" copies/mL	**HHV7 DNA was detected** in the sample within the assay measurement range, its concentration is shown.
HHV7:DNA Detected, quantity below "LLoQ" copies/mL	**HHV7 DNA was detected** in the sample, its concentration is below the assay -Lower Limit of Quantification
HHV7:DNA Detected, quantity beyond "ULoQ" copies/mL	**HHV7 DNA was detected** in the sample, its concentration is above the assay Upper Limit of Quantification
HHV7:DNA Not detected or below the "LoD" copies/mL	**HHV7 DNA** was **not detected** in the sample. The sample is negative for the target DNA, or its concentration is below the assay **Limit of Detection.**
Invalid-Retest Sample	**Not valid assay result** caused by Internal Control failure (due to e.g. incorrect extraction or inhibitors carry-over). The test should be repeated.

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

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in "PCR Only" mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using "Extract + PCR" mode (see "Troubleshooting")

Samples reported as "HHV7:DNA Not detected or below "LoD" copies/mL" are suitable for analysis but HHV7 was not detected. In this case the sample may be either negative for HHV7 DNA or the HHV7 DNA is present at a concentration below the Limit of Detection of the assay (see "Performance Characteristic).

HHV7 DNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as "HHV7:DNA Detected, quantity below "LLoQ" copies/mL" (see "Performance characteristics").

HHV7 DNA positive samples within the Linear Measuring Range (see "Performance characteristics") are detected and are reported as "HHV7: DNA Detected, quantity equal to "XXX" copies / mL".

HHV7 DNA positive samples that are above the Upper Limit of Quantification are reported as "HHV7: DNA Detected, quantity beyond "ULoQ" copies/mL" and they are not suitable for quantification. If needed the sample may be diluted before extraction or PCR and retested to yield results within the Linear Measuring Range of the assay.

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

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

D. Sample result reporting

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

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

The "Track Report" shows the results details by selected Track.

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

ELITe BeGenius PROCEDURE

The procedure to use the **HHV7 ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

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

STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in "**CLOSED**" mode,

- in the "Calibrations" menu on the Home page, verify the Calibrators (**HHV7 Q - PCR Standard**) are approved and valid (Status) for the **HHV7 PCR Mix** lot to be used. If no valid Calibrators are available for the **HHV7 PCR Mix** lot, perform calibration as described in the following sections,

- in the "Controls" menu on the Home page, verify the PCR Controls (**HHV7 Positive Control, HHV7 Negative Control**) are approved and valid (Status) for the **HHV7 PCR Mix** lot to be used. If no valid PCR Controls are available for the **HHV7 PCR Mix** lot, run the PCR Controls as described in the following sections,

choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "Specimens and Controls").

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

Protocols for qualitative analysis are available on request.

STEP 2 – Session Setup

The **HHV7 ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),
B. Eluted sample run (PCR Only),
C. Calibration run (PCR Only),
D. Positive Control and Negative Control run (PCR Only).

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

Note: The **ELITe BeGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **HHV7 PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

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

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

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

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) for 30 minutes at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw the Positive Control tubes** at room temperature for 30 minutes. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block. **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen
3	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode: PCR Only".	Select the "Run mode": "PCR Only".
5	**Load the Q-PCR** Standard tubes into the "Elution Rack".	**Load** the **Positive Control and Negative Control tubes** into the "Elution Rack".
6	**Insert** the **"Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	**Insert** the **"Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" (600 µL) and the "Extracted Elute Volume" (50 µL).	Ensure the "Extraction Input Volume" (600 µL) and the "Extracted Elute Volume" (50 µL).
9	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	**Load** the **PCR Mix** into "Reagent/Elution Rack".	**Load** the **PCR Mix** into "Reagent/Elution Rack".
12	**Insert** the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
13	Click "Next" to continue.	Click "Next" to continue.
14	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.
15	Click "Next" to continue.	Click "Next" to continue.
16	**Load** the "**PCR Basket**" with "**PCR Cassette**" in the Inventory Area.	**Load** the "**PCR Basket**" with "**PCR Cassette**" in the Inventory Area.
17	Click "Next" to continue.	Click "Next" to continue.
18	Close the instrument door.	Close the instrument door.
19	Press "Start".	Press "Start".

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

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

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

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

Note: The **HHV7 Q-PCR Standard** can be used for 4 separate sessions of 2 hours each.

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

Note: The **HHV7 Positive Control** can be used for 4 separate sessions of 3 hours each.

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

STEP 3 -Review and approval of results

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

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

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

The **ELITe BeGenius** generates the results with the **HHV7 ELITe MGB Kit** through the following procedure:

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

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

PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius

Analytical sensitivity: Limit of Blank with Whole Blood

Due to the high prevalence of HHV7 in the population (about 80%) reported in literature (Michael Kidd et al.), a certain percentage of low positive results clinically non-significant is expected when analyzing Whole Blood samples. In order to obtain the negativity of the assay with these samples it was necessary to assess a HHV7 Ct cut-off equal to 35 **on ELITe InGenius and ELITe BeGenius**.

The results on ELITe InGenius are shown in the table below:

Limit of Blank of Whole Blood collected in EDTA and ELITE InGenius			
Samples	**N**	**positive**	**negative**
Whole Blood collected in EDTA negative for HHV7 DNA	35	0	35

The results on ELITe BeGenius are shown in the table below:

Limit of Blank of Whole Blood collected in EDTA and ELITE BeGenius			
Samples	**N**	**positive**	**negative**
Whole Blood collected in EDTA negative for HHV7 DNA	20	0	20

In the Limit of Blank test, the HHV7 ELITe MGB Kit correctly detect all the tested sample as expected within the Ct cut-off set for the target.

Analytical sensitivity: Limit of Detection (LoD)

The 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 on ELITe InGenius using plasmid DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmid DNA was diluted to a titre of 10 copies / 10 µL in presence of plasmid DNA containing the internal control at a titre of 20,000 copies / 10 µL.

The results are reported in the following table.

Samples	N	positive	negative
10 copies HHV7 plasmid DNA + 20,000 copies of internal control	18	18	0

The theoretical LoD value was verified by testing on ELITe InGenius and ELITe BeGenius a pool of EDTA Plasma and a EDTA Whole Blood spiked with HHV7 reference material (ZeptoMetrix, ref. PINATHHV7-ST) at the claimed concentration.

The results obtained confirmed the claimed concentration for the target of HHV7 ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius.

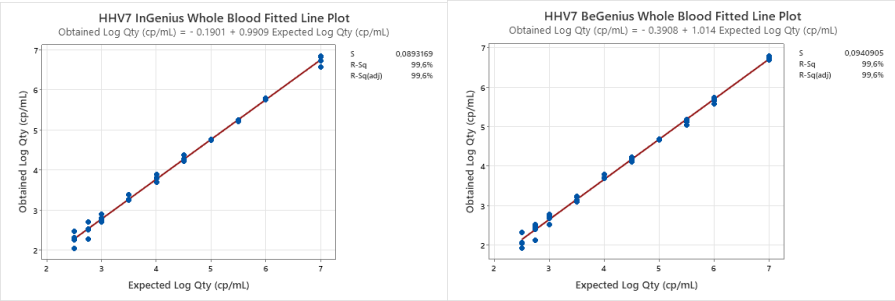
Linear measuring range

The Linear measuring range of HHV7 ELITe MGB Kit was determined with Whole Blood and Plasma samples on ELITe InGenius and ELITe BeGenius.

<u>For Whole Blood</u>**:**

The linear measuring range was determined using a panel of dilutions of plasmid containing HHV7 target sequence in negative EDTA Whole Blood samples.

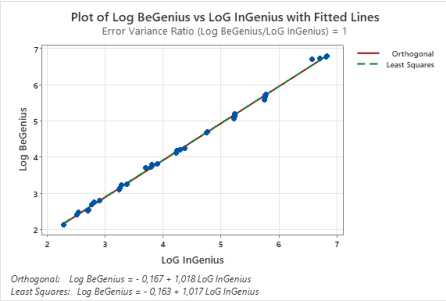
The results are reported in the following figure.



The linear measuring range as copies/mL for EDTA Plasma is calculated by applying the specific conversion factor reported in the following section.

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

The results are summed up in the following figure.

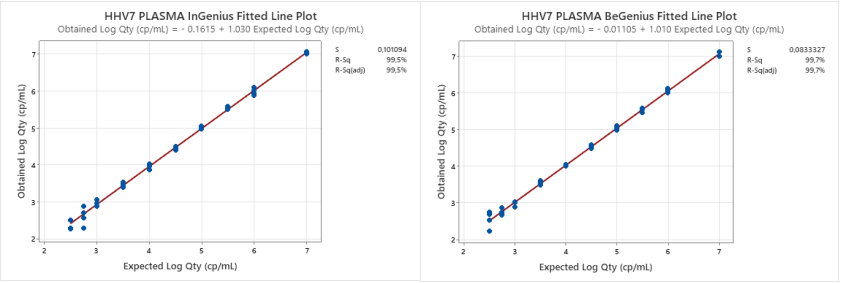


The Orthogonal Regression analysis generated an intercept equal to -0.167 (95% CI: -0.2256, -0.1075) and a slope equal to 1.018 (95% CI: 1.0048, 1.0307). The Linear regression analysis generated a R2 of 0.999.

<u>For Plasma collected in EDTA:</u>

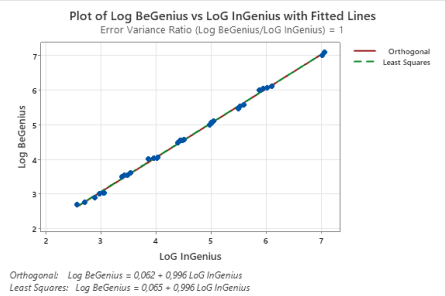
The linear measuring range was determined using a panel of dilutions of plasmid containing HHV7 target sequence in negative EDTA Plasma samples.

The results are reported in the following figure.



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.



The Orthogonal Regression analysis generated an intercept equal to 0.062 (95% CI:0.0053; 0.1194) and a slope equal to 0.996 (95% CI: 0.9845; 1.0082). The Linear regression analysis generated a R2 of 0.999.

The Linear Measuring Range for **Whole Blood and Plasma** collected in EDTA specimens covers a range of concentration as reported in the following table:

Linear Measuring Range for HHV7 ELITe MGB Kit and ELITe InGenius and ELITe BeGenius		
Matrix	**Lower Limit**	**Upper Limit**
Whole Blood	500 copies / mL	10,000,000 copies / mL
Plasma	500 copies / mL	10,000,000 copies / mL

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Repeatability

The Repeatability of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of whole blood specimens collected in EDTA negative or spiked with HHV7 (ZeptoMetrix, ref. PINATHHV7-ST).

An example of Intra-Session Repeatability (on one day) on ELITe InGenius is shown in the table below.

Intra - Session Repeatability on ELITe InGenius					
Sample	**HHV7**				**%Agreement**
	Pos. / Neg.	**Mean Ct**	**SD**	**% CV**	
Negative	0 / 8	NA	NA	NA	100%
3X LoD	8 / 8	32.97	0.38	1.14	100%
10X LoD	8 / 8	31.18	0.29	0.92	100%

An example of Intra-Session Repeatability (on one day) on ELITe BeGenius is shown in the table below.

Intra - Session Repeatability on ELITe BeGenius					
Sample	**HHV7**				**%Agreement**
	Pos. / Neg.	**Mean Ct**	**SD**	**% CV**	
Negative	0 / 8	NA	NA	NA	100%
3X LoD	8 / 8	34.52	0.30	0.88	100%
10X LoD	8 / 8	32.36	0.22	0.69	100%

Results of Inter-Session Repeatability (on two days) on ELITe InGenius are shown in the table below.

Inter - Session Repeatability on ELITe InGenius					
Sample	**HHV7**				**%Agreement**
	Pos. / Neg.	**Mean Ct**	**SD**	**% CV**	
Negative	0 / 16	NA	NA	NA	100%
3X LoD	16 / 16	33.07	0.36	1.09	100%
10X LoD	16 / 16	31.17	0.24	0.77	100%

Results of Inter-Session Repeatability (on two days) on ELITe BeGenius are shown in the table below.

Inter - Session Repeatability on ELITe BeGenius					
Sample	**HHV7**				**%Agreement**
	Pos. / Neg.	**Mean Ct**	**SD**	**% CV**	
Negative	0 / 16	NA	NA	NA	100%
3X LoD	16 / 16	34.41	0.49	1.42	100%
10X LoD	16 / 16	32.34	0.30	0.92	100%

In the Repeatability test, the HHV7 ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 1.42%.

Reproducibility

The Reproducibility of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a HHV7 DNA negative whole blood specimens collected in EDTA negative or spiked with HHV7 (Zeptometrix, ref. PINATHHV7-ST).

The results of the Inter-Batch Reproducibility (two lots) on ELITe InGenius are shown in the table below.

Inter-Batch Reproducibility on ELITe InGenius					
Sample	**HHV7**				**%Agreement**
	Pos. / Rep.	**Mean Ct**	**SD**	**%CV**	
Negative	0 / 8	-	-	-	100%
3 X LoD	8 / 8	33.39	0.20	0.59	100%
10 X LoD	8 / 8	31.39	0.18	0.57	100%

The results of the Inter-Batch Reproducibility (two lots) on ELITe BeGenius is shown in the table below.

Inter-Batch Reproducibility on ELITe BeGenius					
Sample	**HHV7**				**%Agreement**
	Pos. / Rep.	**Mean Ct**	**SD**	**%CV**	
Negative	0 / 8	-	-	-	100%
3 X LoD	8 / 8	34.58	0.14	0.42	100%
10 X LoD	8 / 8	32.66	0.24	0.75	100%

The results of Inter-Instrument Reproducibility (on two days, two lots and two instruments) on ELITe InGenius are shown in the table below.

Inter-Instrument Reproducibility on ELITe InGenius					
Sample	HHV7				%Agreement
	Pos. / Rep.	Mean Ct	SD	%CV	
Negative	0 / 8	-	-	-	100%
3 X LoD	8 / 8	34.50	0.31	0.90	100%
10 X LoD	8 / 8	32.61	0.23	0.69	100%

The results of Inter-Instrument Reproducibility (on two days, two lots and two instruments) on ELITe BeGenius are shown in the table below.

Inter-Instrument Reproducibility on ELITe BeGenius[e]					
Sample	HHV7				%Agreement
	Pos. / Rep.	Mean Ct	SD	%CV	
Negative	0 / 8	-	-	-	100%
3 X LoD	8 / 8	33.25	0.26	0.79	100%
10 X LoD	8 / 8	31.26	0.21	0.66	100%

In the Reproducibility test, the HHV7 ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 0.90%.

Diagnostic Specificity: Confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative samples, was evaluated in association with **ELITe InGenius** by analyzing clinical samples of Whole Blood and Plasma collected in EDTA.

As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic specificity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius.**

The results are summed up in the following table.

Samples	N	positive	negative	% Diagnostic Specificity
Whole Blood samples collected in EDTA	38	0	38	100%
Plasma samples collected in EDTA	33	0	33	100%

All Whole Blood and Plasma samples were valid for the analysis. The Ct cut-off for the HHV7 target was applied only for Whole Blood samples.

The Diagnostic Specificity of the HHV7 ELITe MGB Kit in association to Whole Blood and Plasma collected EDTA was equal to 100%.

The IC Ct cut-off value is set at 35 for Whole Blood and for Plasma samples collected in EDTA for both InGenius and BeGenius.

Diagnostic Sensitivity: Confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with **ELITe InGenius** by analyzing clinical samples of Whole Blood and Plasma collected in EDTA.

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 HHV7 negative Whole Blood and Plasma samples collected in EDTA spiked with “Human Herpes Virus Type 7 Stock –(NATHHV7-ST)” (ZeptoMetrix Corporation) at 1000 copies/mL.

The results are summed up in the following table.

Samples	N	positive	negative	%Diagnostic Sensitivity
Whole Blood collected in EDTA spiked for HHV7 DNA	34	34	0	100%
Plasma collected in EDTA spiked for HHV7 DNA	33	33	0	100%

All samples were correctly detected as positive.

The Diagnostic Sensitivity of the HHV7 ELITe MGB Kit in association to Whole Blood and Plasma collected EDTA was equal to 100%.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File “HHV7 ELITe MGB Kit”, FTP037PLD.

SPECIMENS AND CONTROLS FOR OTHER SYSTEMS

Samples

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

Whole blood collected in EDTA

The whole blood samples for DNA extraction must be collected in EDTA and identified according to laboratory guidelines, transported and stored at room temperature (+16 / +26 °C) for a maximum of 24 hours, 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.

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 **NucliSENS® 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 **5 µL** of **CPE** for the internal control and 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.

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. Add **5 µL** of **CPE** for each requested sample to the ATE buffer. Load on the instrument, in the "internal control" slot, the tubes containing the solution, as indicated in the user manual of the kit; indicate the position where eluates will be dispensed and specify the elution volume of **60 µL**. For details on the extraction procedure follow indications in the user manual of the kit.

Cerebrospinal fluid

The cerebrospinal fluid 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.

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 molecular biology grade water (not provided with this kit) added to the reaction in place of the DNA extracted from the sample.

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For the positive control, use the **HHV7 - ELITe Positive Control** product or the **HHV7 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.

OTHER SYSTEMS PROCEDURE

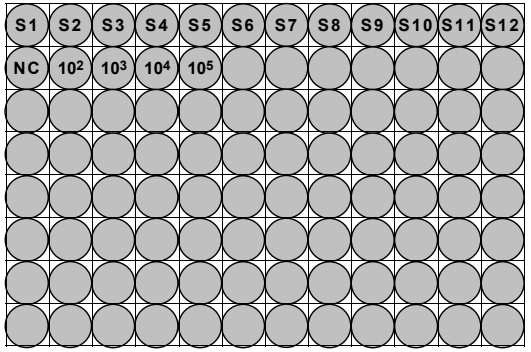
Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

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 HHV7 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "HHV7";
- 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 the 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 HHV7 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “HHV7”;
- 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 12 samples is shown, by way of example, in the previous paragraph describing the procedure for the **7300 Real Time PCR System** instrument.

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

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 the dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**.

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

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

Before starting the session, it is necessary to:
- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **HHV7 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 **HHV7 - Positive Control** or the **HHV7 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 powderless gloves and not to damage the wells.
- take the **Amplification Sealing Sheet** that will be used during the session, being careful to handle it with powderless gloves and not to damage it.

1. Accurately pipet **20 µL** of **HHV7 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, **10 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, 10 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:

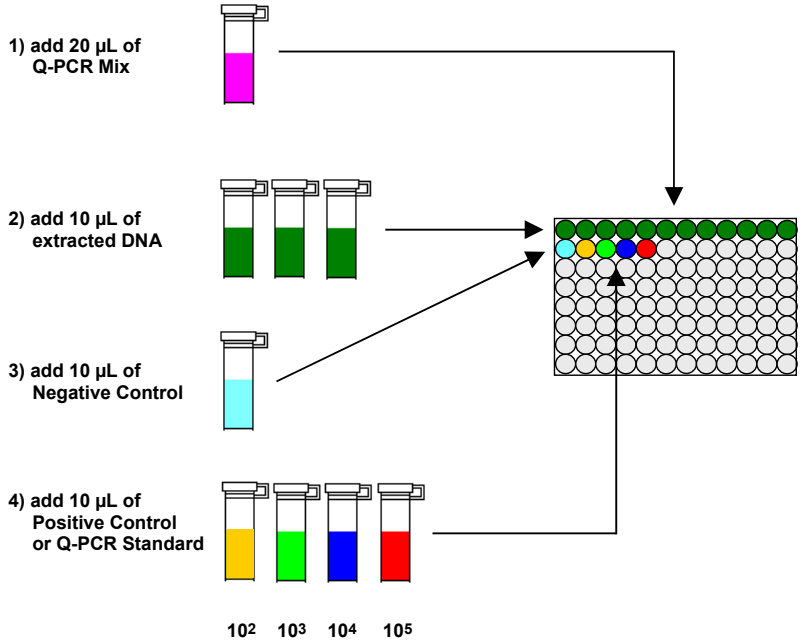
   - When a **qualitative** result is required (detection of HHV7 DNA): accurately pipet, by placing into the reaction mixture, **10 µL** of **HHV7 - Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the volume of 10 µL three times into the reaction mixture. Avoid creating bubbles.

   - When a **quantitative** result is required (quantification of HHV7 DNA): accurately pipet, by placing into the reaction mixture, **10 µL** of **HHV7 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 10 µL three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the **HHV7 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-HHV7-ELITECHGROUP").

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 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 is performed with the instrument **QIAsymphony® SP/AS**, insert the microplate containing the extracts, 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 HHV7 probe (FAM detector "HHV7") 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 HHV7 DNA, the FAM fluorescence of the HHV7 specific probe may begin to increase before the cycle 15. In this case the calculation range for the **Baseline** must be adapted from cycle 6 to the cycle in which the FAM fluorescence of the sample begins to increase, as detected by the instrument software (Results > Component).

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

- set manually the **Threshold** for the FAM detector “HHV7” 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 “HHV7” 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 HHV7 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Positive Control reaction detector FAM "HHV7"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for HHV7, 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 HHV7 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 HHV7 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Negative control reaction detector FAM "HHV7"	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the amplification reaction for the **Negative control** is different from **Ct Undetermined (Undertermined)** for HHV7, the target DNA has been detected. This means that problems have 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 HHV7 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).

This product is able to detect a minimal quantity of about 10 copies of DNA for the region of a capsid protein gene (U57) of HHV7 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	HHV7 DNA
detector FAM "HHV7"	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 HHV7 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 have occurred during the amplification step (inefficient or absent amplification) or during the extraction step (degradation of internal control DNA, sample with too low cell number, loss of DNA during extraction or presence of inhibitors in the extracted DNA) 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 HHV7 and **Ct ≤ 35** for the Internal Control, it means that the HHV7 DNA is not detected in the DNA extracted from the sample; but it can not be excluded that the HHV7 DNA has a lower titre than the detection limit of the product (see 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 concerning the patient.

N.B.: When in the amplification reaction of a sample the HHV7 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 HHV7 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.

The HHV7 **Ct** values in the amplification reactions of the four **Q - PCR standards**, are used to calculate the **Standard Curve** (Results > Standard Curve) for the amplification session, to validate the amplification and the detection as described in the following table:

Standard Curve detector FAM "HHV7"	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 HHV7 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.

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This product is able to quantify from 1,000,000 to 10 copies of DNA for the region of a capsid protein gene (U57) of HHV7 in the amplification reaction, corresponding to the genome Equivalents per reaction (linear measuring range, see Performance Characteristics paragraph), as described in the following table:

Sample result detector FAM "HHV7"	HHV7 genome Equivalents per reaction
Quantity > 1 x 10^6	MORE THAN 1,000,000
1 x 10^1 ≤ Quantity ≤ 1 x 10^6	= Quantity
Quantity < 1 x 10^1	LESS THAN 10

The results (**Quantity**) of each **sample** (Results > Report) are used to calculate the genome Equivalents (**gEq**) of HHV7 present in the sample used in the extraction (**Nc**) according to this formula:

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

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 **NucliSENS® easyMAG®** extraction system is used with samples from whole blood collected in EDTA and the result is required **ingEq / mL**, the formula becomes:

Simplified formula for whole blood and NucliSENS® easyMAG®
Nc (gEq / mL) = 100 x Quantity

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

Simplified formula for cerebrospinal fluid and NucliSENS® easyMAG®
Nc (gEq / mL) = 20 x Quantity

When **QIAsymphony® SP/AS** 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 QIAsymphony® SP/AS
Nc (gEq / mL) = 45 x Quantity

Calculation of the linear measuring range limits

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

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

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

When **NucliSENS® easyMAG®** extraction system is used with whole blood samples collected in EDTA, the formula becomes:

Linear measuring range limits (gEq / mL) with NucliSENS® easyMAG®
Lower limit (gEq / mL) = 100 x 10 gEq Upper limit (gEq / mL) = 100 x 1,000,000 gEq
from 1000 to 100,000,000 gEq / mL

When **NucliSENS® easyMAG®** extraction system is used with Cerebrospinal fluid, the formula becomes:

Linear measuring range limits (gEq / mL) with NucliSENS® easyMAG®
Lower limit (gEq / mL) = 20 x 10 gEq Upper limit (gEq / mL) = 20 x 1,000,000 gEq
from 200 to 20,000,000 gEq / mL

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

Linear measuring range limits (gEq / mL) with QIAsymphony® SP/AS
Lower limit (gEq / mL) = 45 x 10 gEq Upper limit (gEq / mL) = 45 x 1,000,000 gEq
from 450 to 45,000,000 gEq / mL

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PERFORMANCE CHARACTERISTICS WITH OTHER SYSTEMS

Analytical sensitivity: detection limit

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

The analytical sensitivity of this assay, as detection limit, was tested using plasmidic DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmidic DNA was diluted to a titre of 10 copies / 10 µL with IC-DNA, diluted to a titre of 20.000 copies / 10 µL, in human genomic DNA at a titre of 500 ng / 10 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 plasmidic DNA + 20.000 copies IC-DNA + 500 ng of human genomic DNA	50	50	0

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 10 µL of DNA added to the amplification reaction.

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

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

The upper limit of the linear measuring range was set at 10^6 molecules per reaction, corresponding to Equivalent genome per reaction, within 1 logarithm from the highest concentration Q - PCR Standard amplification standard (10^5 molecules / 10 µL).

The lower limit of the linear measuring range was set at 10 molecules per reaction, corresponding to Equivalent genome per reaction, within 1 logarithm from the lowest concentration Q - PCR Standard amplification standard (10^2 molecules / 10 µL).

The final results are summed up in the following table.

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

The linear measurement range limits as **gEq / mL** referring to the used extraction kit are calculated at page 26.

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 session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 25,9% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 10 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 tested within the same session and the theoretical concentration of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 9,0% of measured quantities, within the range from 10^6 molecules to 10 molecules in the 10 µL of DNA added to the amplification reaction.

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

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

The analytical 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 **U57** gene of the HHV7, showed conservation and absence of significant mutations.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested analyzing some HHV7 DNA positive samples.

The diagnostic sensitivity was evaluated using as reference material 23 negative whole blood samples collected in EDTA (tested with a CE IVD nested amplification product) that were spiked to a title equal to three folds the detection limit for HHV7 DNA with the certified reference sample "HHV7 Culture Fluid", (Ref. 0810071CF, ZeptoMetrix, USA). Each sample was tested carrying out the whole analysis procedure, extraction and amplification, with the 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 HHV7 DNA	23	23	0

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

The diagnostic sensitivity was evaluated using as reference material 25 cerebrospinal fluid samples negative for HHV7 DNA (tested with a CE IVD nested amplification product) that were spiked to a title equal to three folds the detection limit for HHV7 DNA with the certified reference sample "HHV7 Culture Fluid", (Ref. 0810071CF, ZeptoMetrix, USA). Each sample was tested carrying out the whole analysis procedure: extraction, with automatic system NucliSENS® easyMAG® and amplification, with the ELITechGroup S.p.A.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid spiked for HHV7 DNA	25	25	0

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

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 HHV7, including the CMV, EBV, HHV6 complete genome, the human viruses more similar to HHV7, 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 verified using some clinical samples negative for HHV7 DNA but positive for other pathogens.

The analytical specificity was verified using as reference material 12 whole blood samples collected in EDTA tested negative for the DNA of HHV7 DNA (tested with a CE IVD nested amplification product), but positive for the DNA of other pathogens (CMV, EBV and HHV6). Each sample was tested carrying out the whole analysis procedure: extraction and amplification, with the ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA CMV positive	4	0	4
Whole blood collected in EDTA EBV positive	6	0	6
Whole blood collected in EDTA HHV6 positive	1	0	1
Whole blood collected in EDTA HHV6 and EBV positive	1	0	1

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Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was tested analyzing some HHV7 DNA negative clinical samples.

The diagnostic specificity was evaluated using as reference material 23 whole blood samples collected in EDTA negative for HHV7 DNA (tested with a CE IVD nested amplification product). Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by the ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
HHV7 DNA negative whole blood collected in EDTA	23	0	23

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

The diagnostic specificity was evaluated using as reference material 26 cerebrospinal fluid samples that were negative for HHV7 DNA (tested with a CE IVD nested amplification product). Each sample was tested carrying out the whole analysis procedure: extraction, with automatic system NucliSENS® easyMAG® and amplification, with the ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
HHV7 DNA negative cerebrospinal fluid	26	1	25

One sample gave a discordant positive result to the HHV7 DNA, with titre lower than 1 copy / reaction. The discrepancy can be explained by considering that samples with titres such low can give alternately and randomly positive and negative results.

The diagnostic specificity of the assay in this test was 96,1%.

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 "HHV7 ELITe MGB® Kit", FTP RTS037PLD.

REFERENCES

F. Drago et al. (1997) *Lancet* 349: 1367 - 1368 (allegato n° 1, 2 pagine);
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30
Michael Kidd et al. (1996) *The Journal of Infectious Diseases* 174: 396-401

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

PROCEDURE LIMITATIONS

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

Currently there are no data available concerning product performance with other clinical samples.

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

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

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

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

There are no data available concerning product performances with DNA extracted from the following clinical samples: suspension of leukocytes and granulocytes, amniotic fluid.

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

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

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

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

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

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

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

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

A negative result obtained with this product indicates that the target DNA is not detected in the DNA extracted from the sample; however it cannot be excluded that the target DNA has a lower titer than the product detection limit (see “Performance Characteristics”). In this case the result could be a false negative.

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

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

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

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

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TROUBLESHOOTING

ELITe InGenius and ELITE BeGenius

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

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

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

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

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

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

Open Platform:

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.
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.
Error while setting the instrument	Check the position settings of the samples, negative controls, positive controls or standards on the instrument
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of molecular biology grade water.	Use a new aliquot of sterile water.
Contamination of the reaction mixture.	Use a new aliquot of reaction mixture.
Contamination of the extraction / preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

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

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

SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch code.


Use by (last day of month).

IVD *in vitro* diagnostic medical device.

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


Contains sufficient for "N" tests.


Caution, consult instructions for use.

CONT Contents.


Keep away from sunlight.


Manufacturer.

HHV7 ELITe MGB® Kit
reagents for DNA Real-Time PCR

REF RTS037PLD

NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Thermo Fisher Scientific and are sold under licensing arrangements between EG SpA and its Affiliates and Thermo Fisher Scientific. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Thermo Fisher Scientific. Email: outlicensing@thermofisher.com.

ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers 6972339, 7112684, 7319022, 7348146, 7381818, 7541454, 7582739, 7601851, 7671218, 7718374, 7723038, 7759126, 7767834, 7851606, 8008522, 8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1430147, 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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

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

HHV7 ELITe MGB® kit
used in association with Genius series® platforms

Ref: RTS037PLD

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

Intended use

The HHV7 ELITe MGB® Kit product is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids Real-Time PCR assay for the detection and the quantification of the DNA of **Human Herpes Virus 7** (HHV7), extracted from clinical specimens.

The assay is validated in association with **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of whole blood and plasma collected in EDTA.

The product is intended for use in the diagnosis and monitoring of HHV7 infections.

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

Amplified sequence

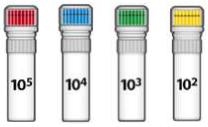
	Gene	Fluorophore	Channel
Target	capsid protein gene U57	FAM	HHV7
Internal Control	IC2	AP525 (VIC)	IC

Validated matrix

› **Whole blood** EDTA

› **Plasma** EDTA

Kit content and related products

HHV7 ELITe MGB KIT RTS037PLD	HHV7 ELITe Standard STD037PLD	HHV7 - ELITe Positive Control CTR037PLD
 X 4	 X 2	 X 1
Ready-to-use PCR Mix 4 tubes of 540 µL 96 reactions per kit 5 freeze-thaw cycles	Ready-to-use 4 levels: 10^5, 10^4, 10^3, 10^2 2 set of 4 tubes of 160 µL 4 freeze-thaw cycles each set (4 separate sessions on board)	Ready-to-use PC 1 tube of 160 µL 4 reactions per kit 4 freeze-thaw cycles (4 separate sessions on board)
Maximum shelf-life:	**24 months**	
Storage temperature	**≤ -20°C**	

Other products required not provided in the kit

› **ELITe InGenius instrument:** INT030
› **ELITe BeGenius instrument:** INT040
› **ELITe InGenius SP200 Extraction Cartridge:** INT032SP200
› **ELITe InGenius PCR Cassette:** INT035PCR
› **ELITe InGenius SP200 Consumable Set:** INT032CS
› **CPE - Internal Control:** CTRCPE
› **HHV7 ELITe Standard:** STD037PLD
› **HHV7 - ELITe Positive Control:** CTR037PLD
› **ELITe InGenius Waste Box:** F2102-000
› **300 µL Filter Tips Axygen:** TF-350-L-R-S
› **1000 µL Filter Tips Tecan:** 30180118

ELITe InGenius and ELITe BeGenius Protocol

›	Sample volume	200 µL	
›	CPE Internal Control volume	10 µL	
›	Total eluate volume	100 µL	
›	PCR eluate input volume	10 µL	
›	HHV7 Q-PCR Mix volume	20 µL	
›	Unit of quantitative result	cp/mL	
›	Frequency of controls	15 days	
›	Frequency of calibration	60 days	

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Whole Blood	**500 cp/mL**	**100%** 34/34*	**100%** 38/38*
Plasma	**500 cp/mL**	**100%** 33/33*	**100%** 33/33*

**confirmed samples/ tested samples*

Sample preparation

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

Sample type	Transport/Storage conditions			
	+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ±10 °C**	**-70 ±15 °C**
Whole Blood collected in EDTA	≤ 24 hours	≤ 72 hours	≤ 1 month	> 1 month
Plasma collected in EDTA	≤ 24 hours	≤ 72 hours	≤ 1 month	> 1 month

Do not use Plasma collected in heparin to prevent inhibition of amplification reaction and frequent invalid results.

ELITe InGenius Procedures

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

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify calibrators: **HHV7 Q-PCR Standard** in the "Calibration menu" Verify controls: **HHV7 Positive Control** and **HHV7 Negative Control** in the "Control menu" *Note:* All must have been run, approved and not expired	**3.** Thaw the **HHV7 PCR Mix** and the **CTRCPE** tubes Vortex gently Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL"	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay protocol" of interest: HHV7 ELITe_PL_200_100 or HHV7 ELITe_WB_200_100	**5.** Select the method "Extract + PCR" and the sample position: Primary tube or Extraction Tube	**6.** Load the PCR Mix and Internal Control in the Inventory Block
7. Load: PCR cassette, Extraction cartridge, Elution tube, Tips, Extraction Tube racks and primary sample racks	**8.** Close the door Start the run	**9.** View, approve and store the results

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

Procedure 2 - PCR only (e.g., eluates, standards, controls)

1 Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL"	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay protocol" of interest: HHV7 ELITe_PC and HHV7 ELITe_NC or HHV7 ELITe_STD)	**5.** Select the method "PCR only" andset the sample position "Elution tube"	**6.** Load the PCR Mix in the inventory block
7. Load: PCR cassette rack and the Elution tube rack with the extracted nucleic acid	**8.** Close the door Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

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

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify calibrators: **HHV7 Q-PCR Standard** in the "Calibration" menu. Verify controls: **HHV7 Positive Control** and **HHV7 Negative Control** in the "Controls" menu. *Note:* All must have been run, approved and not expired.	**3.** Thaw the **HHV7 PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: "200 µL", Elution: "100 µL"
4. Select the "Assay protocol" of interest (HHV7 ELITe_Be_PL_200_100 or HHV7 ELITe_Be_WB_200_100) **Note**: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR-Mix and the Internal Control in Reagent Rack/Elution Rack and insert it in the Cooler Unit
7 Load "PCR Basket" with "PCR Cassette" and the "Extraction Basket" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

Procedure 2 - PCR only (e.g., eluates, standards, controls)

1. Select "Perform Run" on the touch screen and the click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls or standards barcoded tubes in the Elution Rack and insert it in the Cooler Unit	**3.** Verify the extraction volumes: Input: "200 µL", Eluate: "100 µL"
4Select the "Assay protocol" of interest (HHV7 ELITe_Be_PC and HHV7 ELITe_Be_NC or HHV7 ELITe_Be_STD)	**5. .** Load the PCR-Mix in Reagent/ Elution Rack and insert it in the Cooler Unit	**6.** Load "PCR Basket" with "PCR Cassette"
7. Close the door. Start the run	**8.** View, approve and store the results	