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EMPOWERING IVD

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NOTICE of CHANGE dated 22/12/2022

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«EBV ELITe MGB Kit»
Ref. RTS020PLD

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

- *Description of IC cut off value already adopted in the Assay protocol of the product (section "Diagnostic specificity: confirmation of negative samples", page 31)*

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
	DIESE FASSUNG DER GEBRAUCHSANLEITUNG IST KOMPATIBEL MIT DER VORHERIGEN VERSION DES TESTKITS

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EBV ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS020PLD CE IVD -20°C

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INTENDED USE

The **«EBV ELITe MGB® Kit»** product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of Epstein-Barr human herpesvirus (EBV)** in DNA samples extracted from whole blood collected in EDTA, plasma collected in EDTA and cerebrospinal fluid (CSF).

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

ASSAY PRINCIPLES

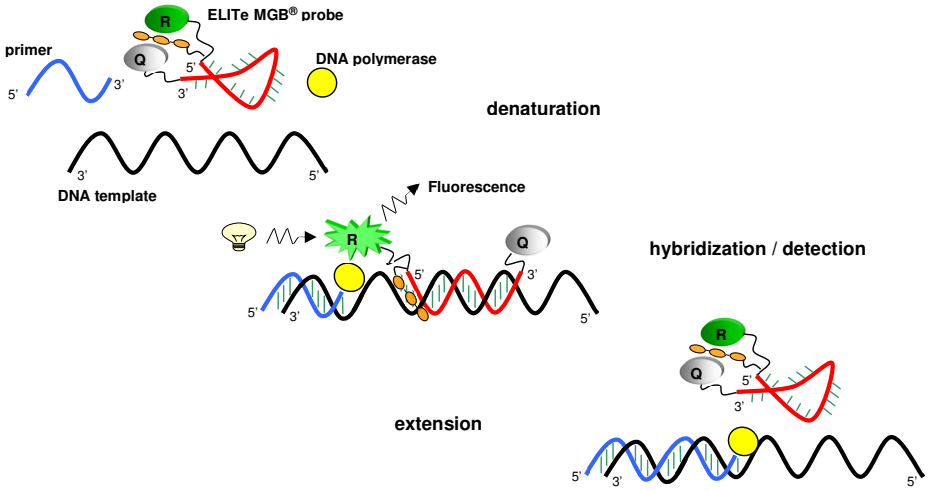
The assay consists of a real time amplification reaction with a programmable thermostat provided with a fluorescence detection optical system.

In each well, two amplification reactions are performed starting from DNA extracted from the samples being tested: a specific reaction for a region of the **EBNA-1 gene** of EBV genome and a specific reaction for a region of the **human beta Globin gene** (Internal Control of inhibition). The EBV specific ELITe MGB® probe, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the EBV amplification reaction. The Internal Control specific ELITe MGB® probe, labelled with AP525 fluorophore (analogue to VIC), is activated when hybridizes with the specific product of the amplification reaction for the Internal Control. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data allows detecting the presence and the titre of EBV DNA into the starting sample.

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

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

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



PRODUCT DESCRIPTION

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

The primers and the EBV specific probe (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non-fluorescent molecule) are specific for a region of the **EBNA-1 gene** of EBV.

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

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

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

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

MATERIALS PROVIDED IN THE PRODUCT

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

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

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

OTHER PRODUCTS REQUIRED

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

For automatic sample analysis with the instrument **«ELITe InGenius®»** (ELITechGroup S.p.A., ref. INT030) the following generic products are validated: the extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200) or «**ELITe InGenius® SP 1000**» (ELITechGroup S.p.A., ref. INT033SP1000), the consumables for extraction and amplification of nucleic acids from biological samples **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS), **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000), **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR) and **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, code TF-350-L-R-S).

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

for the calibrators **«EBV ELITe_STD»** or **«EBV ELITe_STD_1000_100»**,

for the positive control of amplification **«EBV ELITe_PC»** or **«EBV ELITe_PC_1000_100»**,

for negative control of amplification **«EBV ELITe_NC»** or **«EBV ELITe_NC_1000_100»**,

for samples analysis **«EBV ELITe_WB_200_100», «EBV ELITe_PL_200_100»**, and **«EBV ELITe_PL_1000_100».**

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

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

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

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

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

for samples analysis **«EBV ELITe_Be_WB_200_100» and «EBV ELITe_Be_PL_200_100»**.

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

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

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

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

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

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

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

When a 7500 Fast Dx Real-Time PCR Instrument is used, it is recommended the use of generic product: **«MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL»** (Life Technologies, ref. 4346906), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

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

If detection of EBV DNA is required (qualitative analysis), use the product

«EBV - ELITe Positive Control» (ELITechGroup S.p.A., ref. CTR020PLD) or the product **«EBV - ELITe Positive Control RF»** (ELITechGroup S.p.A., ref. CTR020PLD-R) specific for the use with cobas z 480 analyzer, positive control of plasmid DNA.

If detection and quantification of EBV DNA is required (quantitative analysis), use the product **«EBV ELITe Standard»** (ELITechGroup S.p.A., ref. STD020PLD), four dilutions of known quantity plasmid DNA to obtain the standard curve.

A conversion factor allows to express the results of the quantitative analysis in International Units of EBV of "1st WHO International Standard for Human Epstein Barr Virus for Nucleic Acid Amplification Techniques" (NIBSC ref. 09/260, United Kingdom).

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

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

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

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

Warnings and precautions for molecular biology

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

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

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

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

The extraction products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The reagents required for amplification

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

Amplification products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination.

Warnings and precautions specific for the components

The **EBV Q - PCR Mix** must be stored at temperature lower than -20°C in the dark.

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

The **EBV Q - PCR Mix** can be kept on board in the inventory area cool block up to five independent work sessions of three hours each (“Extraction+PCR” run mode) or for three consecutive work sessions of three hours each (“Extraction+PCR” run mode).

ELITe InGenius AND ELITe BeGenius

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Whole blood collected in EDTA

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

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

Note: when the DNA extraction from whole blood is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version **1.3** (or later equivalent versions), use the extraction protocol **EBV ELITe _WB_200_100**. This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL of water.

Note: when the DNA extraction from whole blood is carried out with the **ELITe BeGenius** and with **ELITe BeGenius Software** version **2.0** (or later equivalent versions), use the extraction protocol **EBV ELITe_Be_WB_200_100.** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

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

Plasma collected in EDTA

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

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

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

Note: when the DNA extraction from 200 µL of plasma is carried out with the **ELITe BeGenius** and with **ELITe BeGenius Software** version **2.0** (or later equivalent versions), use the extraction protocol **EBV ELITe_Be_PL_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

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

Note: when the DNA extraction from 1000 µL of plasma is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version **1.3** (or later equivalent versions), use the extraction protocol **EBV ELITe _PL_1000_100**. These protocols process 1000 µL of sample, add the **CPE** at 10 µL / extraction and elute the nucleic acids in 100 µL.

Primary tube can NOT be used in association with the assay protocol EBV ELITe_PL_1000_100.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in "Potential Interfering substances" paragraph of "Performance characteristics" chapter.

Do not use Whole Blood or Plasma collected in heparin in order to avoid inhibition of amplification reaction and frequent invalid results.

Amplification calibrators and amplification controls

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

as calibrator set, use the four concentration levels of the **EBV ELITe Standard**, in association with the protocol **«EBV ELITe_STD»** or **«EBV ELITe_STD_1000_100»** for **ELITe InGenius**, and **«EBV ELITe_Be_STD»** for **ELITe BeGenius**,

as amplification Positive Control use the **EBV - ELITe Positive Control**, in association with the protocol **«EBV ELITe_PC»** or **«EBV ELITe_PC_1000_100»** for **ELITe InGenius**, and **«EBV ELITe_Be_PC»** for **ELITe BeGenius**,

as amplification Negative Control, use Molecular biology grade water (not provided with this kit), in association with protocol **«EBV ELITe_NC»** or **«EBV ELITe_NC_1000_100»** for **ELITe InGenius**, and **«EBV ELITe_Be_NC»** for **ELITe BeGenius**.

Note: ELITe InGenius with **ELITe InGenius Software** and **ELITe BeGenius** with **ELITe BeGenius Software** allow generation of the calibration curve and the validation of amplification Controls for each lot of amplification reagent to be stored in its database.
Calibration curves, approved and stored in the database, will expire after **60 days**. At expiration date it is necessary to re-run the calibrator set.
Amplification validation Control results, approved and stored in the database, will expire after **15 days**. At expiration date it is necessary to re-run the Positive and Negative Controls.

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

Quality controls

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

PROCEDURE ELITe InGenius

The procedure to use the **«EBV - ELITe MGB® Kit»** with the system **ELITe InGenius** consists of three steps:
- Verification of the system readiness
- Set up of the session
- Review and approval of results

Verification of the system readiness

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

- switch on the **ELITe InGenius** and select the mode "**CLOSED**";
- verify that the Calibrators (Calibration - **EBV Q-PCR Standard**) are run, approved and not expired (status). This can be checked under the "Calibration" menu in the Home page;
- verify (Controls) that the amplification Controls (**EBV Positive Control**, EBV Negative Control) are run, approved and not expired (status). This can be checked under the "Control" menu in the Home page;

- choose the type of run and set up the run, following the instructions of Graphical User Interface (GUI) for the session set up and using the Assay Protocols provided by ELITechGroup. These IVD protocols were specifically validated with ELITe MGB Kits, matrices and ELITe InGenius instrument and **ELITe InGenius** instrument and the cited matrix.

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

Assay protocols for EBV ELITe MGB® kit and ELITe InGenius

Name	Matrix	Report unitage	Characteristics
EBV ELITe_WB_200_100	Whole Blood	Copies/mL or IU/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
EBV ELITe_PL_200_100	Plasma	Copies/mL or IU/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
EBV ELITe_PL_1000_100	Plasma	Copies/mL or IU/mL	Extraction Input Volume: 1000 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Protocols for qualitative analysis are available on request.

Setup of the session

The **EBV ELITe MGB® Kit** in association to the **ELITe InGenius** can be used in in order to perform:
A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Calibration run (PCR only),
D. Amplification Positive and/ or Positive Control run (PCR only),

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

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

The main operations for setting the four types of run are described below.

A. Integrated run

To set up the integrated run, carry out the steps below following the **SW Graphical User Interface (GUI)**:

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw a sufficient number of CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home screen”.
4. Select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
5. For each Track of interest fill in the “SampleID” (SID) by typing or by scanning the sample barcode.
6. Select the assay protocol to be used in the “Assay” column (i.e. EBV ELITe_WB_200_100).
7. Ensure that the “Protocol” displayed is: “Extract + PCR”.
8. Select the sample loading position in the “Sample Position” column:
   - if a primary tube is used select “Primary Tube”, the Primary tube can be use only starting from 200 µL of samples;
   - if a secondary tube is used select “Extraction Tube”.

   Click “Next” to continue the setup.
9. Load CPE and EBV Q-PCR Mix on the Inventory Block selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the “PCR Cassette”, the “ELITe InGenius SP 200” or “ELITe InGenius SP1000” extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

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

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

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

Note: The **EBV Q - PCR Mix** can be kept on board in the inventory area cool block up to five independent work sessions of three hours each (“Extraction+PCR” run mode) or for three consecutive work sessions of three hours each (“Extraction+PCR” run mode).

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B. Amplification run

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

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home screen”.
3. Even if the extraction will not be performed, select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 200 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
4. For each Track of interest type the “SampleID” (SID) by typing or by scanning the sample barcode.
5. Select the assay protocol to be used in the “Assay” column (i.e. EBV ELITe _WB_200_100).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure the Eluted sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load EBV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the “PCR Cassette” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

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

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

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

Note: The **EBV Q - PCR Mix** can be kept on board in the inventory area cool block up to five independent work sessions of three hours each (“Extraction+PCR” run mode) or for three consecutive work sessions of three hours each (“Extraction+PCR” run mode).

C. Calibration run

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

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw EBV Q - PCR Standard tubes (Cal1: EBV Q-PCR Standards 10^2, Cal2: EBV Q-PCR Standards 10^3, Cal3: EBV Q-PCR Standards 10^4, Cal4: EBV Q-PCR Standards 10^5). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home screen”.
4. Even if the extraction will not be performed, select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
5. Starting from the Track of interest, select the assay protocol to be used in the “Assay” column (i.e. EBV ELITe_STD or EBV ELITe_STD_1000_100) and fill with the lot number and expiry date for the EBV Q - PCR Standard. Click “Next” button to continue the setup.
6. Load the EBV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
7. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load the Calibrator tubes and “PCR Cassettes” on board following the GUI instruction. Click “Next” to continue the setup. Take care to load the PCR Standard fluids to the correct tracks as indicated in the GUI.
9. Close the instrument door.
10. Press “Start” to start the run.

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

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

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

Note: The **EBV Q - PCR Mix** can be kept on board in the inventory area cool block up to five independent work sessions of three hours each (“Extraction+PCR” run mode) or for three consecutive work sessions of three hours each (“Extraction+PCR” run mode).

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D. Amplification run for Positive Control and Negative Control

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

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the product EBV - ELITe Positive Control, for Positive Control amplification. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water for the sessions in one Elution tube, provided with the ELITe InGenius SP Consumable Set.
4. Select “Perform Run” from the “Home screen”.
5. Even if the extraction will not be performed, select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
6. For the positive control, select EBV ELITe_PC or EBV ELITe_PC_1000_100 for the positive control and fill in the lot number and expiry date for the EBV Positive Control.
7. For the negative control, select EBV ELITe_NC or EBV ELITe_NC_1000_100 and fill in the lot number and expiry date for the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load EBV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the amplification PCR cassette, the Positive Control and/or the Negative Control, following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

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

Note: The Positive Control and the Negative Control must be run as amplification control, to set up the “Control Charts”. Four Positive Control and Negative Control results, from 4 different runs are requested to set up the control chart. After that, the results of Positive control and Negative Control are used for monitoring the amplification step performances. Refer to the user’s manual of the instrument for more details.

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

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

Note: The **EBV Q - PCR Mix** can be kept on board in the inventory area cool block up to five independent work sessions of three hours each (“Extraction+PCR” run mode) or for three consecutive work sessions of three hours each (“Extraction+PCR” run mode).

Review and approval of results

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

Note: For detailed information refer to the **ELITe InGenius** instrument user manual.

The **ELITe InGenius** generates results using the **EBV ELITe MGB® Kit** through the following procedure:

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

A. Validation of Calibration curve

The fluorescence signals emitted by the specific EBV probe ("EBV") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocols "EBV ELITe_STD" and "EBV ELITe_STD_1000_100".

The Calibration curve, specific for the amplification reagent lot, is stored in the database (Calibration) after the approval of the "Administrator" or "Analyst" personnel by following the GUI instruction.

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

Before analysing any sample, it is absolutely mandatory to generate and to approve the Calibration curve for the lot of amplification reagent used. The availability of Calibration curve results with "Approved" (Status) is shown in the "Calibration" window of the ELITe InGenius software.

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

Note: When the Calibration Curve is run together with samples and its result is invalid, the entire session is invalid and the amplification of all samples must be repeated.

B. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the specific EBV probe ("EBV") in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocols "EBV ELITe_PC", "EBV ELITe_PC_1000_100", EBV ELITe_NC" and "EBV ELITe_NC_1000_100".

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, are stored in the database (Controls) after the approval of the "Administrator" or "Analyst" personnel by following the GUI instruction.

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

Before analysing any sample and after approval of the Calibration curve, it is absolutely mandatory to generate and to approve an amplification Positive Control and Negative Control results for the lot of amplification reagent used. The availability of an amplification Positive Control and Negative Control results with "Approved" (Status) is shown in the "Controls" window of the ELITe InGenius software. If the amplification Positive Control and Negative Control results are missing, generate them as described above.

Note: When the Positive Control or Negative Control result does not meet the acceptance criteria, the "not passed" message is shown on the "Controls" menu and it is not possible to approve the result. The Positive Control or Negative Control amplification reaction has to be repeated.

Note: When the Positive Control or Negative Control are run as an amplification control together with samples and its result is invalid, the entire session is invalid and the amplification of all samples must be repeated.

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C. Validation of Samples results

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

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

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

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

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

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

For each Sample the calculation of the viral load is automatically performed by the system. The measure is expressed as "copies / mL" or "IU / mL" as set in the assay protocol.

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

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

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

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

Samples suitable for analysis but in which it was not possible to detect resistance gene DNA are reported as: "DNA Not Detected or below LoD". In this case it cannot be excluded that the resistance gene DNA is present at a concentration below the limit of detection of the assay (see "Performance characteristics).
Note: The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes concerning the patient.

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

D. Samples result reporting

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

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

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

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

PROCEDURE ELITe BeGenius

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

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

System readiness verification

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

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

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

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

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

Qualitative Assay Protocols are available upon request.

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Setup of the session

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

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

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

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

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

A. Sample run

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

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw a sufficient number of CPE tubes for the session. Each new tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home screen”.
4. Remove all the Racks from the “Cooler Unit” and place them on the preparation table.
5. Select the “run mode”: “Extract + PCR”.
6. Load the samples into the cooling area starting from the L5 Sample Rack.
7. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.

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

8. Check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
9. Select the assay protocol to be used in the “Assay” column (i.e. EBV ELITe_Be_WB_200_100). Click “Next” to continue the setup.
10. If a second extraction has to be performed, repeat steps 6 to 9 using the L4 Sample Rack.
11. Load the barcoded eluate tubes into cooling area starting from L3 Elution Rack.

Note: Elution tubes can be labelled to improve traceability.

12. Insert the L3 Elution Rack into the “Cooler Unit”. Click “Next” to continue the setup.
13. Repeat steps 11 and 12 using the L2 Reagent/Elution Rack.
14. Load CPE and EBV Q-PCR Mix into the into cooling area.
15. Insert the L1 Reagent Rack into the “Cooler Unit”. Click “Next” to continue the setup.
16. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
17. Load the PCR Rack with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
18. Load the Extraction Rack with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables by following the GUI instruction. Click “Next” to continue the setup.
19. Close the instrument door.
20. Press “Start” to start the run.

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

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

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

B. Amplification run

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

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Select "Perform Run" from the "Home screen".
3. Remove Racks 1, 2 and 3 from the "Cooler Unit" and place them on the preparation table.
4. Select the "run mode": "PCR Only".
5. Load the samples into the cooling area starting from the L3 Elution Rack.
6. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
7. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
8. Select the assay protocol to be used in the "Assay" column (e.g. EBV ELITe_Be_WB_200_100). Click "Next" to continue the setup.
9. Load EBV Q-PCR Mix into the cooling area.
10. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
11. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
12. Load the PCR Rack with "PCR Cassette" in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
13. Close the instrument door.
14. Press "Start" to start the run.

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

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

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

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C. Calibration run

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

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the EBV Q - PCR Standard tubes (Cal1: EBV Q-PCR Standards 10^2, Cal2: EBV Q-PCR Standards 10^3, Cal3: EBV Q-PCR Standards 10^4, Cal4: EBV Q-PCR Standards 10^5). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. Remove Racks 1, 2 and 3 from the "Cooler Unit" and place them on the preparation table.
5. Select the "run mode": "PCR Only".
6. Load the Calibrator tubes into the L3 Elution Rack.
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
8. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
9. Select the assay protocol "EBV ELITe_Be_STD" to be used in the "Assay" column. Click "Next" button to continue the setup.
10. Load EBV Q-PCR Mix into the L2 Reagent/Elution Rack.
11. Insert the L2 Reagent/Elution Rack into the "Cooler Unit". Click "Next" to continue the setup.
12. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
13. Load the PCR Rack with "PCR Cassette" in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
14. Close the instrument door.
15. Press "Start" to start the run.

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

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

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

D. Positive Control and Negative Control run

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

1. Thaw a sufficient number of EBV Q - PCR Mix tubes for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the product EBV - ELITe Positive Control, for Positive Control amplification. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of the molecular biology grade water (as Negative Control) for the sessions in one Elution tube, provided with the ELITe InGenius SP Consumable Set.
4. Select “Perform Run” from the “Home screen”.
5. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
6. Select the “run mode”: “PCR Only”.
7. Load the Positive Control and Negative Control tubes into the L3 Elution Rack.
8. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
9. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
10. Select the assay protocol EBV ELITe_Be_PC and EBV ELITe_Be_NC to be used in the “Assay” column. Click “Next” button to continue the setup.
11. Load EBV Q-PCR Mix into the L2 Reagent/Elution Rack.
12. Insert the L2 Reagent/Elution Rack into the “Cooler Unit”. Click “Next” to continue the setup.
13. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
14. Load the PCR Rack with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
15. Close the instrument door.
16. Press “Start” to start the run.

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

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

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

Review and approval of results

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

The **ELITe BeGenius** generates the results using the EBV ELITe MGB Kit through the following procedure:

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

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

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PERFORMANCE CHARACTERISTICS

Analytical sensitivity: Limit of Detection

The analytical sensitivity of this assay, as Limit of Detection (LoD), in association with whole blood and plasma collected in EDTA and **ELITe InGenius** was verified with a panel of EBV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, United Kingdom) in EBV DNA - negative matrix. The panel consisted of at least six points around the limit concentration. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, run set up, extraction of nucleic acids, real time amplification and data interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The final results are reported in the following tables.

Limit of Detection with ELITe InGenius (IU / mL)				
Sample volume	Matrix	LoD	95% confidence range	
			lower limit	lower limit
200 µL	**Whole bood**	**104 IU / mL**	75 IU / mL	175 IU / mL
	Plasma	**124 IU / mL**	77 IU / mL	290 IU / mL
1000 µL	**Plasma**	**18 IU / mL**	13 IU / mL	28 IU / mL

The analytical sensitivity as copies / mL for each matrix and **ELITe InGenius** is calculated by applying the specific conversion factor reported at page 30.

The analytical sensitivity as copies / mL is reported below.

Limit of Detection with ELITe InGenius (copies / mL)				
Sample Volume	Matrix	LoD	95% confidence range	
			lower limit	upper limit
200 µL	**Whole blood**	36 copies / mL	26 copies / mL	60 copies / mL
	Plasma	65 copies / mL	41 copies / mL	153 copies / mL
1000 µL	**Plasma**	11 copies / mL	8 copies /mL	18 copies /mL

The calculated LoD value was verified in association to **ELITe InGenius** and **ELITe BeGenius** by testing 20 replicates of Whole Blood collected in EDTA and 20 replicates of Plasma collected in EDTA samples spiked by EBV certified reference material (1st WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 18 out of 20 replicates give a positive result as per CLSI standard EP17-A.

The results are reported in the following tables.

Limit of Detection for Whole Blood and Plasma samples and ELITe InGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Whole Blood collected in EDTA	104 IU / mL	EBV	20	20	0
Plasma collected in EDTA	124 IU / mL	EBV	20	20	0

Limit of Detection for Whole Blood and Plasma samples and ELITe BeGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Whole Blood collected in EDTA	104 IU / mL	EBV	20	19	1
Plasma collected in EDTA	124 IU / mL	EBV	20	20	0

The LoD value for EBV target was confirmed at 104 IU / mL for Whole Blood collected in EDTA, at 124 IU / mL for Plasma collected in EDTA.

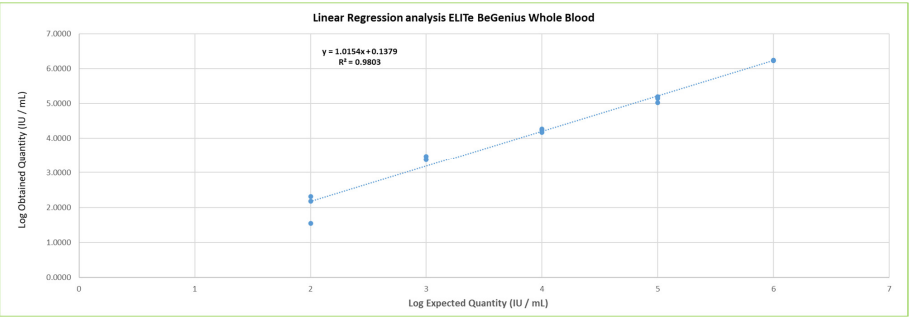
Linear measuring range and Limits of quantification

The linear measuring range of EBV ELITe MGB Kit used in association with Whole Blood and Plasma collected in EDTA (sample volume 200 µL) and **ELITe InGenius and ELITe BeGenius** was verified with a panel of EBV dilutions. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, United Kingdom) in EBV DNA - negative matrix. The panel consisted of five dilution points (1 $\log_{10}$ dilution steps) from 10^6 IU / mL to 10^2 IU / mL. Each sample of the panel was tested in 3 replicates. The analysis of the obtained data, performed by linear regression, demonstrated that the assay shows a linear response for all the dilution levels.

For Whole Blood:

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Whole Blood samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.999 for **ELITe InGenius** and 0.980 for **ELITe BeGenius**.

The results are reported in the following tables.



The Lower Limit of Quantification (LLoQ) was set at 104 IU / mL, the LoD concentration, that gives quantitative results precise (Standard Deviation equal to 0.2968 Log IU / mL for **ELITe InGenius** and 0.2486 Log IU / mL for **ELITe BeGenius**) and accurate (Bias equal to 0.4035 Log IU / mL for **ELITe InGenius** and 0.1329 Log IU / mL for **ELITe BeGenius**).

The Upper Limit of Quantification (ULoQ) was set at 1,000,001 IU / mL, the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0299 Log IU / mL for **ELITe InGenius** and 0.0079 Log IU / mL for **ELITe BeGenius**) and accurate (Bias equal to 0.3459 Log IU / mL for **ELITe InGenius** and 0.2311 Log IU / mL for **ELITe BeGenius**).
The linear measuring range as copies / mL for Whole Blood is calculated by applying the specific conversion factor reported at page 30.

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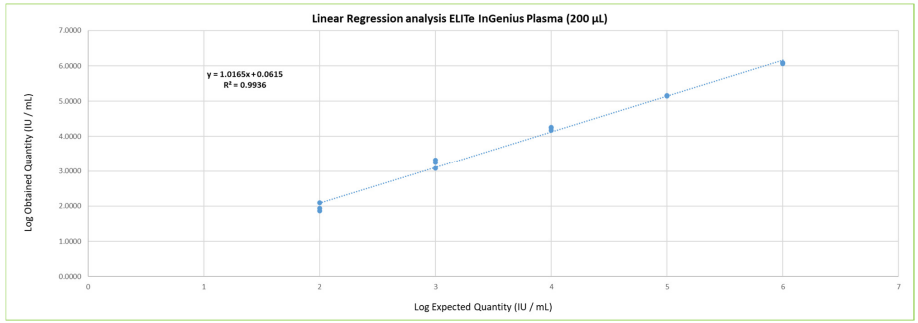
The final results are summarized in the following table.

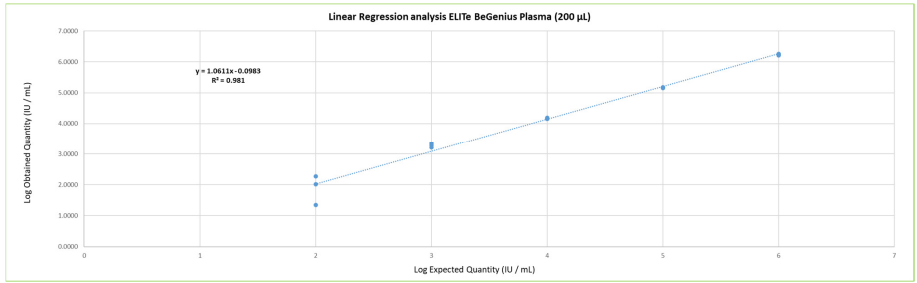
Linear measuring range for Whole Blood samples and ELITe InGenius and ELITe BeGenius			
Sample volume	Unit of measure	lower limit	upper limit
200 µL	**IU / mL**	**104**	**1,000,001**
	copies / mL	**36**	**344,828**

For plasma (sample volume 200 µL):

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Plasma collected in EDTA samples shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.994 for **ELITe InGenius** and 0.981 for **ELITe BeGenius**.

The results are reported in the following tables.





The Lower Limit of Quantification (LLoQ) was set at the LoD concentration that gives quantitative results precise (Standard Deviation = 0.2728 Log IU / mL for **ELITe InGenius** and Standard Deviation = 0.3457 Log IU / mL for **ELITe BeGenius**) and accurate (Bias = 0.0556 Log IU / mL for **ELITe InGenius** and Bias = 0.1089 Log IU / mL for **ELITe BeGenius**) within ±0.5 Log IU / mL: 124 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration that gives quantitative results precise (Standard Deviation = 0.0154 Log IU / mL for **ELITe InGenius** and Standard Deviation = 0.0252 Log IU / mL for **ELITe BeGenius**) and accurate (Bias = 0.0761 Log IU / mL for **ELITe InGenius** and Bias = 0.2348 Log IU / mL for **ELITe BeGenius**) within ±0.5 Log IU / mL: 1,000,000 IU / mL.

The linear measuring range as copies / mL for Plasma collected in EDTA is calculated by applying the specific conversion factor reported at page 30.

The final results are summarized in the following table.

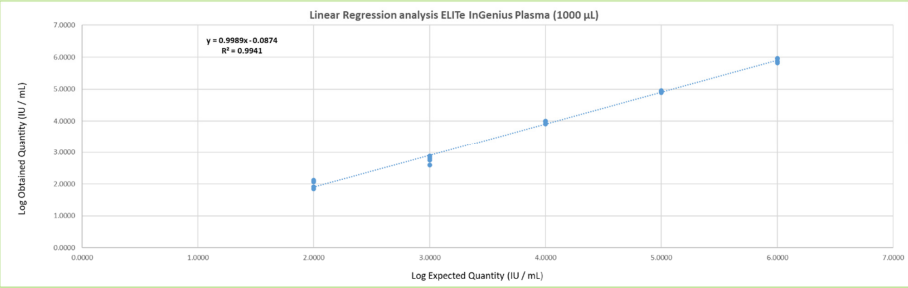
Linear measuring range for Plasma samples and ELITe InGenius and ELITe BeGenius (200 µL)		
Unit of measure	lower limit	upper limit
IU / mL	**124**	**1,000,000**
copies / mL	**65**	**526,316**

For plasma (sample volume 1000 µL):

The linear measuring range of EBV ELITe MGB Kit used in association with Plasma collected in EDTA (sample volume 1000 µL) and **ELITe InGenius** was verified with a panel of EBV dilutions. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, United Kingdom) in EBV DNA - negative matrix. The panel consisted of five dilution points (1 $\log_{10}$ dilution steps) from 10^6 IU / mL to 10^2 IU / mL. Each sample of the panel was tested in 3 replicates. The analysis of the obtained data, performed by linear regression, demonstrated that the assay shows a linear response for all the dilution levels.

The analysis of the obtained data, performed by linear regression analysis, demonstrated that the assay in association with Plasma samples (sample volume 1000 µL) shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.994 for **ELITe InGenius**.

The results are reported in the following tables.



The Lower Limit of Quantification (LLoQ) was set at the lower concentration that gives quantitative results precise (Standard Deviation = 0.126 Log IU / mL for **ELITe InGenius**) and accurate (Bias = -0.015 Log IU / mL for **ELITe InGenius**) within ±0.5 Log IU / mL: 99 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration that gives quantitative results precise (Standard Deviation = 0.064 Log IU / mL for **ELITe InGenius**) and accurate (Bias = -0.102 Log IU / mL for **ELITe InGenius**) within ±0.5 Log IU / mL: 1,000,000 IU / mL.

The linear measuring range as copies / mL for Plasma collected in EDTA is calculated by applying the specific conversion factor reported at page 30.

The final results are summarized in the following table.

Linear measuring range for Plasma samples and ELITe InGenius (1000 µL)			
Sample volume	Unit of measure	lower limit	upper limit
1000 µL	**IU / mL**	**99**	**1,000,000**
	copies / mL	**62**	**625,000**

The lower limit of the linear measuring range was set at the lowest concentration that gives 100% of positivity and quantitative results sufficiently accurate and precise.

The upper limit of the linear measuring range was set at the highest concentration that gives quantitative results sufficiently accurate and precise.
The linear measuring range as copies / mL for each matrix is calculated by applying the specific conversion factor reported at page 30.

Repeatability

The Repeatability of results obtained by the product EBV ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Whole Blood samples collected in EDTA. The panel included one negative sample and two samples spiked by EBV certified reference material (1st WHO EBV International Standard, NIBSC) at concentration of 3 x LoD (about 312 IU / mL) and of 10 x LoD (about 1040 IU / mL).

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

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

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

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe InGenius Lot U0521-016								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	23.97	0.38	1.60
3 x LoD	8 / 8	35.68	0.57	1.60				
10 x LoD	8 / 8	34.22	0.24	0.70				

Inter – Session Repeatability ELITe InGenius Lot U0521-016								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 14	N.A.	N.A.	N.A.	46 / 46	24.21	0.46	1.91
3 x LoD	16 / 16	35.72	0.53	1.48				
10 x LoD	16 / 16	34.39	0.37	1.07				

In the Repeatability test on **ELITe InGenius**, the assay detected the EBV target as expected and showed low %CV of Ct values that did not exceed 1.6% for EBV and 1.9% for Internal Control.

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

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

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

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe BeGenius Lot U0521-016								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24/24	27.13	0.76	2.80
3 x LoD	8 / 8	37.32	0.49	1.30				
10 x LoD	8 / 8	35.97	0.43	1.19				

Inter – Session Repeatability ELITe BeGenius Lot U0521-016								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 14	N.A.	N.A.	N.A.	46 / 46	27.32	0.69	2.53
3 x LoD	16 / 16	37.29	0.67	1.79				
10 x LoD	16 / 16	35.82	0.67	1.86				

In the Repeatability test on **ELITe BeGenius**, the assay detected the EBV target as expected and showed low %CV of Ct values that did not exceed 1.9% for EBV and 2.8% for Internal Control.

Reproducibility

The Reproducibility of results obtained by the product EBV ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of whole blood samples. The panel included one negative sample and two samples spiked with EBV certified reference material (1st WHO EBV International Standard, NIBSC) at concentration of 3 x LoD (about 312 IU / mL) and of 10 x LoD (about 1040 IU / mL).

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

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

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

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe InGenius								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	25.25	0.70	2.77
3 x LoD	8 / 8	35.78	0.44	1.24				
10 x LoD	8 / 8	30.38	0.36	1.17				

Inter – Batch Reproducibility ELITe InGenius								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	25.25	0.70	2.77
3 x LoD	8 / 8	35.91	0.38	1.06				
10 x LoD	8 / 8	34.48	0.15	0.43				

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In the Reproducibility test on **ELITe InGenius**, the assay detected the EBV target as expected and showed low %CV of Ct values that did not exceed 1.24% for EBV and 2.77%for Internal Control.

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

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

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

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe BeGenius								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 7	N.A.	N.A.	N.A.	23 / 23	28.39	0.61	2.14
3 x LoD	8 / 8	36.79	0.86	2.32				
10 x LoD	8 / 8	35.15	0.65	1.84				

Inter – Batch Reproducibility ELITe BeGenius								
Sample	**EBV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 7	N.A.	N.A.	N.A.	23 / 23	28.23	0.57	2.02
3 x LoD	8 / 8	37.45	0.65	1.72				
10 x LoD	8 / 8	35.57	0.42	1.18				

In the Reproducibility test on **ELITe BeGenius**, the assay detected the EBV target as expected and showed low %CV of Ct values that did not exceed 2.32% for EBV and 2.14 %for Internal Control.

Reproducibility with certified reference material

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

The results, obtained starting from 200 µL of sample, are reported in the following table.

Tests with calibrated reference materials and ELITe InGenius				
Sample	Nominal titre IU / mL	Nominal titre Log_{10} IU / mL	Positive / Replicates	Mean results Log_{10} IU / mL
EBVMQP01-High	36,577	4.560	2/2	4.835
EBVMQP01-Medium	3,657	3.560	2/2	3.843
EBVMQP01-Low	365	2.560	2/2	2.899
EBVMQP01-Negative	Negative	-	0/2	-

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

The results, obtained starting from 1000 µL of sample, are reported in the following table.

Tests with calibrated reference materials and ELITe InGenius				
Sample	Nominal titre IU / mL	Nominal titre Log IU / mL	Positive / Replicates	Mean results Log IU / mL
EBVMQP01-High	36,577	4.560	2/2	4.765
EBVMQP01-Medium	3,657	3.560	2/2	3.795
EBVMQP01-Low	365	2.560	2/2	2.592
EBVMQP01-Negative	negativo	-	0/2	-

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

Further tests were carried out using as reference material the calibrated panel «AcroMetrix EBV Plasma Panel» (Life Technologies). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** System and ELITechGroup S.p.A. products.

The results, obtained starting from 200 µL of sample, are reported in the following table.

Tests with calibrated reference materials and ELITe InGenius				
Sample	Nominal titre IU / mL	Nominal titre Log_{10} IU / mL	Positive / Replicates	Mean results Log_{10} IU / mL
Acrometrix EBV 1E6	10^6	6.000	2/2	5.791
Acrometrix EBV 1E5	10^5	5.000	2/2	5.044
Acrometrix EBV 1E4	10^4	4.000	2/2	3.776
Acrometrix EBV 1E3	10^3	3.000	2/2	2.541
Acrometrix EBV 1E2	10^2	2.000	2/2	2.034

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

Further tests were carried out using as reference material QCMD 2014 Epstein-Barr Virus DNA EQA Panel (Qnostics Ltd, Scotland, UK) a panel of EBV dilutions. Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, using **ELITe InGenius** and ELITechGroup S.p.A. products.

The results, obtained starting from 200 µL of sample, are reported in the following table.

Tests with calibrated reference materials and ELITe InGenius				
Sample	Consensus virus conc. Log_{10} IU / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
EBVDNA14-01	3.504	0.212	2/2	3.439
EBVDNA14-02	3.169	0.295	2/2	2.876
EBVDNA14-03	2.500	0.310	2/2	2.275
EBVDNA14-04	3.956	0.208	2/2	4.190
EBVDNA14-05	negative	-	0/2	-
EBVDNA14-06	3.957	0.259	2/2	3.999
EBVDNA14-07	2.962	0.220	2/2	2.953
EBVDNA14-08	3.465	0.221	2/2	3.419

All samples were correctly detected. Six (6) out of seven positive samples were quantified within the range defined by the Consensus ± 1 Standard Deviation (SD) and one sample (EBVDNA14-04) was quantified within ± 2 SD. However, this sample is slightly over quantified (+0.234 Log IU /mL while the SD is equal to 0.208 Log IU / mL).

Further tests, starting from 1000 µL of sample, were carried out using as calibrated reference material the panel « QCMD 2015 Epstein-Barr virus DNA EQA Panel» (Qnostics Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products.

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The results, obtained starting from 1000 µL of sample, are reported in the following table.

Tests with calibrated reference materials and ELITe InGenius				
Sample	Consensus virus conc. Log_{10} IU / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
EBVDNA15C1-01	3.418	0.343	2/2	3.220
EBVDNA15C1-02	3.415	0.345	0/2	3.098
EBVDNA15C1-03	Negative	-	2/2	-
EBVDNA15C1-04	3.955	0.305	2/2	3.697
EBVDNA15C1-05	2.493	0.516	2/2	2.136
EBVDNA15C2-01	3.956	0.350	2/2	3.662
EBVDNA15C2-02	3.942	0.347	2/2	3.697
EBVDNA15C2-03	2.886	0.374	2/2	2.622
EBVDNA15C2-04	3.952	0.377	2/2	3.732
EBVDNA15C2-05	2.912	0.340	2/2	2.723

All samples were correctly detected. All positive samples were quantified within the range defined by the Consensus ± 1 Standard Deviation (SD)

Further tests were carried out using as reference material QCMD 2014 Epstein-Barr Virus Whole Blood EQA Panel (Qnostics Ltd, Scotland, UK) a panel of EBV dilutions. Each sample of the panel was tested in 4 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, using **ELITe InGenius** and ELITechGroup S.p.A. products.

The results in IU/mL were calculated applying the conversion factor for **ELITe InGenius** System and whole blood samples and are reported in the following table.

Tests with calibrated reference materials and ELITe InGenius				
Sample	Consensus virus conc. Log_{10} IU / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
EBVWB14-01	3.361	0.439	4/4	3.242
EBVWB14-02	2.960	0.641	4/4	2.037
EBVWB14-03	3.841	0.367	4/4	3.860
EBVWB14-04	3.845	0.362	4/4	3.786
EBVWB14-05	3.441	0.343	4/4	3.161
EBVWB14-06	4.255	0.451	4/4	4.466
EBVWB14-07	negative	-	0/4	-
EBVWB14-08	4.889	0.290	4/4	4.955

All samples were correctly detected. Six (6) out of seven positive samples were quantified within the range defined by the Consensus ± 1 Standard Deviation (SD) and one sample (EBVWB14-02) was quantified within ± 2 SD. However, this sample had a low titre and showed a high SD in the proficiency study.

Conversion factor to International Units

The conversion factor to be used with this assay to transform the quantitative result from copies / mL into International Units / mL was determined using a panel of calibrated reference material approved by the WHO ("1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques", NIBSC, United Kingdom, code 09/162) in the negative Whole Blood and Plasma collected in EDTA for EBV DNA and in association with **ELITe InGenius**. The panel had at least 3 dilution steps of 1 Log. Each point of the panel was tested in at least 10 replicates carrying out the whole analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products.

A summary of results is shown in the tables below.

Conversion factor to International Units Whole Blood, Fc = 2.9 IU / copy						
Sample			Result			Log difference
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	(ref. - test)
100000	5.000	10	47041	135479	5.108	- 0.108
10000	4.000	10	4509	12987	4.063	- 0.063
1000	3.000	10	223	641	2.746	+ 0.254

Conversion factor to International Units Plasma (sample volume 200 µL), Fc = 1.9 IU / copy						
Sample			Result			Log difference
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	(ref. - test)
100000	5.000	10	72352	137469	5.128	- 0.128
10000	4.000	10	5092	9674	3.967	+ 0.033
1000	3.000	10	435	826	2.904	+ 0.096

Conversion factor to International Units Plasma (sample volume 1000 µL), Fc = 1.6 IU / copy						
Sample			Result			Log difference
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	(ref. - test)
316228	5.5	16	182001	291201	5.459	+ 0.041
100000	5	16	57197	91515	4.953	+ 0.047
31623	4.5	16	20626	33002	4.510	- 0.010
10000	4	16	6911	11058	4.028	- 0.028
3162	3.5	16	2086	3338	3.514	- 0.014
1000	3	16	604	966	2.965	+ 0.035

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

Conversion factor to International Units with ELITe InGenius		
Sample volume	Matrix	Fc (IU / copies)
200 µL	**Whole Bood**	**2.9**
	Plasma	**1.9**
1000 µL	**Plasma**	**1.6**

The conversion factor, to convert a quantitative result from copies / mL to International Units / mL, was verified for **ELITe InGenius** and **ELITe BeGenius** analysing the results obtained during the Linearity test.

The target quantification precision, as Standard Deviation of Log IU/mL, was lower than 0.5 Log for both Whole Blood and Plasma and meet the acceptance criteria for **ELITe InGenius** and **ELITe BeGenius**.

The target quantification accuracy, as difference between the Theoretical and Measured concentrations in Log IU / mL, was lower than 0.5 Log for both Whole Blood and Plasma and meet the acceptance criteria for **ELITe InGenius** and **ELITe BeGenius.**

These results confirmed the Conversion factors calculated for each matrix with **ELITe InGenius**.

Diagnostic sensitivity: confirmation of positive samples

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analysing some clinical samples of Whole Blood collected in EDTA and Plasma collected in EDTA positive for EBV DNA in association with **ELITe InGenius**. As **ELITe BeGenius** showed equivalent analytical performances to **ELITe InGenius**, it can be assumed that the results of Diagnostic sensitivity obtained in association with **ELITe InGenius** are applicable also to **ELITe BeGenius**.

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The test, starting from 200 µL of sample, was performed on:

- 30 whole blood samples collected in EDTA that were positive for EBV DNA (tested with a real time amplification CE IVD product).
- 12 plasma samples collected in EDTA from patients that were positive for EBV DNA (tested with a real time amplification CE IVD product) and on 35 samples of plasma collected in EDTA negative for EBV DNA, that were spiked for EBV DNA adding “1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques” (NIBSC code 09/260, United Kingdom).

The test, starting from 1000 µL of sample, was performed on 30 plasma samples collected in EDTA negative for EBV DNA, that were spiked for EBV DNA adding “1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques” (NIBSC code 09/260, United Kingdom).

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

The results are summed up in the following table.

Sample volume	Samples	N	positive	negative
200 µL	Whole Blood collected in EDTA and positive for EBV DNA	30	30	0
	Plasma collected in EDTA and positive for EBV DNA	12	12	0
	Plasma collected in EDTA and spiked with EBV DNA	35	35	0
1000 µL	Plasma collected in EDTA and spiked with EBV DNA	30	29	1

All Whole Blood sampleswere confirmed to be valid positive for EBV DNA. The diagnostic sensitivity of the assay in this test was equal to 100%.

All Plasma samples (200 µL) were confirmed to be valid positive for EBV DNA. The diagnostic sensitivity of the assay in this test was equal to 100%.

Twenty-nine (29) out of 30 Plasma samples (1000 µL) were confirmed to be valid positive for EBV DNA, one sample was discrepant negative. The diagnostic sensitivity of the assay in these tests was equal to 96.7%.

All samples, analyzed starting from 1000 µL of sample, were valid for the analysis, 29 out of 30 plasma samples were confirmed positive, one sample was discrepant negative. The diagnostic sensitivity of the assay in these tests was equal to 96.7%.

The total diagnostic sensitivity of the assay in these tests was equal to 99%.

Diagnostic specificity: confirmation of negative samples

The Diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analysing some clinical samples of Whole Blood and Plasma negative for EBV DNA in association with **ELITe InGenius**. As **ELITe BeGenius** showed equivalent analytical performances to **ELITe InGenius**, it can be assumed that the results of Diagnostic specificity obtained in association with **ELITe InGenius** are applicable also to **ELITe BeGenius**.

The test, starting from 200 µL of sample, was performed on:

- 32 whole blood samples collected in EDTA that were negative for EBV DNA (tested with a CE IVD real time amplification product).
- 61 plasma samples collected in EDTA that were negative for EBV DNA (tested with a CE IVD real time amplification product).

The test, starting from 1000 µL of sample, was performed on 62 plasma samples collected in EDTA that were presumably negative for EBV DNA.

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

The results are summed up in the following table.

Sample volume	Samples	N	positive	negative
200 µL	Whole Blood collected in EDTA and negative for EBV DNA	32	3	29
	Plasma collected in EDTA and negative for EBV DNA	61	1	60
1000 µL	Plasma collected in EDTA and presumably negative for EBV DNA	62	2	60

Twenty-nine (29) out of 32 Whole Blood samples were confirmed to be valid negative for EBV - DNA, three samples were discrepant positive at low titer. These samples had a titre below the limit of detection of the method for EBV - DNA; these samples can randomly test either negative or positive. The discrepant results may be explained considering that EBV is a virus largely widespread in the population in a latent form.

The diagnostic specificity of the assay in this test with whole blood was equal to 90.6%.

Sixty (60) out of 61 Plasma samples (200 µL) were confirmed to be valid negative for EBV - DNA, one sample was discrepant positive at low titer. This sample had a titre close to the limit of detection of the method for EBV - DNA; this sample can randomly test either negative or positive. The discrepant result may be explained considering that EBV is a virus largely widespread in the population in a latent form.

The diagnostic specificity of the assay in this test with plasma was equal to 98.4%.

Sixty (60) out of 62 Plasma samples (1000 µL) were confirmed to be valid negative for EBV - DNA, two samples were discrepant positive at low titer. This sample had a titre close to the limit of detection of the method for EBV - DNA; this sample can randomly test either negative or positive. The discrepant result may be explained considering that EBV is a virus largely widespread in the population in a latent form.

The diagnostic specificity of the assay in this test was equal to 96.8%.

The total diagnostic specificity of the assay in these tests was equal to 96%.

The Internal Control Ct (IC Ct) cut-off value is set at 35.

Note: The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instruments are recorded in Section 7 of the Product Technical File for the "EBV ELITe MGB® Kit", FTP RTS020PLD.

EBV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF **RTS020PLD**

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

SAMPLES AND CONTROLS

Samples

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

Whole blood collected in EDTA

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

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

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

Note: when you carry out the DNA extraction from whole blood with the **ELITe STAR** and with **software version 3.4.13** (or later equivalent versions), use the extraction protocol **UUNI_E100_S200_ELI**, that uses 200 µL of sample and elutes the extract in 100 µL. Samples in primary tubes can be directly loaded on **«ELITe STAR»**. A minimum volume of 700 µL is always required for each sample. Add **200 µL** of **CPE** into Proteinase-Carrier tube as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

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

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

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

Plasma collected in EDTA

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

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

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

Note: when you carry out the DNA extraction from plasma with the **ELITe STAR** and with **software version 3.4.13** (or later equivalent versions), use the extraction protocol **UUNI_E100S_200_ELI**, that uses 200 µL of sample and elutes the extract in 100 µL (the elution takes actually place in 115 µL of which 100 µL are recovered). Samples in primary tubes can be directly loaded on **«ELITe STAR»**. A minimum volume of 400-600 µL is always required for each sample. Add **200 µL** of **CPE** into Proteinase-Carrier tube as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

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

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

Cerebrospinal fluid (CSF)

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

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

Interfering substances

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

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

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

Amplification controls

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

For the negative control, use molecular biology grade water (not provided with this product) added to the reaction in place of the DNA extracted from the sample.

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

Quality controls

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

External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable. Example of commercially available external controls is the "EBV Molecular Q Panel" (code EBVMQP01 by Qnostics Ltd, UK).

PROCEDURE

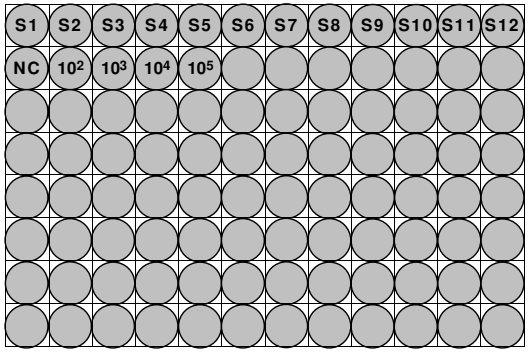
Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

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 EBV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "EBV";
- set (Detector Manager) the "detector" for the Internal Control 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 the "detector" (type of fluorescence that is to be measured), the "passive reference" "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

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

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



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

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

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

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

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (data collection)	30 sec.
	72 °C	20 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	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 EBV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “EBV”;
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "VIC" (AP525 is similar to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”;
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "Cy5" (AP593 is used instead of Cy5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

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

The set up of the quantitative analysis of 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**;

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

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

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

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

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

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

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

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

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

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

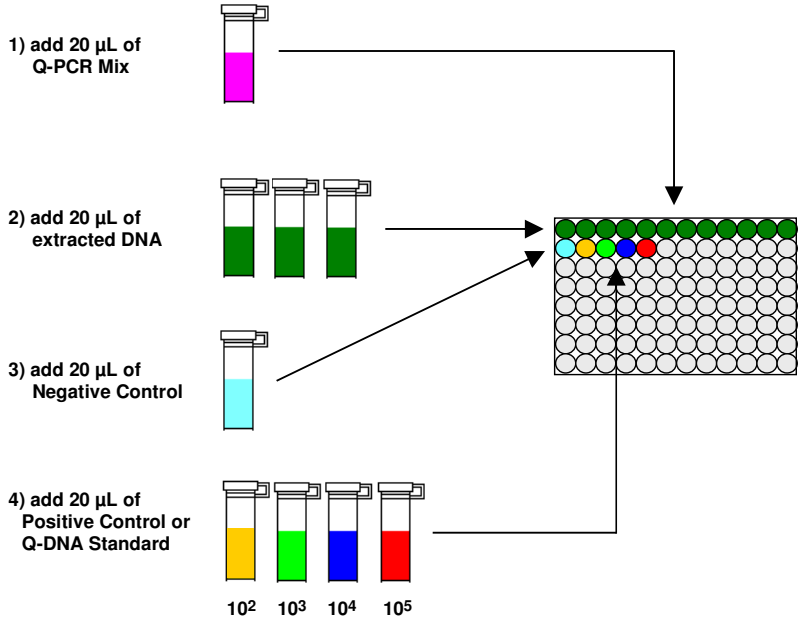
   - When a **qualitative** result is required (detection of EBV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **EBV - ELITe Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control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

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

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

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



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

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

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reagent for DNA Real Time amplification

REF RTS020PLD

Qualitative analysis of the results

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

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

Note: In the case of a positive sample with a high titre of EBV DNA, the FAM fluorescence of the EBV specific probe may begins 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 “EBV” 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 “EBV” 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 to determine the **Threshold cycle (Ct)**, the cycle in which the fluorescence reached the **Threshold** value.

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

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

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

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

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

Negative control reaction detector FAM "EBV"	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 EBV, the target DNA was detected. This means that problems occurred during the amplification step (contamination), which may lead to incorrect results and false positives. The session is not valid and needs to be repeated starting from the amplification step.

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

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

This product is able to detect a minimal quantity of about 10 copies of DNA of the EBNA-1 gene of EBV in the amplification reaction, corresponding to 10 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	EBV DNA
detector FAM "EBV"	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 EBV and **Ct > 35** or **Ct Undetermined** for the Internal Control, it means that it was impossible to detect efficiently the DNA for the Internal Control. In this case problems occurred during the amplification step (inefficient or absent amplification) or during the extraction step (degradation of sample 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 EBV and **Ct ≤ 35** for the Internal Control, it means that the EBV DNA is not detected in the DNA extracted from the sample; but it can not be excluded that the EBV DNA has a lower titre than the detection limit of the product (see the paragraph about Performance Characteristics). In this case the result could be a false negative.

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

***Note:** When in the amplification reaction of a sample the EBV 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 EBV DNA. In this case the sample is nevertheless suitable and the positive result of the assay is valid.

Quantitative analysis of the results

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

In the amplification reactions of the four **Q - PCR standards**, the **Ct** values of EBV 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 "EBV"	Acceptability range	Amplification / Detection
Correlation coefficient (R2)	0.990 ≤ R2 ≤ 1.000	CORRECT

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

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

Sample result detector FAM "EBV"	EBV genome Equivalents per reaction
Quantity > 1×10^6	MORE THAN 1,000,000
$1 \times 10^1 \leq$ Quantity $\leq 1 \times 10^6$	= Quantity
Quantity < 1×10^1	LESS THAN 10

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

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

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

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

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

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

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

When **«ELITe GALAXY»** 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 plasma and ELITe GALAXY
Nc (gEq / mL) = 35 x Quantity

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

Simplified formula for whole blood and «NucliSENS® easyMAG®»
Nc (gEq / mL) = 50 x Quantity

When «**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) = 23 x Quantity

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

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

Calculation of the measuring range limits

When a particular extraction assay method is used, the measuring range limits may be calculated from the measuring range of the amplification reaction according to the following formula:

$\text{Lower limit (gEq / mL)} = \frac{\text{Ve x 10 gEq}}{\text{Vc x Va x Ep}}$
$\text{Upper limit (gEq / mL)} = \frac{\text{Ve x 1,000,000 gEq}}{\text{Vc x Va x Ep}}$

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

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

When «**ELITe STAR**» extraction system is used with cellular and non-cellular samples, the formula becomes:

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

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When «**ELITe GALAXY**» extraction system is used with cellular and non-cellular samples, the formula becomes:

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

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

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

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

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

When «**QIAsymphony® SP/AS**» extraction system is used with non-cellular samples, the formula becomes:

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

Convertion of results to International Units (IU)

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

Simplified formula for whole blood and «EXTRAblood»
Fc = 2.0 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 50 x Quantity

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

Simplified formula for whole blood and «ELITe STAR»
Fc = 2.09 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 58.2 x Quantity

When **«ELITe STAR»** extraction system is used with plasma samples collected in EDTA and the result **expressed in IU / mL** is required, the formula becomes:

Simplified formula for plasma and «ELITe STAR»
Fc = 2.15 IU / gEq
Nc (IU / mL) = Nc (gEq / mL) x Fc
Nc (IU / mL) = 60.2 x Quantity

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

Simplified formula for whole blood and «ELITe GALAXY»
Fc = 0.89 IU / gEq
Nc (IU / mL) = Nc (gEq / mL) x Fc
Nc (IU / mL) = 31.2 x Quantity

When **«ELITe GALAXY»** extraction system is used with plasma samples collected in EDTA and the result **expressed in IU / mL** is required, the formula becomes:

Simplified formula for plasma and «ELITe GALAXY»
Fc = 0.76 IU / gEq
Nc (IU / mL) = Nc (gEq / mL) x Fc
Nc (IU / mL) = 26.6 x Quantity

When «**NucliSENS® easyMAG®**» extraction system is used with whole blood samples collected in EDTA and the result **expressed in IU / mL** is required, the formula becomes:

Simplified formula for whole blood and «NucliSENS® easyMAG®»
Fc = 1.7 IU / gEq
Nc (IU / mL) = Nc (gEq / mL) x Fc
Nc (IU / mL) = 85 x Quantity

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

Simplified formula for whole blood and «QIAsymphony® SP/AS»
Fc = 1.8 IU / gEq
Nc (IU / mL) = Nc (gEq / mL) x Fc
Nc (IU / mL) = 41 x Quantity

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

Simplified formula for plasma and «QIAsymphony® SP/AS»
Fc = 2.3 IU / gEq
Nc (IU / mL) = Nc (gEq / mL) x Fc
Nc (IU / mL) = 28 x Quantity

Where **Fc** is the conversion factor established using the reference calibrated material approved by WHO "1st WHO International Standard for Epstein Barr virus for Nucleic Acid Amplification Techniques", NIBSC code 09/260, United Kingdom (see Performance Characteristics paragraph).

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PERFORMANCE CHARACTERISTICS

Analytical sensitivity: detection limit

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

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

Samples	**N**	**positive**	**negative**
10 copies plasmidic DNA + 500 ng of human genomic DNA	50	50	0

The analytical sensitivity of this assay used in association to whole blood samples and **ELITe STAR** was verified with a panel of EBV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, UK) in EBV DNA - negative EDTA whole blood. The viral concentrations ranged from 3.160 IU / mL to 1000 IU / mL. Each sample of the panel was tested in 8 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **ELITe STAR** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.
The final results are reported in the following tables.

Limit of Detection for whole blood samples and ELITe STAR (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**212 UI / mL**	113 UI / mL	805 UI / mL

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for whole blood samples and ELITe STAR (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**101 gEq / mL**	54 gEq / mL	385 gEq / mL

The analytical sensitivity of this assay used in association to plasma samples and **ELITe STAR** was verified with a panel of EBV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, United Kingdom) in EBV DNA - negative EDTA plasma. The viral concentrations ranged from 3.160 IU / mL to 1000 IU / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **ELITe STAR** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.
The final results are reported in the following tables.

Limit of Detection for plasma samples and ELITe STAR (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**229 UI / mL**	108 UI / mL	1571 UI / mL

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for plasma samples and ELITe STAR (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**107 gEq / mL**	50 gEq / mL	731 gEq / mL

The analytical sensitivity of this assay used in association to whole blood samples and **ELITe GALAXY** was verified with a panel of EBV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, UK) in EBV DNA - negative EDTA whole blood. The viral concentrations ranged from 10 IU / mL to 560 IU / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The final results are reported in the following tables.

Limit of Detection for whole blood samples and ELITe GALAXY (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**99 UI / mL**	57 UI / mL	376 UI / mL

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for whole blood samples and ELITe GALAXY (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**111 gEq / mL**	64 gEq / mL	422 gEq / mL

The analytical sensitivity of this assay used in association to plasma samples and **ELITe GALAXY** was verified with a panel of EBV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, UK) in EBV DNA - negative EDTA plasma. The viral concentrations ranged from 10 IU / mL to 560 IU / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The final results are reported in the following tables.

Limit of Detection for plasma samples and ELITe GALAXY (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**97 IU / mL**	66 IU / mL	284 IU / mL

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for plasma samples and ELITe GALAXY (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**128 gEq / mL**	87 gEq / mL	374 gEq / mL

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Analytical sensitivity: linear measuring range

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

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

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

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

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

The final results are summed up in the following table.

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

The measuring range limits in gEq / mL and referred to the used extraction kit are calculated at page 34.

Analytical sensitivity: Precision and Accuracy

The precision of the assay, as the variability of results obtained with several replicates of the same sample tested within the same session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 21.0% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The accuracy of the assay, as the difference between the mean of results obtained with several replicates of a sample within the same session and the theoretical concentration of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 11.1% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

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

Analytical sensitivity: reproducibility with calibrated reference material

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

The tests were carried out using as calibrated reference material a panel of dilutions of EBV within the concentration limit (QCMD 2008 Epstein-Barr Virus DNA EQA Panel, Qnostics Ltd, Scotland, UK). Each sample was tested in duplicates carrying out the whole analysis, extraction with «**EXTRAblood**» and amplification with ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials				
Sample	Commercial assay virus conc. consensus Log_{10} gEq / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
EBV08-01	EBV, 2.394	0.473	2/2	1.937
EBV08-02	EBV, 3.177	0.476	2/2	3.185
EBV08-03	EBV, 3.443	0.400	2/2	3.021
EBV08-04	EBV, 4.159	0.391	2/2	4.089
EBV08-05	EBV, 2.707	0.504	2/2	2.408
EBV08-06	Negative, NA	-	0/2	-
EBV08-07	EBV, 3.857	0.349	2/2	3.796
EBV08-08	EBV, 5.131	0.361	2/2	4.930
EBV08-09	EBV, 4.414	0.358	2/2	4.186
EBV08-10	EBV, 2.651	0.456	2/2	2.458

All samples were correctly detected. The quantitative results are within the range defined by the commercial assay Consensus ± 1 Standard Deviation, excepted for sample EBV08-03.

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

The results in gEq/mL are reported in the following table.

Tests with calibrated reference materials and ELITe STAR				
Sample	Commercial assay virus conc. consensus Log_{10} gEq / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
EBV12-01	EBV, 2.719	0.446	1/2	1.885
EBV12-02	EBV, 3.802	0.417	2/2	3.794
EBV12-03	EBV, 5.173	0.358	2/2	5.168
EBV12-04	EBV, 4.790	0.421	2/2	4.569
EBV12-05	EBV, 4.313	0.371	2/2	4.064
EBV12-06	EBV, 4.458	0.373	2/2	4.334
EBV12-07	EBV, 4.769	0.384	2/2	4.416
EBV12-08	EBV, 3.471	0.403	2/2	3.324
EBV12-09	EBV, 3.313	0.446	2/2	3.128
EBV12-10	Negative, NA	-	0/2	-

All negative samples were correctly detected. In the quantitative analysis, 8/9 positive samples were correctly quantified within the range defined by the commercial assay Consensus ± 1 Standard Deviation. One sample (EBV12-01) was quantified within ± 2 SD. This result may be explained because titre of the sample was close than the detection limit of method used.

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The results in IU/mL were calculated applying the conversion factor for **ELITe STAR** and plasma and are reported in the following table.

Tests with calibrated reference materials and ELITe STAR				
Sample	Commercial assay virus conc. consensus Log_{10} UI / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
EBV12-01	EBV, 2.473	0.386	1/2	2.217
EBV12-02	EBV, 3.635	0.422	2/2	4.126
EBV12-03	EBV, 4.987	0.307	2/2	5.500
EBV12-04	EBV, 4.646	0.295	2/2	4.901
EBV12-05	EBV, 4.138	0.300	2/2	4.396
EBV12-06	EBV, 4.345	0.333	2/2	4.666
EBV12-07	EBV, 4.631	0.270	2/2	4.749
EBV12-08	EBV, 3.470	0.442	2/2	3.657
EBV12-09	EBV, 3.161	0.394	2/2	3.460
EBV12-10	Negative, NA	-	0/2	-

All negative samples were correctly detected. In the quantitative analysis, 7/9 positive samples were correctly quantified within the range defined by the commercial assay Consensus ± 1 Standard Deviation. Two samples (EBV12-02 and EBV12-03) was quantified within ± 2 SD.

Further tests were carried out using as calibrated reference material a panel of dilutions of EBV within the concentration limit (QCMD 2012 Epstein-Barr Virus DNA EQA Panel, Qnostics Ltd, UK). Each sample was tested in duplicates carrying out the whole analysis procedure: extraction ad PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The results in gEq/mL are reported in the following table.

Tests with calibrated reference materials and ELITe GALAXY				
Sample	Commercial assay virus conc. consensus Log_{10} gEq / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
EBV12-01	EBV, 2.719	0.446	2/2	2.763
EBV12-02	EBV, 3.802	0.417	2/2	3.638
EBV12-03	EBV, 5.173	0.358	2/2	5.060
EBV12-04	EBV, 4.790	0.421	2/2	4.598
EBV12-05	EBV, 4.313	0.371	2/2	4.063
EBV12-06	EBV, 4.458	0.373	2/2	4.319
EBV12-07	EBV, 4.769	0.384	2/2	4.597
EBV12-08	EBV, 3.471	0.403	2/2	3.258
EBV12-09	EBV, 3.313	0.446	2/2	3.224
EBV12-10	Negative, NA	-	0/2	-

All negative samples were correctly detected as negatives and all positive samples were detected as positives in agreement with quantitative results defined by commercial assays consensus.

The results in IU/mL were calculated applying the conversion factor for **ELITe GALAXY** and plasma and are reported in the following table.

Tests with calibrated reference materials and ELITe GALAXY				
Sample	Commercial assay virus conc. consensus Log_{10} UI / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
EBV12-01	EBV, 2.473	0.386	2/2	2.644
EBV12-02	EBV, 3.635	0.422	2/2	3.518
EBV12-03	EBV, 4.987	0.307	2/2	4.941
EBV12-04	EBV, 4.646	0.295	2/2	4.479
EBV12-05	EBV, 4.138	0.300	2/2	3.944
EBV12-06	EBV, 4.345	0.333	2/2	4.200
EBV12-07	EBV, 4.631	0.270	2/2	4.478
EBV12-08	EBV, 3.470	0.442	2/2	3.139
EBV12-09	EBV, 3.161	0.394	2/2	3.105
EBV12-10	Negative, NA	-	0/2	-

All negative samples were correctly detected as negatives and all positive samples were detected as positives in agreement with quantitative results defined by commercial assays consensus.

Analytical sensitivity: Conversion factor to International Units

The conversion factor to be used with this assay to convert quantitative result from gEq / mL to International Units / mL was defined as 2.0 International Units / gEq when whole blood samples and «EXTRAblood» manual extraction kit are used; as 2.2 International Units / gEq when whole blood samples and automatic extraction system ELITe STAR are used; as 0.8 International Units / gEq when whole blood samples and automatic extraction system ELITe GALAXY are used; as 1.7 International Units / gEq when whole blood samples and «NucliSENS® easyMAG®» automatic extraction system are used; as 1.8 International Units / gEq when whole blood samples and «QIAsymphony® SP/AS» automatic extraction system are used; as 2.0 International Units / gEq when plasma samples and ELITe STAR are used; as 0.7 International Units / gEq when plasma and automatic extraction system ELITe GALAXY are used; as 2.3 International Units / gEq when plasma and «QIAsymphony® SP/AS» automatic extraction system are used.

Whole blood collected in EDTA

The conversion factor was calculated using a panel of four dilutions (0.5 Log_{10} between dilutions) of calibrated reference material approved by WHO ("1st WHO International Standard for Epstein Barr virus for Nucleic Acid Amplification Techniques", NIBSC code 09/260, United Kingdom) in whole blood collected in EDTA.

Each point of the panel was tested in 8 replicates carrying out the whole analysis, extraction with «**EXTRAblood**» and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 2.0 International Units (IU) per gEq of EBV detected with whole blood samples.

The final results are reported in the following table.

Conversion to International Units with whole blood and «EXTRAblood» (Fc = 2.0 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
316,255	5.500	154,718	30,4034	5.48
100,000	5.000	51,264	100,737	5.00
31,625	4.500	15,602	30,660	4.49
10,000	4.000	5,438	10,686	4.03

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Each point of the panel was tested in 15 replicates carrying out the whole analysis, extraction and PCR Setup with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 2.2 International Units (IU) per gEq of EBV detected with whole blood samples.

The final results are reported in the following table.

Conversion to International Units with whole blood and ELITe STAR (Fc = 2.09 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
3,162	3.500	1,295	2,709	3.339
10,000	4.000	5,116	10,703	3.976
31,623	4.500	18,300	38,283	4.559
100,000	5.000	55,188	115,453	5.034
316,228	5.500	177,128	370,551	5.537

Each point of the panel was tested in 15 replicates carrying out the whole analysis, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 0.8 International Units (IU) per gEq of EBV detected with whole blood samples.

The final results are reported in the following table.

Conversion to International Units with whole blood and ELITe GALAXY (Fc = 0.89 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
3,162	3.500	3,821	3,400	3.518
10,000	4.000	13,623	12,124	4.101
31,623	4.500	32,547	28,967	4.460
100,000	5.000	120,239	107,013	5.028
316,228	5.500	281,782	250,786	5.390

Each point of the panel was tested in 8 replicates carrying out the whole analysis procedure: extraction with «NucliSENS® easyMAG®» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allowed to calculate a mean conversion factor (Fc) equal to 1.7 International Units (IU) per gEq of EBV detected with whole blood samples.

The final results are reported in the following table.

Conversion to International Units with whole blood and «NucliSENS® easyMAG®» (Fc = 1.7 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
316,255	5.500	212,198	366,796	5.56
100,000	5.000	56,930	98,407	4.99
31,625	4.500	20,334	35,148	4.55
10,000	4.000	4,734	8,183	3.91

Each point of the panel was tested in 8 replicates carrying out the whole analysis procedure: extraction with «QIAsymphony® SP/AS» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allowed to calculate a mean conversion factor (Fc) equal to 1.8 International Units (IU) per gEq of EBV detected with whole blood samples.

The final results are reported in the following table.

Conversion to International Units with whole blood and «QIAsymphony® SP/AS» (Fc = 1.8 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
316,255	5.500	203251	365852	5.563
100,000	5.000	61830	111294	5.046
31,625	4.500	18174	32713	4.515
10,000	4.000	4546	8183	3.913
3,162	3.500	1850	3330	3.522
1,000	3.000	575	1035	3.015

Plasma collected in EDTA

The conversion factor was calculated using a panel of four dilutions (0.5 Log_{10} between dilutions) of calibrated reference material approved by WHO ("1st WHO International Standard for Epstein Barr virus for Nucleic Acid Amplification Techniques", NIBSC code 09/260, United Kingdom) in plasma collected in EDTA.

Each point of the panel was tested in 15 replicates carrying out the whole analysis procedure: extraction with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 2.0 International Units (IU) per gEq of EBV detected with plasma samples.

The final results are reported in the following table.

Conversion to International Units with plasma and ELITe STAR (Fc = 2.15 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
316,255	5.500	167,105	359,275	5.537
100,000	5.000	45,185	97,147	4.961
31,625	4.500	17,428	37,470	4.555
10,000	4.000	4,536	9,753	3.993
3,162	3.500	1,435	3,084	3.454

Each point of the panel was tested in 15 replicates carrying out the whole analysis, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 0.7 International Units (IU) per gEq of EBV detected with plasma samples.

The final results are reported in the following table.

Conversion to International Units with plasma and ELITe GALAXY (Fc = 0.76 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
3,162	3.500	5,610	4,263	3.608
10,000	4.000	15,554	11,821	4.050
31,623	4.500	39,837	30,276	4.451
100,000	5.000	148,584	112,924	5.035
316,228	5.500	308,566	234,510	5.334

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Each point of the panel was tested in 8 replicates carrying out the whole analysis procedure: extraction with «QIAsymphony® SP/AS» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allowed to calculate a mean conversion factor (Fc) equal to 2.3 International Units (IU) per gEq of EBV detected with plasma samples.

The final results are reported in the following table.

Conversion to International Units with plasma and «QIAsymphony® SP/AS» (Fc = 2.3 IU / gEq)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity gEq / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
316,255	5.500	110,437	249,588	5.39
100,000	5.000	37,691	85,181	4.93
31,625	4.500	14,498	32,765	4.51
10,000	4.000	7,442	16,819	4.22

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

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

The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probe in the alignment of the sequences available in the database for the EBNA-1 gene of EBV showed conservation and absence of significant mutations.

Diagnostic sensitivity: confirmation of positive samples

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

The diagnostic sensitivity was evaluated using as reference material 21 whole blood samples collected in EDTA, all positive for EBV DNA (tested with a CE IVD real time amplification product) and 21 samples of cerebrospinal fluid negative for EBV DNA and spiked with samples EBV09-04, EBV09-05 and EBV09-06 from QCMD 2009 Epstein-Barr Virus DNA EQA Panel (Qnostics Ltd, Scotland, United Kingdom). Each sample was tested carrying out the whole analysis, extraction and amplification by ELITechGroup S.p.A. products.

The results are summed in the following table.

Samples	**N**	**positive**	**negative**
Whole blood collected in EDTA positive for EBV DNA	21	21	0
Cerebrospinal fluid spiked with EBV DNA	21	21	0

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

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

The diagnostic sensitivity was evaluated using 31 plasma samples collected in EDTA that were positive for EBV DNA and 31 whole blood samples collected in EDTA that were positive for EBV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure: extraction with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	**N**	**positive**	**negative**
Plasma collected in EDTA positive for EBV DNA	31	29	2
Whole blood collected in EDTA positive for EBV DNA	31	30	1

Three samples reported a negative result with ELITechGroup S.p.A. products. This discordance may be explained by the EBV titre of the samples, that was estimated close to the detection limit of method.

The diagnostic sensitivity of the assay in this test was equal to 95.2%.

The diagnostic sensitivity was evaluated using 30 plasma samples collected in EDTA that were positive for EBV DNA (tested with a real time amplification CE IVD product) and 32 whole blood samples collected in EDTA that were positive for EBV DNA (tested with a real time amplification CE IVD product). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA positive for EBV DNA	30	29	1
Whole blood collected in EDTA positive for EBV DNA	32	32	0

One plasma sample reported a negative result with ELITechGroup S.p.A. products. This discordance may be explained by a probably bad preservation of the sample.

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

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

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

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

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

The analytical specificity was checked using as reference material 20 whole blood samples collected in EDTA negative for EBV DNA but positive for CMV DNA or HHV6 DNA (tested with CE IVD real time amplification products). 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
Whole blood collected in EDTA positive for CMV DNA	11	0	11
Whole blood collected in EDTA positive for HHV6 DNA	9	0	9

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

Diagnostic specificity: confirmation of negative samples

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

The diagnostic specificity was evaluated using as reference material 29 whole blood samples collected in EDTA and 21 cerebrospinal fluid samples that were negative for EBV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis, extraction and amplification by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood negative for EBV DNA	29	1	28
Cerebrospinal fluid negative for EBV DNA	21	0	18

One whole blood sample gave a positive result for EBV DNA with a very low titre (about 3 copies / reaction) in the first session of analysis. The same sample resulted negative, valid in the second session. This discordant result may be explained by very low titre of EBV DNA, below the limit of detection of the reference method.

Three samples of cerebrospinal fluid gave an invalid result, possibly for the presence of an inhibitor and were not used to calculate the specificity. The diagnostic specificity of the assay in this test was equal to 97.9%.

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The diagnostic specificity was evaluated using 40 plasma samples collected in EDTA that were negative for EBV DNA and 60 whole blood samples collected in EDTA that were negative for EBV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure: extraction and PCR Setup with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA negative for EBV DNA	40	0	40
Whole blood collected in EDTA negative for EBV DNA	60	3	53

Four whole blood samples are not valid. Three whole blood samples resulted discrepant positive (58 gEq/mL, 107 gEq/mL and 37 gEq/mL respectively). These samples had a titre below the limit of detection of the method for EBV - DNA; these samples can randomly test either negative or positive. The discrepant results may be explained considering that EBV is a virus largely widespread in the population in a latent form.

The diagnostic specificity of the assay in this test was equal to 96.9%.

The diagnostic specificity was evaluated using 30 plasma samples collected in EDTA that were negative for EBV DNA and 32 whole blood samples collected in EDTA that were negative for EBV DNA (tested with a real time amplification CE IVD product). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA negative for EBV DNA	30	0	30
Whole blood collected in EDTA negative for EBV DNA	32	1	31

One whole blood sample resulted discrepant positive (18 gEq/mL). This sample titre was below the limit of detection of the method for EBV - DNA, this sample can randomly test either negative or positive. The discrepant result may be explained considering that EBV is a virus largely widespread in the population in a latent form.

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

Note: The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instruments are recorded in Section 7 of the Product Technical File for the "EBV ELITe MGB® Kit", FTP RTS020PLD.

Roche cobas z 480 analyzer

SAMPLES AND CONTROLS

Samples

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

Whole blood collected in EDTA

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

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

Plasma collected in EDTA

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

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

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

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

Interfering substances

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

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

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

Amplification controls

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

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

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

Quality controls

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

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PROCEDURE

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

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

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

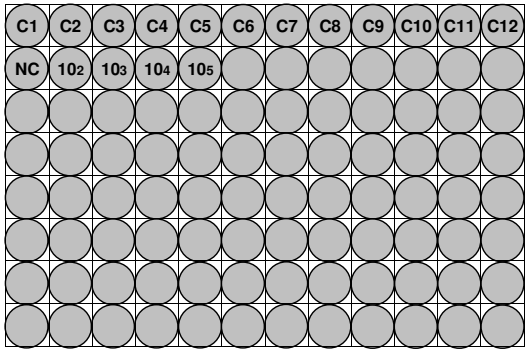
Note: fluorescence acquisition occurs individually, set Ramp Rate (°C/sec) to 4.4°C/sec.

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

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

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

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



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

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

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

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

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

2. Depositing it precisely into the reaction mixture, transfer **20 µL** of **extracted DNA** from the first sample in the corresponding well on the **AD-plate** as previously established in the **Work Plan**. Mix the sample well by pipetting the **extracted DNA** three times into the reaction mixture. Be sure not to create any bubbles. Proceed in the same manner with all the other **extracted DNA**.
3. Depositing it precisely into the reaction mixture, transfer **20 µL** of **ultra-pure molecular biology grade water** (not supplied with the product) into the well on the **AD-plate** containing the negative amplification control as previously established in the **Work Plan**. Mix the negative control well by pipetting the **ultra-pure molecular biology grade water** three times into the reaction mixture. Be sure not to create any bubbles.
4. On the basis of the result required (qualitative or quantitative), one of these two options must be followed:

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

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

5. Carefully seal the **AD-plate** using the **Sealing Film.**
6. Transfer the **AD-plate** into the real-time Thermal Cycler in the amplification/detection of amplification products area and start the amplification thermal cycle, saving the session settings under a unique and recognizable identifier (e.g. "year-month-day-EBV-EGSpA").

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

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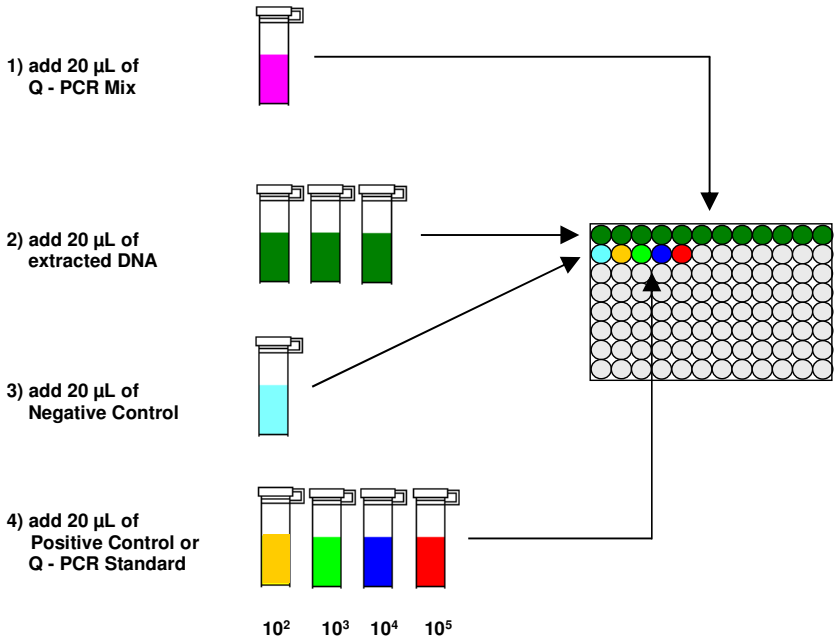
The following figure shows synthetically the preparation of the amplification reaction..



Qualitative results analysis

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

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

Select the group of samples to be analyzed

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

For **Plasma** Samples:

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

For **Whole Blood** Samples:

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

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

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

Reaction Positive Control "EBV" detector	Assay result	Amplification / Detection
Ct ≤ 25	**POSITIVE**	**CORRECT**

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

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

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

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

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

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

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

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

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

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

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

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

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

Quantitative results analysis

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

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

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

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

Sample result FAM "EBV" detector	EBV copies per reaction
Quantity > 1×10^6	**GREATER THAN 1,000,000**
1.0×10^1 ≤ Quantity ≤ 1×10^6	**= Quantity**
Quantity < 1.0×10^1	**LESS THAN 10**

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

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

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

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

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

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: limit of detection

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

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

Samples	N	positives	negatives
10 copies of plasmid DNA + 150,000 copies of pBETAGLOBIN	18	18	0

The analytical sensitivity of this assay used in association with whole blood and plasma and **MagNA Pure 24** was verified with a panel of EBV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, United Kingdom) in EBV DNA - negative matrix. The panel consisted of six points around the limit concentration. Each sample of the panel was tested in 12 replicates carrying out carrying out the extraction using the automanic system **MagNA Pure 24** and amplification using ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The final results are reported in the following tables.

Limit of Detection with MagNA Pure 24 (IU / mL)			
Matrix	95% positivity	95% confidence range	
		lower limit	lower limit
whole bood	**143 IU / mL**	87 IU / mL	413 IU / mL
plasma	**163 IU / mL**	82 IU / mL	1137 IU / mL

The analytical sensitivity as copies / mL for each matrix is calculated by applying the specific conversion factor reported at page 51.

The analytical sensitivity as copies / mL is reported below.

Limit of Detection with MagNA Pure 24 (copies / mL)			
Matrix	95% positivity	95% confidence range	
		lower limit	upper limit
whole blood	102 copies / mL	62 copies / mL	295 copies / mL
plasma	125 copies / mL	63 copies / mL	875 copies / mL

Analytical sensitivity: linear measuring range

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

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

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

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

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The results are shown in the following table.

Linear measuring range using MagNA Pure 24		
	Lower limit	Upper limit
copies / mL	**250**	**25,000,000**
copies / reaction	**10**	**1,000,000**

Conversions from copies / mL to copies / reaction and vice versa were calculated as shown on page 51.

The linearity of this assay used in association with different matrices and **MagNA Pure 24** was verified with a panel of EBV dilutions. The panel was prepared by diluting the "1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques" (NIBSC code 09/260, United Kingdom) in EBV DNA - negative matrix. The panel consisted of five dilution points (1 log_{10} dilution steps) from 10^6 IU / mL to 10^2 IU / mL. Each sample of the panel was tested in four replicates carrying out the extraction using the automanic system **MagNA Pure 24** and amplification using 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 above the LoD.

Limit of quantification

The lower limit of the linear measuring range was set at the lowest concentration that gives 100% of positivity and quantitative results sufficiently accurate and precise. The upper limit of the linear measuring range was set at the highest tested concentration that gives quantitative results sufficiently accurate and precise.

The linear measuring range as copies / mL for each matrix is calculated by applying the specific conversion factor reported at page 51.

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

Linear measuring range for whole blood samples and MagNA Pure 24		
Unit of measure	lower limit	upper limit
IU / mL	**178**	**1,000,000**
copies / mL	**127**	**714,286**

Linear measuring range for plasma samples and MagNA Pure 24		
Unit of measure	lower limit	upper limit
IU / mL	**178**	**1,000,000**
copies / mL	**137**	**769,231**

Analytical sensitivity: Precision and Accuracy

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

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

The accuracy of this assay, in terms of the difference between the mean of the results obtained in the same amplification session using different replicates of a sample and the sample's theoretical concentration value, allowed to obtain a mean Inaccuracy percentage of the measured Log quantity of around 1.1% in the range from 10^6 molecules to 10^1 molecules in 20 µL of DNA added to the amplification reaction.

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

Reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of value of calibrated reference material, was evaluated using as reference material the calibrated panel «EBV Molecular "Q" Panel» (Qnostics, Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the extraction using the automanic system **MagNA Pure 24** and amplification using ELITechGroup S.p.A. products.

The results in IU/mL were calculated applying the conversion factor for **MagNA Pure 24** System and plasma and are reported in the following table.

Tests with calibrated reference materials and MagNA Pure 24				
Sample	Nominal titre IU / mL	Nominal titre Log_{10} IU / mL	Positive / Replicates	Mean results Log_{10} IU / mL
EBVMQP01-High	36,577	4.560	2/2	4.586
EBVMQP01-Medium	3,657	3.560	2/2	3.553
EBVMQP01-Low	365	2.560	2/2	2.664
EBVMQP01-Negative	negative	-	0/2	-

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

Further tests were carried out using as reference material the calibrated panel «AcroMetrix EBV Plasma Panel» (Life Technologies). Each sample of the panel was tested in 2 replicates carrying out the extraction using the automanic system **MagNA Pure 24** and amplification using ELITechGroup S.p.A. products.

The results in IU/mL were calculated applying the conversion factor for **MagNA Pure 24** System and plasma and are reported in the following table.

Tests with calibrated reference materials and MagNA Pure 24				
Sample	Nominal titre IU / mL	Nominal titre Log_{10} IU / mL	Positive / Replicates	Mean results Log_{10} IU / mL
Acrometrix EBV 1E6	10^6	6.000	2/2	5.987
Acrometrix EBV 1E5	10^5	5.000	2/2	5.152
Acrometrix EBV 1E4	10^4	4.000	2/2	4.208
Acrometrix EBV 1E3	10^3	3.000	2/2	3.147
Acrometrix EBV 1E2	10^2	2.000	2/2	2.246

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

Further tests were carried out using as reference material QCMD 2017 Epstein-Barr virus DNA EQA Panel (Qnostics Ltd, Scotland, UK) a panel of EBV dilutions. Each sample of the panel was tested in 2 replicates carrying out the extraction using the automanic system **MagNA Pure 24** and amplification using ELITechGroup S.p.A. products.

The results in IU/mL were calculated applying the conversion factor for **MagNA Pure 24** System and plasma and are reported in the following table.

Tests with calibrated reference materials and MagNA Pure 24			
Sample	Consensus virus conc. Log_{10} IU / mL	Positive / Replicates	Mean results Log_{10} IU / mL
EBVDNA17S-01	3.885	2/2	3.884
EBVDNA17S-02	3.906	2/2	3.742
EBVDNA17S-03	2.903	2/2	2.928
EBVDNA17S-04	2.952	2/2	2.819
EBVDNA17S-05	2.181	2/2	1.817
EBVDNA17S-06	3.215	2/2	3.188
EBVDNA17S-07	3.899	2/2	3.974
EBVDNA17S-08	2.315	2/2	1.908
EBVDNA17S-09	-	0/2	-
EBVDNA17S-10	2.333	2/2	2.184

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

Conversion factor to International Units

The conversion factor to be used with this assay to transform the quantitative result from copies / mL into International Units / mL was determined using a panel of calibrated reference material approved by the WHO ("1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques ", NIBSC, United Kingdom, code 09/162) in the different negative matrices for EBV DNA and in association with **MagNA Pure 24**. The panel had 6 dilution steps of 1 Log. Each point of the panel was tested in 16 replicates carrying out the whole analysis procedure: extraction using the **MagNA Pure 24** automatic extraction system and amplification using ELITechGroup S.p.A. products.

The analysis of the data obtained allowed to calculate a mean conversion factor (Fc) equal to **1.4** International Units (IU) per copies of EBV detected with **whole blood** samples.

The final results are reported in the following table.

Conversion to International Units with whole blood and « MagNA Pure 24» (Fc = 1.4 IU / copies)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity copies / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
111,055	5.046	81,828	119,952	5.074
34,903	4.543	26,663	38,395	4.575
10,970	4.040	7,705	11,095	4.033
3,448	3.538	2,275	3,276	3.503
1,084	3.035	711	1,024	2.994
341	2.532	275	395	2.572

The analysis of the data obtained allowed to calculate a mean conversion factor (Fc) equal to **1.3** International Units (IU) per copies of EBV detected with **plasma** samples.

The final results are reported in the following table.

Conversion to International Units with plasma and « MagNA Pure 24» (Fc = 1.3 IU / copies)				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity copies / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
111,055	5.046	77,250	101,313	4.993
34,903	4.543	26,743	34,766	4.523
10,970	4.040	8,462	11,000	4.028
3,448	3.538	2,616	3,401	3.519
1,084	3.035	687	893	2.937
341	2.532	255	332	2.486

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity was evaluated using as reference material 34 samples of whole blood collected in EDTA negative for EBV DNA which were spiked for EBV DNA adding "1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques" (NIBSC, United Kingdom, code 09/260) and 30 samples of plasma collected in EDTA negative for EBV DNA which were spiked for EBV DNA adding "1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques" (NIBSC, United Kingdom, code 09/260).

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

Samples	N	positives	negatives
Whole blood collected in EDTA spiked for EBV DNA	34	34	0
Plasma collected in EDTA spiked for EBV DNA	30	30	0

All samples were valid at first test.

All whole blood and plasma samples were confirmed positive for EBV DNA. The diagnostic sensitivity of the assay associated to whole blood and plasma samples was 100%.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity was evaluated using as reference material 34 samples of whole blood collected in EDTA presumably negative for EBV DNA and 31 samples of plasma collected in EDTA presumably negative for EBV DNA.

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

Samples	N	positives	negatives
Whole blood collected in EDTA presumably negative for EBV DNA	34	0	34
Plasma collected in EDTA presumably negative for EBV DNA	31	1	30

All whole blood samples were valid at first test and were confirmed negative for EBV DNA.
The diagnostic specificity of the assay associated to whole blood samples was 100%.

All plasma samples were valid at first test. Thirty (30) out of 31 plasma samples were confirmed negative for EBV DNA, while one sample showed positive discrepant result.
The diagnostic specificity of the assay associated to plasma samples was 96.8%.

The total diagnostic specificity of the assay was 98.5%.

Note: The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instruments are recorded in Section 7 of the Product Technical File for the "EBV ELITe MGB® Kit", FTP RTS020PLD.

REFERENCES

S. W. Aberle et al. (2002) *J Clin Virology* 25: S79 - S85
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30

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PROCEDURE LIMITATIONS

Use this product only with DNA extracted from the following human samples: whole blood collected in EDTA, plasma collected in EDTA and cerebrospinal fluid.

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 or Ficoll® 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: suspensions of leucocytes, suspensions of lymphomonocytes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TROUBLESHOOTING

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

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

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

Irregular or high levels of 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 9 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.

Error 30103 on ELITe InGenius	
Possible causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of sample in an “Extract + PCR” session.

SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch code.


Use by (last day of month).

IVD *in vitro* diagnostic medical device.

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


Contains sufficient for "N" tests.


Attention, consult instructions for use.

CONT Contents.


Keep away from sunlight.


Manufacturer.

EBV ELITe MGB® Kit
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NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Life Technologies Corporation and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Life Technologies Corporation. 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, Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008. Phone: +1(760)603-7200. Fax: +1(760)602-6500. Email: outlicensing@thermofisher.com.

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

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

EBV ELITe MGB® kit used with Genius series platforms

Ref: RTS020PLD

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

A. Intended use

The EBV ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **Epstein-Barr human herpesvirus**. The assay is CE-IVD validated in combination with the instruments **ELITe InGenius** and **ELITe BeGenius**.

B. Amplified sequence

Target	Gene	Fluorophore
EBV	EBNA-1	FAM
Internal Control	Human beta globin gene	AP525 (VIC)

C. Validated matrix

› **Whole blood** EDTA

› **Plasma** EDTA

D. Kit content

EBV Q-PCR Mix
4 tubes of 540 µL

X 4

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

E. Material required not provided in the kit

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

› **EBV ELITe Standard :** STD020PLD
› **EBV - ELITe Positive Control :** CTR020PLD
› **ELITe InGenius Waste Box :** F2102-000
› **300 µL Filter Tips Axygen :** TF-350-L-R-S
› **1000 µL Filter Tips Tecan :** 30180118

F. Protocol

› Sample volume	200 µL
› CPE Internal Control volume	10 µL
› Total eluate volume	100 µL
› PCR eluate input volume	20 µL
› EBV Q-PCR Mix volume	20 µL

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

G. Performance

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Whole Blood	**104 IU/mL – 36 cp/mL**	**100%** 30/30*	**90.6%** 29/32*
Plasma (200 µL)	**124 IU/mL – 65 cp/mL**	**100%** 47/47*	**98.4%** 60/61*

**confirmed samples/ tested samples*

Matrix	Linearity (copies/mL)	Linearity (IU/mL)	Conversion factor cp/mL to IU/mL
Whole Blood	**36 - 344,828**	**104 – 1,000,001**	**2.9**
Plasma (200 µL)	**65 - 526,316**	**124– 1,000,000-**	**1.9**

H. Reference material tested with ELITE InGenius

Panel name	Provider	Qualitative results	Quantitative results
EBV Molecular Q Panel	Qnostics	Concordance 100% (4/4)*	Titre as expected value ± 0.5 log
AcroMetrix EBV Plasma Panel	Life Technologies	Concordance 100% (5/5)*	Titre as expected value ± 0.5 log
QCMD 2014 Epstein-Barr Virus DNA EQA Panel	Qnostics	Concordance 100% (8/8)*	Titre as expected value ± 1 log 1 sample Titer as expected value ± 2 log

**confirmed samples/ tested samples*

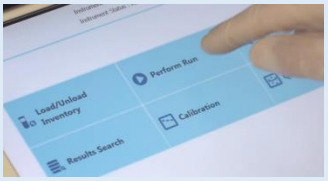
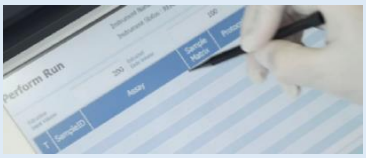
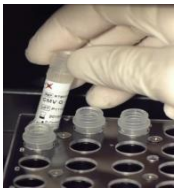
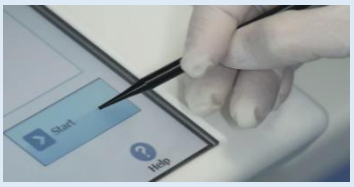
I. Procedures ELITe InGenius

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

Before analysis

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

Procedure 1 - Complete run: Extraction + PCR

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

Procedure 2 - PCR only

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

Procedure 3 - Extraction only

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

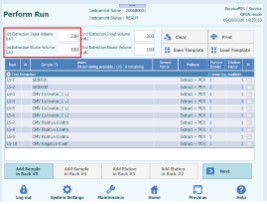
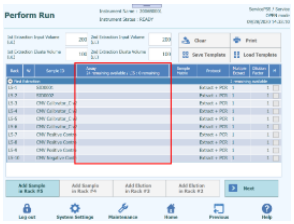
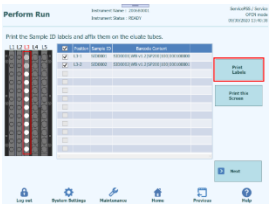
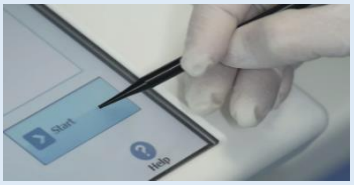
L. Procedures ELITe BeGenius

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

Before analysis

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

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen and then click on the run mode «Extraction and PCR» 	**2.** Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active 	**3.** Verify the extraction volumes: Input: "200 µL", Eluate: "100 µL" 
4. Select the "Assay protocol" of interest  **Note**: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area 	**6.** Load the Q-PCR-Mix and the CPE Internal Control in Reagent Rack and insert it in the cooling area 
7. Load: Filter Tips, Extraction rack, and PCR rack 	**8.** Close the door. Start the run 	**9.** View, approve and store the results 

Procedure 2 - PCR only

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

Procedure 3 - Extraction only

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

EBV ELITe MGB® kit used with ELITe InGenius®

Code: RTS020PLD

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

A. Intended use

The EBV ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **Epstein-Barr human herpesvirus**. The assay is CE-IVD validated in combination with the instrument **ELITe InGenius**.

B. Amplified sequence

	Gene	Fluorophore
Target	EBNA-1	FAM
Internal Control	Human beta globin gene	AP525 (VIC)

C. Validated matrix

› **Whole blood** EDTA

› **Plasma** EDTA

D. Kit content

EBV Q-PCR Mix
4 tubes of 540 µL

X 4

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

E. Material required not provided in the kit

› **ELITe InGenius instrument:** INT030
› **ELITe InGenius SP1000** Extraction Cartridge**:** INT033SP1000
› **ELITe InGenius PCR Cassette** amplification cartridges**:** INT035PCR
› **ELITe InGenius SP200 Consumable Set** consumables for extraction**:** INT032CS

› **EBV ELITe Standard :** STD020PLD
› **EBV ELITe Positive Control:** CTR020PLD
› **CPE Internal Control:** CTRCPE
› **ELITe InGenius Waste Box:** F2102-000
› **Filter Tips 300:** TF-350-L-R-S

F. ELITe InGenius protocol

› Sample volume	1000 µL
› CPE Internal Control volume	10 µL
› Total eluate volume	100 µL
› PCR eluate input volume	20 µL
› EBV Q-PCR Mix volume	20 µL

› Unit of quantitative result	International Unit: IU/mL copies/mL
› Frequency of controls	15 days
› Frequency of calibration	60 days

G. Performance

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Plasma (1000 µL)	**18 IU/mL – 11 cp/mL**	**96.7%** 29/30*	**96.8%** 60/62 **confirmed samples/ tested samples*

Matrix	Linearity (copies/mL)	Linearity (IU/mL)	Conversion factor cp/mL to IU/mL
Plasma (1000 µL)	**62 - 625,000**	**99 - 1,000,000**	**1.6**

H. Reference material tested

Panel name	Provider	Qualitative results	Quantitative results
EBV Molecular Q Panel	Qnostics	Concordance 100% (4/4)*	Titre as expected value ± 0.5 log
QCMD 2015 Epstein-Barr virus DNA EQA Panel	Qnostics	Concordance 100% (10/10)*	Titre as expected value ± 1 log

*confirmed *samples/ tested samples*

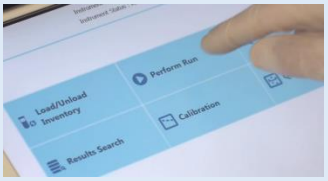
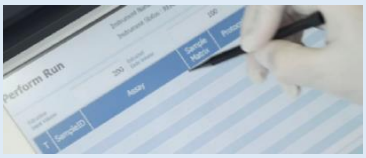
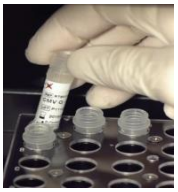
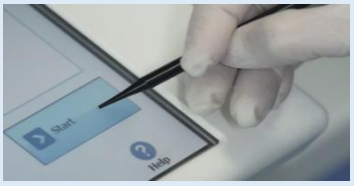
I. Procedures

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

Before analysis

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

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen 	**2.** Verify the extraction volumes: Input:"1000 µL", eluate: "100 µL" 	**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID 
4. Select the "Assay protocol" of interest 	**5.** Select the sample position: Primary tube or sonication tube 	**6.** Load the Q-PCR-Mix and the CPE Internal Control in the inventory block 
7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, sonication tube and primary sample racks 	**8.** Close the door Start the run 	**9.** View, approve and store the results 

Procedure 2 - PCR only

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

Procedure 3 - Extraction only

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

EBV ELITe MGB® Kit used with ABI PCR instrument

Code: RTS020PLD

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

A. Intended use

The EBV ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **Epstein-Barr human herpesvirus**.

The assay is CE-IVD validated in combination with **ABI PCR thermal cyclers** (Thermo-Fisher) and the following extraction systems: **ELITe STAR** (ELITechGroup), **ELITe GALAXY** (ELITechGroup), **easyMAG** (BioMérieux) or **QIAsymphony** (Qiagen).

B. Amplified sequence

Target	Gene	Fluorophore
EBV	EBNA-1	FAM
Internal Control	Human beta globin gene	AP525 (VIC)

C. Validated matrix

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

D. Kit content

EBV Q-PCR Mix
4 tubes of 540 µL

X 4

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

E. Material required not provided in the kit

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

› **EBV ELITe Positive Control:** CTR020PLD
› **EBV ELITe Standard:** STD020PLD
› **CPE Internal Control:** CTRCPE
› **easyMAG** - Generic protocol 2.0.1
› **QIAsymphony** - DNA Mini kit or DSP Virus/Pathogen Midi kit
› **Molecular biology grade water**

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
ELITe STAR - ABI	Whole Blood	**212 IU/mL – 101 gEq/mL**	**95.2%** (30/31)*	**96.9%** (53/60)*
	Plasma	**229 IU/mL – 107 IU/mL-**	**95.2%** (29/30)*	**100%** (40/40)*
ELITe GALAXY - ABI	Whole Blood	**99 IU/mL – 111 gEq/mL**	**100%** (32/32)*	**96.9%** (31/32)*
	Plasma	**97 IU/mL – 128 gEq/mL**	**96.67%** (29/30)*	**100%** (30/30)*

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

**confirmed samples/tested samples*

G. Procedure

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

Extraction - Validated systems

Extraction	Validated matrix	Sample volume processed	Min. sample volume	Total eluate volume	CPE Internal Control volume
ELITe Star	Whole Blood, Plasma	200 µL	700 µL	100 µL	200µL
ELITe Galaxy	Whole Blood, Plasma	300 µL	400 µL	200 µL	10 µL
EasyMAG	Whole Blood, Plasma	500 µL	-	100 µL	5 µL
QIAsymphony	Whole Blood, Plasma	500 µL	600 µL	85 µL	6 µL

Amplification - Settings of 7500 Fast Dx and 7300 PCR instruments

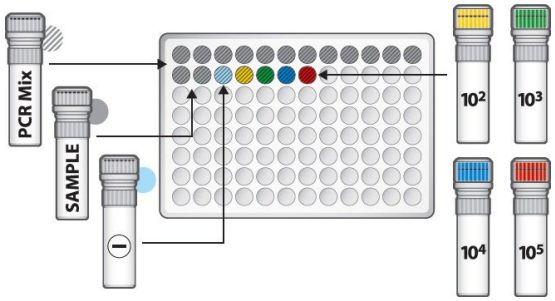
1. Switch on the thermal-cycler
2. Set "EBV" detector with "FAM" and quencher "none"
3. Set "Internal Control" detector with "VIC" and quencher "none"
4. Set passive fluorescence as "Cy5" with 7500 Fast Dx and as "ROX" with 7300 instrument
5. Set up the thermal profil as indicated. Fluorescence acquisition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

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

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

Amplification - PCR Set -up

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



Amplification - Threshold for qualitative analysis

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

Interpretation - Qualitative results

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

**Repeat the assay starting from the extraction*

Interpretation - Quantitative results

The EBV ct value obtained for each sample and the standard curve generated are used to calculate the quantity of target DNA in the reaction.
The sample quantification ranges from approximately 10 to 10^6 gEq/reaction.

EBV ELITe MGB® kit used with Cobas-Z 480 PCR instruments

Code: RTS020PLD

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

A. Intended use

The EBV ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **Epstein-Barr human herpesvirus.**

The assay is CE-IVD validated in combination with **Cobas – Z 480 analyzer (Roche)** and the following extraction systems: **MagNA Pure 24 System**.

B. Amplified sequence

Target	Gene	Fluorophore
EBV	**EBNA-1**	**FAM (465 – 510)**
Internal Control	**Human beta globin gene**	**VIC (540 - 580)**

C. Validated matrix

› **Whole Blood** › **Plasma EDTA**

D. Kit content

EBV Q-PCR Mix 4 tubes of 540 µL	X 4	› Ready to use complete mixture › Number of tests per kit: 96 › Freeze-thaw cycles per tube: 5 › Maximum shelf-life: 24 months › Storage Temperature: - 20°C

E. Material required not provided in the kit

› **Cobas – Z 480 analyzer PCR Instrument**
› **MagNA Pure 24 System = software 1.0**
› **EBV - ELITe Positive Control:** CTR020PLD
EBV – ELITe Positive Control RF:CTR020PLD-R
› **EBV ELITe Standard:** STD020PLD
› **CPE Internal Control:** CTRCPE
› **Molecular biology grade water**

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
MagNA Pure 24	Whole Blood	**143 IU/mL - 10 cp/rxn**	**100%** (34/34)*	**100%** (34/34)*
	Plasma	**163 IU/mL - 10 cp/rxn**	**100%** (30/30)*	**96.8%** (30/31)*

**confirmed samples/tested samples*

G. Procedure

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

Extraction - Validated systems

Extraction	Validated matrix	Sample volume processed	Min. sample volume	Total eluate volume	CPE Internal Control volume
MagNA Pure 24	Whole Blood, Plasma	200 µL	350 µL	100 µL	20 µL diluted 1:2

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

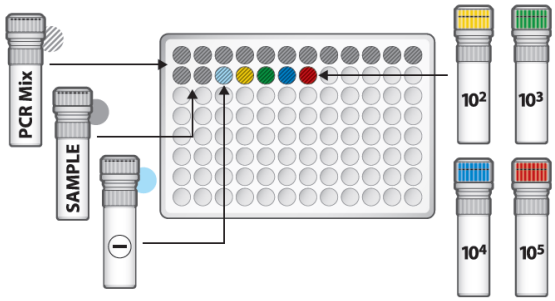
1. Switch on the thermal-cycler
2. Set "EBV" detector with "FAM (465 -510)".
3. Set "Internal Control" detector with "VIC (540 -580)".
4. Set up the thermal profile as indicated. Fluorescence acquisition must be set during hybridation step at 60°C

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

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

Amplification - PCR Set-up

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



Amplification - Threshold for qualitative analysis*

Instrument	Matrix	Background Fluorescence Level FAM	EBV FAM	Background Fluorescence Level VIC	Internal Control VIC
Cobas-Z 480 PCR instruments	Whole Blood	from cycle 2 to cycle 6	0.80	from cycle 6 to cycle 10	1.5
Cobas-Z 480 PCR instruments	Plasma	from cycle 2 to cycle 6	0.55	from cycle 6 to cycle 10	1.2

**manually set the Threshold and Noiseband*

Interpretation - Qualitative results

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

**Repeat the assay starting from the extraction*

Interpretation - Quantitative results

The EBV Ct value obtained for each sample and the standard curve generated are used to calculate the quantity of target DNA in the reaction. The sample quantification ranges from approximately 10 to 10^6 copies/reaction or approximately from 250 to 2.5 10^7 copies/mL.

WORK SHEET

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