ELITechGroup
EMPOWERING IVD

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NOTICE of CHANGE dated 08/06/2022

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«CMV ELITe MGB® Kit»
Ref. RTK015PLD

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

- *Extension of the use of the product in association with «ELITe BeGenius®» instrument (REF INT040), Plasma and Whole Blood*
- *Update of PERFORMANCE CHARACTERISTICS:*
  - *Change in Limit of Detection (LoD)*
  - *Change in Linear measuring range*
  - *Addition of Repeatability*
  - *Addition of Reproducibility*
- *Introduction of IC cut-off value*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

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

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CMV ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTK015PLD

CE 0344

IVD

-20 °C

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INTENDED USE

The **«CMV ELITe MGB® Kit»** product is a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of Human Cytomegalovirus (CMV)** in DNA samples extracted from whole blood collected in EDTA, plasma collected in EDTA, cerebrospinal fluid (CSF), urine, buccal swab, amniotic fluid and bronchoalveolar lavage (BAL) / bronchial aspirate (BA).

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

ASSAY PRINCIPLES

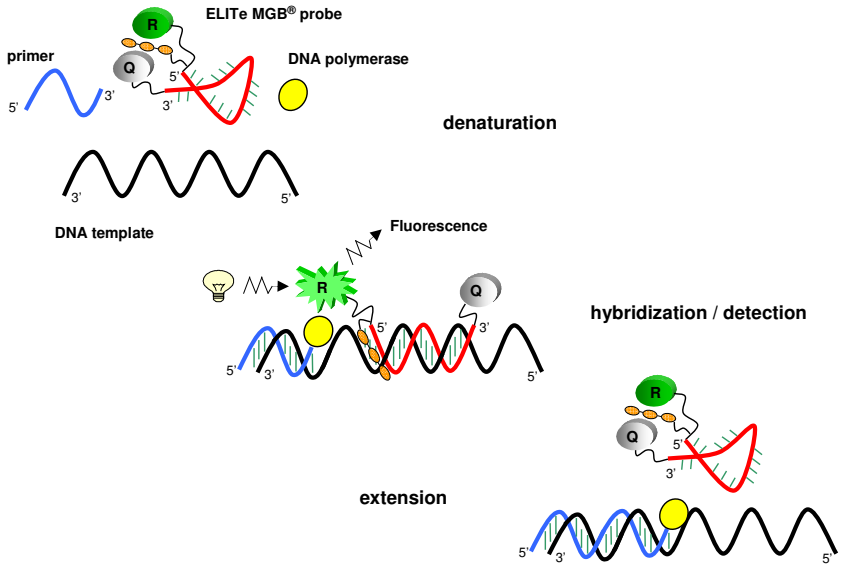
The assay consists of a real time amplification reaction with a programmable thermostat provided with a fluorescence detection optical system.

In each well, two amplification reactions are performed starting from DNA extracted from the samples being tested: a specific reaction for the **exon 4 region of the CMV MIEA gene** (major immediate early antigen, HCMVUL123) and a specific reaction for a region of the **human beta Globin gene** (Internal Control of inhibition). The CMV specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the CMV amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with AP525 fluorophore (analogous to VIC), is activated when hybridizes with the specific product of the Internal Control amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data allows detecting the presence and the titre of CMV DNA in the starting sample.

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

The assay is validated with the systems described in this user manual.

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



PRODUCT DESCRIPTION

The **«CMV ELITe MGB® Kit»** product supplies the **ready to use complete mixture** "CMV 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 CMV specific probe (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non-fluorescent molecule) are specific for the **exon 4 region of the CMV MIEA gene** (major immediate early antigen, HCMVUL123).

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 KIT

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

MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

- 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 kit.

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

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For automatic sample analysis with the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) the following generic products are required: the extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200) 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, ref. TF-350-L-R-S).

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

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

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

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

for samples analysis **«CMV ELITe_WB_200_100»**, **«CMV ELITe_PL_200_100»**, **«CMV ELITe_PL_1000_100»**, **«CMV ELITe_CSF_200_100»**, **«CMV ELITe_U_200_100»**, **«CMV ELITe_BS_200_100»**, **«CMV ELITe_AF_200_100»** and **«CMV ELITe_BAL_200_100».**

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

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

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

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

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

for samples analysis **«CMV ELITe_Be_WB_200_100»** and **«CMV 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), kit 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.

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

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

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 CMV DNA is required (qualitative analysis), use the product **«CMV - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR015PLD) or the product **«CMV - ELITe Positive Control RF»** (ELITechGroup S.p.A., ref. CTR015PLD-R), positive control of plasmid DNA.

If detection and quantification of CMV DNA is required (quantitative analysis), use the product **«CMV ELITe Standard»** (ELITechGroup S.p.A., ref. STD015PLD), four dilutions of known quantity plasmid DNA to obtain the standard curve.

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.

A conversion factor allows to express the results of the quantitative analysis in International Units of CMV of the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, 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 acids extraction, amplification and detection, require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

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

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

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

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

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. The pipettes used to handle extraction products must be exclusively used for this purpose.

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

Warnings and precautions specific for the components

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

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

The **CMV Q - PCR Mix** can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each.

ELITe InGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Whole blood collected in EDTA

The whole blood samples for nucleic acid extraction must be collected in EDTA 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 nucleic acid extraction from whole blood is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.3 (or later equivalent versions), use the extraction protocols **CMV ELITe_WB_200_100**. This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elute the nucleic acids in 100 µL of water.

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

Plasma collected in EDTA

The plasma samples for nucleic acid 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 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 protocols **CMV 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 nucleic acid 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 protocols **CMV ELITe_PL_1000_100**. This protocol processes 1000 µL of sample, adds the **CPE** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

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

Cerebrospinal fluid (CSF)

The cerebrospinal fluid (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.

Before the analysis with this product 0.2 mL of sample has to be transferred in the extraction tube provided with «ELITe InGenius® SP 200 Consumable Set».

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 nucleic acid extraction from cerebrospinal fluid is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocols **CMV ELITe_CSF_200_100**. This protocol processes 200 µL of sample, adds the **CPE** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Urine

Urine samples for nucleic acid extraction must be collected in preservative-free containers according to laboratory guidelines, transported and stored at room temperature (+18 / + 25 °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.

Before the analysis with this product 0.2 mL of sample has to be transferred in the extraction tube provided with «ELITe InGenius® SP 200 Consumable Set».

If possible, avoid freezing of first void urine samples. Freezing can cause precipitation of inhibitors and the loss of the DNA titre.

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

Note: when the DNA extraction from urine is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocols **CMV ELITe_U_200_100**. This protocol processes 200 µL of sample, adds the **CPE** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Buccal Swab

The buccal swab samples for nucleic acid extraction should be collected with the collection and transport systems «eSwab Collection Kit» (COPAN Italia S.p.A., ref. 480CE), identified according to laboratory guidelines, and transported and stored at room temperature (+18 / + 25 °C) for a maximum of five days, otherwise at +2 / +8 °C for up to seven days, otherwise they must be frozen and stored at -20 °C for a maximum of six months or at -70 °C for longer periods.

Before the analysis with this product 0.2 mL of sample has to be transferred in the extraction tube provided with «ELITe InGenius® SP 200 Consumable Set».

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.

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Note: when nucleic acid extraction from buccal swab is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocols **CMV ELITe_BS_200_100**. This protocol processes 200 µL of sample, adds the **CPE** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Amniotic fluid

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

Before the analysis with this product 0.2 mL of sample has to be transferred in the extraction tube provided with «ELITe InGenius® SP 200 Consumable Set».

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 nucleic acid extraction from amniotic fluid is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocols **CMV ELITe_AF_200_100**. This protocol processes 200 µL of sample, adds the **CPE** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Bronchoalveolar Lavage (BAL) and Broncho Aspirate (BA)

The BAL / BA samples, intended for DNA extraction, must be collected in sterile physiological solution or sterile PBS according to laboratory guidelines, transported at +2 /+8 °C and stored at +2 / +8 °C for a maximum of one week. Otherwise, they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C up to one year, according to laboratory practice.

Before the analysis with this product 0.2 mL of sample has to be transferred in the extraction tube provided with «ELITe InGenius® SP 200 Consumable Set».

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.

If BAL / BA samples are particularly mucous, they can be liquefied by dithiothreitol based reagents (e.g. Sputasol, Oxoid, Thermo Fisher Scientific) as per laboratory guidelines.

Note: when the DNA extraction from BAL / BA is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the Assay Protocol CMV ELITe_BAL_200_100. This protocol processes 200 µL of sample, adds the CPE at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Other samples:

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

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.

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

Amplification calibrators and amplification controls

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

as calibrator set, use the four concentration levels of the **CMV ELITe Standard**, in association with the protocol **«CMV ELITe_STD»** or **«CMV ELITe_STD_1000_100»**,
as amplification Positive Control use the **CMV - ELITe Positive Control**, in association with the protocol **«CMV ELITe_PC»** or **«CMV ELITe_PC_1000_100»**,
as amplification Negative Control, use Molecular biology grade water (not provided with this kit), in association with protocol **«CMV ELITe_NC»** or **«CMV ELITe_NC_1000_100»**.

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

ELITe InGenius® PROCEDURE

The procedure to use the **CMV ELITe MGB® Kit** with the system **ELITe InGenius** consists of three steps:

- System readiness verification
- 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 (**CMV Q-PCR Standard**) have been run, approved and are not expired (status). This can be checked under the "Calibration" menu in the Home page;
- verify (Controls) that the amplification Controls (**CMV Positive Control, CMV Negative Control**) have been run, approved and are 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.

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

Assay protocols for CMV ELITe MGB® kit

Name	Matrix	Report unitage	Characteristics
CMV ELITe_WB_200_100	Whole Blood	gEq/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
CMV ELITe_PL_200_100	Plasma	gEq/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

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Name	Matrix	Report unitage	Characteristics
CMV ELITe_PL_1000_100	Plasma	gEq/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
CMV ELITe_CSF_200_100	Cerebrospinal Fluid	gEq/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
CMV ELITe_U_200_100	Urine	gEq/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
CMV ELITe_BS_200_100	Buccal swab	gEq/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
CMV ELITe_AF_200_100	Amniotic Fluid	gEq/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
CMV ELITe_BAL_200_100	BAL / BA	gEq/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

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 **CMV 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 run for Positive and Negative Control (PCR only).

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

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

The main operations for setting the four types of runs 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 CMV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in a sufficient number 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.

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

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”.
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. CMV 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 CMV 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 any spilling of the extracted sample.
Note: At the end of the run the “PCR Cassette” containing 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 PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

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B. Amplification run

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

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

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

2. Select “Perform Run” from the “Home”.
3. Even if no extraction will be carried out , ensure that the Extraction Input Volume is200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and 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. CMV 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 raw)”. Click “Next” to continue the setup.
8. Load CMV 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 any 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 PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run

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

1. Thaw CMV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in a sufficient number 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.

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

2. Thaw CMV Q - PCR Standard tubes (Cal1: CMV Q-PCR Standards 10^2, Cal2: CMV Q-PCR Standards 10^3, Cal3: CMV Q-PCR Standards 10^4, Cal4: CMV Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home screen”.
4. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 200 µL 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 (CMV ELITe_STD or CMV ELITe_STD_1000 _100) and fill with the lot number and expiry date for the CMV Q - PCR Standard. Click “Next” button to continue the setup.
6. Load the CMV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
7. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load the **CMV Q-PCR Standard** tubes and “PCR Cassette” 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 PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

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D. Amplification run for Positive Control and Negative Control

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

1. Thaw CMV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in a sufficient number 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.

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

2. Thaw CMV - ELITe Positive Control tubes at room temperature (~+25°C) for 30 minutes, 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 no extraction will be carried out, ensure that 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 CMV ELITe_PC or CMV ELITe_PC_1000_100 and fill in the lot number and expiry date for the CMV Positive Control.
7. For the negative control, select CMV ELITe_NC or CMV ELITe_NC_1000_100and fill in the lot number and expiry date for the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load CMV 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 CMV Positive Control tube and/or the Negative Control tube, 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. The remaining Negative Control must be disposed.

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

Review and approval of results

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

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

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

The **ELITe InGenius** generates results using the **CMV 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 CMV probe ("CMV") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol "CMV ELITe_STD" and "CMV 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: if the Calibration curve does not meet the acceptance criteria, the "Failed" message is shown on the "Calibration" screen and it is not possible to approve curve. The Calibrator amplification reactions have to be repeated.

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

B. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the specific CMV probe ("CMV") 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 protocol "CMV ELITe_PC", "CMV ELITe_PC_1000_100", CMV ELITe_NC" and "CMV ELITe_NC_1000_100".

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

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

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

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

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

Note: 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 CMV probe ("CMV") 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**
CMV Q-PCR Standard	APPROVED
2) Positive Control	**Status**
CMV Positive Control	APPROVED
3) Negative Control	**Status**
CMV 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 "gEq / 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**
CMV: DNA Detected, quantity equal to XXX gEq / mL or IU / mL	**CMV DNA detected** within the measurement range of the assay, quantity as shown.
CMV: DNA Detected, quantity below LLoQ gEq / mL or IU / mL	**CMV DNA detected** below the lower limit of quantification of the assay
CMV: DNA Detected, quantity beyond ULoQ gEq / mL or IU / mL	**CMV DNA detected** beyond the upper limit of quantification of the assay
CMV: DNA Not Detected or below LoD gEq / mL or IU / mL	**CMV 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 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.

ELITe BeGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Whole blood collected in EDTA

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

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

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

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

Plasma collected in EDTA

The plasma samples for nucleic acid 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 200 µL of plasma is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius® Software** version **2.0.0** (or later equivalent versions), use the extraction protocol **CMV 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.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: cerebrospinal fluid (CSF), Urine, Buccal Swab, Amniotic fluid, Bronchoalveolar Lavage (BAL) and Broncho Aspirate (BA), suspensions of leucocytes, suspensions of granulocytes.

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.

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

Amplification calibrators and amplification controls

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

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

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

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.

ELITe BeGenius® PROCEDURE

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

Assay protocols for «CMV ELITe MGB® Kit» and ELITe BeGenius

Name	Matrix	Report unitage	Characteristics
CMV ELITe_Be_WB_200_100	Whole Blood	gEq/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

CMV ELITe_Be_PL_200_100	Plasma	gEq/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.

Protocols for qualitative analysis are available on request.

Setup of the session

The **CMV 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 send 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 as per the **SW Graphical User Interface (GUI)**:

1. Thaw CMV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session 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.

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

2. Thaw the CPE tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session. Each new tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. Remove the Racks from the "Cooler Unit" and place them on the preparation table.
5. Select the "run mode": "Extract + PCR".
6. Load the samples into the Racks 5 and 4 (start always from Rack 5).
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.

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

8. Check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
9. Select the assay protocol to be used in the "Assay" column (i.e. CMV ELITe_Be_PL_200_100). Click "Next" to continue the setup.
10. If used, repeat step 7 to 9 for Rack 4..
11. Load the Elution tubes into the Racks 3 and 2 (start always from Rack 3)..

Note: Elution tubes can be labelled to improve traceability.

12. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
13. If used, repeat step 12 for Rack 2..
14. Load CPE and CMV Q-PCR Mix into the Rack 1..
15. Insert the Rack 1 into the "Cooler Unit". Click "Next" to continue the setup.

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16. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
17. Load the Basket with "PCR Cassette" in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
18. Load the Basket with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables by following the GUI instruction. Click "Next" to continue the setup.
19. Close the instrument door.
20. Press "Start" to start the run.

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

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

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

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

B. Amplification run

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

1. Thaw CMV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number 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.

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

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 Racks 3 and 2 (start always from Rack 3)..
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. CMV ELITe_Be_PL_200_100). Click "Next" to continue the setup.
9. Repeat step from 7 to 9 for Rack 2
10. Load CMV Q-PCR Mix into Rack 1.
11. Insert the 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 Basket 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 Extracted Sample can be removed from the instrument, capped, identified and stored at -20 °C. Avoid the spilling of the Extracted Sample.

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

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

C. Calibration run

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

1. Thaw CMV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number 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.

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

2. Thaw the CMV Q - PCR Standard tubes (Cal1: CMV Q-PCR Standards 10^2, Cal2: CMV Q-PCR Standards 10^3, Cal3: CMV Q-PCR Standards 10^4, Cal4: CMV Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. 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 Rack 3.
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
8. Select the assay protocol to be used in the "Assay" column (CMV ELITe_Be_STD). Click "Next" button to continue the setup. .
9. Load CMV Q-PCR Mix into Rack 2.
10. Insert the Rack 2 into the "Cooler Unit". Click "Next" to continue the setup.
11. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
12. Load the basket with the 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 Calibrators can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Q-PCR Standards.

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

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

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D. Positive Control and Negative Control run

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

1. Thaw CMV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session 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.

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

2. Thaw the CMV - ELITe Positive Control tube at room temperature (~+25°C) for 30 minutes, 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 session 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 Rack 3.
8. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
9. Select the assay protocol to be used "CMV ELITe_Be_PC" and "CMV ELITe_Be_NC" in the "Assay" column. Click "Next" button to continue the setup.
10. Load CMV Q-PCR Mix into the Rack 2.
11. Insert the Rack 2 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 basket 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 Positive Control can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Positive Controls.

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

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

Review and approval of results

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

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

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

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

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

PERFORMANCE CHARACTERISTICS ELITe InGenius and ELITe BeGenius

Analytical sensitivity: Limit of Detection

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

The LoD of this assay was tested using plasmid DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmid DNA was diluted to a titre of 10 copies / 20 µL in human genomic DNA at a titre of 500 ng / 20 µL. This sample was tested in 12 replicates carrying out the amplification by ELITechGroup S.p.A. products on two different ELITe InGenius instruments.

Samples	N	positive	negative
10 copies plasmid DNA + 500 ng of human genomic DNA	24	23	1

The analytical sensitivity of this assay used in association to different matrices and **ELITe InGenius** was verified with a panel of CMV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV DNA - negative matrix. The panel consisted of at least six points around the limit concentration. Each sample of the panel was tested in at least 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 for each matrix are reported in the following tables.

Limit of Detection with ELITe InGenius (IU / mL)				
Sample volume	Matrix	95% positivity	95% confidence range	
			lower limit	upper limit
200 µL	**whole bood**	**109 IU / mL**	71 IU / mL	239 IU / mL
	plasma	**88 IU / mL**	50 IU / mL	291 IU / mL
	cerebrospinal fluid	**58 IU / mL**	48 IU / mL	82 IU / mL
	urine	**151 IU / mL**	119 IU / mL	214 IU / mL
	buccal swab	**44 IU / mL**	36 IU / mL	57 IU / mL
	amniotic fluid	**57 IU / mL**	46 IU / mL	78 IU / mL
	BAL / BA	**97 IU / mL**	58 IU / mL	291 IU / mL
1000 µL	**plasma**	**17 IU / mL**	14 IU / mL	22 IU / mL

The analytical sensitivity as gEq / mL for each matrix is calculated by applying the specific conversion factor reported at page 25.

The analytical sensitivity as gEq / mL is reported below.

Limit of Detection with ELITe InGenius (gEq / mL)				
Sample Volume	Matrix	95% positivity	95% confidence range	
			lower limit	upper limit
200 µL	**whole bood**	**156 gEq / mL**	99 gEq / mL	332 gEq / mL
	plasma	**293 gEq / mL**	167 gEq / mL	970 gEq / mL
	cerebrospinal fluid	**193 gEq / mL**	160 gEq / mL	273 gEq / mL
	urine	**216 gEq / mL**	170 gEq / mL	306 gEq / mL
	buccal swab	**220 gEq / mL**	180 gEq / mL	285 gEq / mL
	amniotic fluid	**285 gEq / mL**	230 gEq / mL	390 gEq / mL
	BAL / BA	**485 gEq / mL**	290 gEq / mL	1455 gEq / mL
1000 µL	**plasma**	**57 gEq / mL**	47 gEq /mL	73 gEq /mL

The calculated LoD value for **whole blood** and **plasma** (sample volume 200 µL) matrices in association to **ELITe InGenius** and **ELITe BeGenius** was verified by testing 20 replicates of whole blood collected in EDTA and 20 replicates of Plasma collected EDTA samples spiked by CMV 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	109 IU / mL	CMV	20	20	0
Plasma collected in EDTA	88 IU / mL	CMV	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	109 IU / mL	CMV	20	20	0
Plasma collected in EDTA	88 IU / mL	CMV	20	19	1

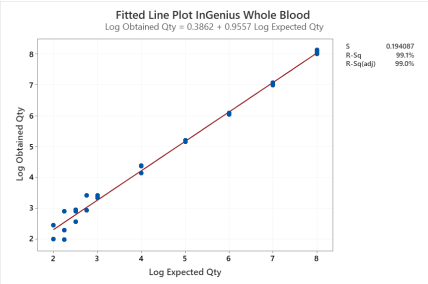
The LoD value for CMV target was confirmed at 109 IU / mL for Whole Blood collected in EDTA and at 88 IU / mL for Plasma collected in EDTA.

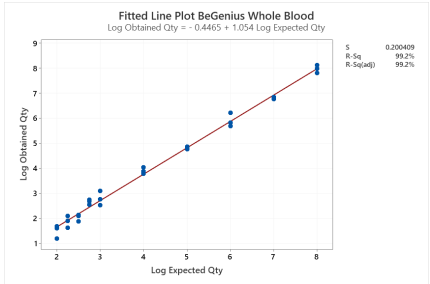
Linear measuring range

Whole Blood:

The linear measuring range of CMV ELITe MGB® Kit used in association with Whole Blood and **ELITe InGenius** and **ELITe BeGenius** was tested using a panel prepared by diluting a CMV reference material (Notovir Italy) in CMV DNA - negative matrix. The panel consisted of eight dilution points from 10^8 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 in association with Whole Blood samples shows a linear response for all the dilution levels with a Square Correlation Coefficient (R2) equal to 0.991 for **ELITe InGenius** and 0.992 for **ELITe BeGenius**.





The Lower Limit of Quantification (LLoQ) was set at the concentration, that gives quantitative results precise (Standard Deviation equal to 0.2702 Log IU / mL for ELITe InGenius and 0.1925 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to 0.3453 Log IU / mL for ELITe InGenius and -0.1462 Log IU / mL for ELITe BeGenius): 178 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0597 Log IU / mL for ELITe InGenius and 0.1590 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to -0.0683 Log IU / mL for ELITe InGenius and 0.0249 Log IU / mL for ELITe BeGenius): 100,000,000 IU / mL.

The linear measuring range as gEq / mL for Whole blood is calculated by applying the specific conversion factor reported at page 32.

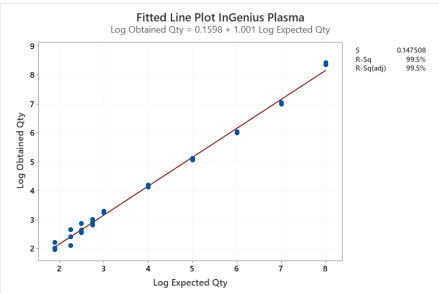
The final results are summarized in the following table.

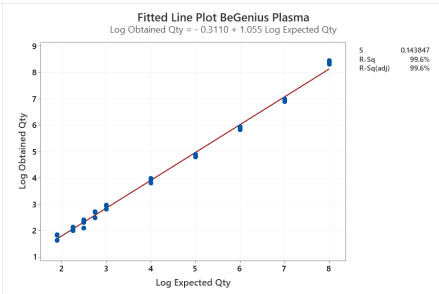
Linear measuring range for whole blood samples and ELITe InGenius and ELITeBeGenius		
Unit of measure	lower limit	upper limit
IU / mL	**178**	**100,000,000**
gEq / mL	**254**	**142,857,143**

Plasma (sample volume 200 µL):

The linear measuring range of CMV ELITe MGB® Kit used in association with Plasma and **ELITe InGenius** and **ELITe BeGenius** was tested using a panel prepared by diluting a CMV reference material (Notovir, Italy) in CMV DNA - negative matrix. The panel consisted of eight dilution points from 10^8 to 80 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 in association with Plasma samples shows a linear response for all the dilution levels with a Square Correlation Coefficient (R2) equal to 0.995 for ELITe InGenius and 0.996 for ELITe BeGenius.





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The Lower Limit of Quantification (LLoQ) was set at the LoD concentration, that gives quantitative results precise (Standard Deviation equal to 0.2701 Log IU / mL for ELITe InGenius and 0.2114 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to 0.3314 Log IU / mL for ELITe InGenius and -0.0619 Log IU / mL for ELITe BeGenius): 88 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0351 Log IU / mL for ELITe InGenius and 0.0675 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to -0.3988 Log IU / mL for ELITe InGenius and -0.3865 Log IU / mL for ELITe BeGenius): 100,000,000 IU / mL.

The linear measuring range as gEq / mL for Plasma is calculated by applying the specific conversion factor reported at page 32.

The final results are summarized in the following table.

Linear measuring range for Plasma samples and ELITe InGenius and ELITe BeGenius		
Unit of measure	lower limit	upper limit
IU / mL	**88**	**100,000,000**
gEq / mL	**293**	**333,333,334**

Other matrices

The linearity of this assay used in association with **ELITe InGenius** was verified with a panel of CMV dilutions in the following different matrices: plasma (sample volume 1000 µL), cerebrospinal fluid, urine, buccal swab, amniotic fluid, BAL / BA.

The linearity in "**PCR Only**" mode of this assay was determined using a panel of dilutions (1 $\log_{10}$ dilution steps) of a plasmid DNA containing the amplification product, whose initial concentration was measured by spectrophotometer. The dilutions from 2 x 10^6 genome equivalent per reaction to 2 x 10^1 genome equivalent per reaction were tested in 5 replicates carrying out the amplification by the ELITechGroup S.p.A. products. The analysis of the obtained data, performed by linear regression, demonstrated that the assay shows a linear response for all the dilutions (square correlation coefficient greater than 0.99).

The linearity in "**Extract+PCR**" mode of this assay used in association to different matrices and **ELITe InGenius** was verified with a panel of CMV dilutions. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV 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 at least 3 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 analysis of the obtained data, performed by linear regression, demonstrated that the assay shows a linear response for all the dilution levels.

Limits of quantification

The lower limit of the linear measuring range was set at the lowest concentration that gives 100% of positivity and quantitative results accurate and precise within ± 0.5 Log IU / mL. The upper limit of the linear measuring range was set at the highest tested concentration that gives quantitative results accurate and precise within ± 0.5 Log IU / mL.

The linear measuring range as gEq / mL for each matrix is calculated by applying the specific conversion factor reported at page 32.

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

Linear measuring range for plasma samples and ELITe InGenius			
Sample volume	Unit of measure	lower limit	upper limit
1000 µL	**IU / mL**	**178**	**1,500,000**
	gEq / mL	**593**	**5,000,000**

Linear measuring range for cerebrospinal fluid samples and ELITe InGenius			
Sample volume	Unit of measure	lower limit	upper limit
200 µL	**IU / mL**	**101**	**15,000,000**
	gEq / mL	**335**	**50,000,000**

Linear measuring range for urine samples and ELITe InGenius			
Sample volume	Unit of measure	lower limit	upper limit
200 µL	**IU / mL**	**316**	**35,000,000**
	gEq / mL	**451**	**50,000,000**

Linear measuring range for Buccal swab samples and ELITe InGenius			
Sample volume	Unit of measure	lower limit	upper limit
200 µL	**IU / mL**	**100**	**10,000,000**
	gEq / mL	**500**	**50,000,000**

Linear measuring range for amniotic fluid samples and ELITe InGenius			
Sample volume	Unit of measure	lower limit	upper limit
200 µL	**IU / mL**	**100**	**10,000,000**
	gEq / mL	**500**	**50,000,000**

Linear measuring range for BAL / BA samples and ELITe InGenius			
Sample volume	Unit of measure	lower limit	upper limit
200 µL	**IU / mL**	**178**	**10,000,000**
	gEq / mL	**890**	**50,000,000**

Repeatability

The Repeatability of results obtained by the product CMV 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 CMV certified reference material "1st WHO International Standard for CM Virus DNA (NIBSC code 09/162, United Kingdom) at concentration of 3 x LoD (about 327 IU / mL) and of 10 x LoD (about 1090 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 on **ELITe InGenius** system in "Extract + PCR" mode.

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 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 Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe InGenius								
Sample	**CMV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	24.18	0.17	0.69
3 x LoD	8 / 8	35.91	0.51	1.42				
10 x LoD	8 / 8	33.98	0.29	0.86				

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Inter – Session Repeatability ELITe InGenius								
Sample	CMV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	24.20	0.22	0.90
3 x LoD	16 / 16	36.09	0.78	2.15				
10 x LoD	16 / 16	34.07	0.25	0.75				

In the Repeatability test on **ELITe InGenius**, the assay detected the CMV target as expected and showed low %CV of Ct values that did not exceed 2.2% for CMV and 0.9% 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, by the same operator, on the same day. Samples were processed in randomized positions on **ELITe BeGenius** system in "Extract + PCR" mode.

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, by the same operator, on two different days. 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 Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe BeGenius								
Sample	**CMV**				**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	27.21	0.38	1.39
3 x LoD	8 / 8	37.21	0.49	1.33				
10 x LoD	8 / 8	35.03	0.52	1.48				

Inter – Session Repeatability ELITe BeGenius								
Sample	CMV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	27.18	0.37	1.38
3 x LoD	16 / 16	37.51	0.61	1.63				
10 x LoD	16 / 16	35.06	0.44	1.25				

In the Repeatability test on **ELITe BeGenius**, the assay detected the CMV target as expected and showed low %CV of Ct values that did not exceed 1.6% for CMV and 1.4% for Internal Control.

Reproducibility

The Reproducibility of results obtained by the product CMV 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 CMV certified reference material "1st WHO International Standard for CM Virus DNA (NIBSC code 09/162, United Kingdom) at concentration of 3 x LoD (about 327 IU / mL) and of 10 x LoD (about 1090 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, using the same lot and two different instruments by two different operators. Samples were processed in randomized positions on **ELITe InGenius** system in "Extract + PCR" mode.

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

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

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe InGenius								
Sample	**CMV**				**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	25.31	0.71	2.82
3 x LoD	8 / 8	35.96	0.81	2.26				
10 x LoD	8 / 8	33.62	0.36	1.07				

Inter – Batch Reproducibility ELITe InGenius								
Sample	**CMV**				**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	25.20	0.65	2.59
3 x LoD	8 / 8	35.97	0.65	1.82				
10 x LoD	8 / 8	33.72	0.29	0.86				

In the Reproducibility test on **ELITe InGenius**, the assay detected the CMV target as expected and showed low %CV of Ct values that did not exceed 2.3% for CMV and 2.8%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, using the same lot and 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, using two different lots and the same instrument by the same operator. 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	**CMV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	28.29	0.48	1.69
3 x LoD	8 / 8	36.06	0.46	1.27				
10 x LoD	8 / 8	34.12	0.23	0.66				

Inter – Batch Reproducibility ELITe BeGenius								
Sample	**CMV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	28.30	0.47	1.65
3 x LoD	8 / 8	36.19	0.51	1.41				
10 x LoD	8 / 8	34.22	0.12	0.36				

In the Reproducibility test on **ELITe BeGenius**, the assay detected the CMV target as expected and showed low %CV of Ct values that did not exceed 1.4% for CMV and 1.7 % for Internal Control.

CMV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF **RTK015PLD**

Reproducibility with certified reference material

The analytical sensitivity of the assay was evaluated using as reference material the calibrated panel «CMV 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 IU / mL	Positive / Replicates	Mean results Log IU / mL
CMVMQP01-High	10^5	5.000	2/2	5.024
CMVMQP01-Medium	10^4	4.000	2/2	3.996
CMVMQP01-Low	10^3	3.000	2/2	3.060
CMVMQP01-Negative	negative	-	0/2	-

All positive samples were detected as positive with a titre 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
CMVMQP01-High	10^5	5.000	2/2	4.679
CMVMQP01-Medium	10^4	4.000	2/2	3.717
CMVMQP01-Low	10^3	3.000	2/2	2.733
CMVMQP01-Negative	negative	-	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 calibrated reference material the panel «QCMD 2014 Human Cytomegalovirus 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.

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

Tests with calibrated reference materials and ELITe InGenius				
Sample	Consensus Log IU / mL	Standard Deviation	Positive / Replicates	Mean results Log IU / mL
CMVDNA14-01	2.468	0.343	2/2	2.256
CMVDNA14-02	3.034	0.281	2/2	2.915
CMVDNA14-03	3.383	0.368	2/2	3.185
CMVDNA14-04	3.014	0.251	2/2	2.976
CMVDNA14-05	1.859	0.462	2/2	1.706
CMVDNA14-06	2.767	0.325	2/2	2.526
CMVDNA14-07	4.030	0.280	2/2	3.924
CMVDNA14-08	Negative	-	0/2	-
CMVDNA14-09	2.065	0.512	2/2	1.273
CMVDNA14-10	3.947	0.278	2/2	3.946

All samples were correctly detected. Eight (8) out of nine positive samples were quantified within the range defined by the EQA Consensus ± 1 Standard Deviation (SD) and one sample (CMVDNA14-09) was quantified within 2 SD. This can be explained because the sample titre is below the lower limit of quantification.

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

The results in IU/mL were calculated applying the conversion factor for **ELITe InGenius** and plasma and 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
CMV DNA 3E6	3,000,000	6.477	2/2	6.386
CMV DNA 3E5	300,000	5.477	2/2	5.444
CMV DNA 3E4	30,000	4.477	2/2	4.473
CMV DNA 3E3	3,000	3.477	2/2	3.441
CMV DNA 3E2	300	2.477	2/2	2.575

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

Further tests, starting from 1000 µL of sample, were carried out using as calibrated reference material the panel «QCMD 2017 Human Cytomegalovirus 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.

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

Tests with calibrated reference materials and ELITe InGenius			
Sample	Consensus Log IU / mL	Positive / Replicates	Mean results Log IU / mL
CMVDNA17S-01	2.431	2/2	2.362
CMVDNA17S-02	3.762	2/2	3.665
CMVDNA17S-03	3.920	2/2	3.822
CMVDNA17S-04	2.847	2/2	2.671
CMVDNA17S-05	2.572	2/2	2.189
CMVDNA17S-06	2.849	2/2	2.658
CMVDNA17S-07	3.902	2/2	3.785
CMVDNA17S-08	3.746	2/2	3.667
CMVDNA17S-09	Negative	0/2	-
CMVDNA17S-10	3.900	2/2	3.707

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

Conversion factor to International Units

The conversion factor, to convert a quantitative result from gEq / mL to International Units / mL, was calculated using a panel of at least three dilutions (1 Log_{10} between dilutions) of calibrated reference material approved by WHO ("1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques", NIBSC code 09/162, United Kingdom) in different matrices tested negative for CMV DNA.

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.

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

Conversion factor to International Units with ELITe InGenius		
Sample volume	Matrix	Fc (IU / gEq)
200 µL	**whole bood**	**0.7**
	plasma	**0.3**
	cerebrospinal fluid	**0.3**
	urine	**0.7**
	buccal swab	**0.2**
	amniotic fluid	**0.2**
	BAL / BA	**0.2**
1000 µL	**plasma**	**0.3**

The Conversion Factor of CMV ELITe MGB® Kit used in association with **Whole Blood** collected in EDTA and **ELITe InGenius** and **ELITe BeGenius** was verified with a panel of CMV dilutions. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom), in CMV DNA - negative matrix. The panel consisted of seven dilution points from about 10^6 IU / mL to $10^{2.5}$ IU / mL. Each sample of the panel was tested in 3 replicates.

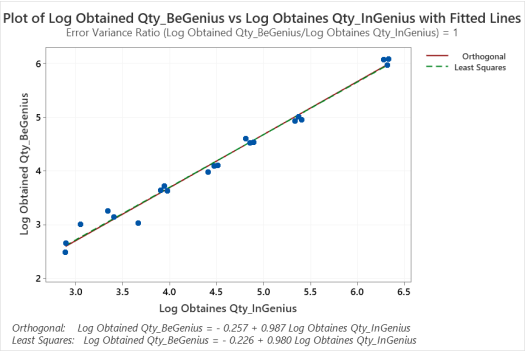
The target quantification precision, as Standard Deviation of Log IU/mL, was lower than 0.5 Log for both **ELITe InGenius** and **ELITe BeGenius**.

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

These results confirmed the Conversion factor calculated for whole blood with **ELITe InGenius**.

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

The results are summed up in the following figure.



The Orthogonal Regression analysis generated an intercept equal to -0.257 (95% CI: -0.503; -0.011) and a slope equal to 0.987 (95% IC: 0.934; 1.040). The linear regression analysis generated a R2 of 0.986.

The Conversion Factor of CMV ELITe MGB® Kit used in association with **Plasma** collected in EDTA (sample volume 200 µL) and **ELITe InGenius** and **ELITe BeGenius** was verified with a panel of CMV dilutions. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom), in CMV DNA - negative matrix. The panel consisted of eight dilution points from about 10^6 IU / mL to 10^2 IU / mL. Each sample of the panel was tested in 3 replicates.

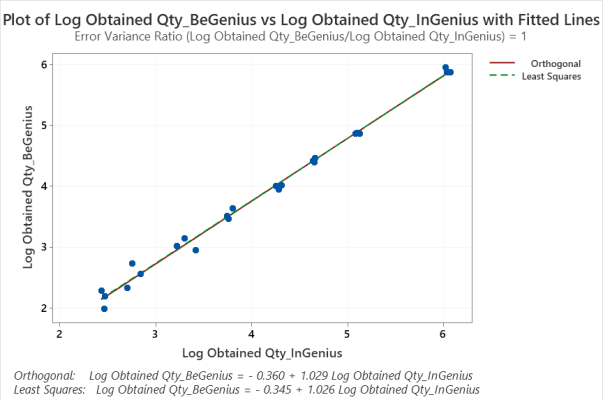
The target quantification precision, as Standard Deviation of Log IU/mL, was lower than 0.5 Log for both **ELITe InGenius** and **ELITe BeGenius**.

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

These results confirmed the Conversion factors calculated for whole blood with **ELITe InGenius**.

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

The results are summed up in the following figure.



In this test, the orthogonal regression analysis generated a slope equal to 1.029 (95% CI: 0.993 - 1.065) and an intercept equal -0.360 (95% CI: - 0.510; -0.209). The linear regression analysis generated a R2 of 0.993.

Robustness: absence of cross-contamination

The assay robustness, as absence of cross-contamination, was verified analysing the results of five sessions in which CMV DNA negative samples were alternated with CMV DNA spiked samples. None of the CMV DNA negative samples resulted positive.

The absence of cross-contamination was verified using a CMV DNA negative whole blood sample spiked with calibrated reference material approved by WHO ("1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques", NIBSC code 09/162, United Kingdom) to a viral load of 10,000 IU / mL and a CMV DNA negative whole blood sample. Five series of 12 samples, alternating a spiked sample with a negative sample, were tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **ELITe InGenius** and with ELITechGroup S.p.A. products.

Samples	N	positive	negative
CMV DNA spiked whole blood collected in EDTA	30	30	0
CMV DNA negative whole blood collected in EDTA	30	0	30

Robustness: whole system failure rate

The assay robustness, as the whole system failure rate leading to false negative results, was verified analysing a panel of CMV DNA spiked at low titre samples and resulted equal to 1.7%.

The whole system failure rate was verified using a CMV DNA negative whole blood samples spiked with calibrated and certified reference material CMVDNA12-01 sample of the “QCMD 2012 Human Cytomegalovirus EQA Panel” (Qnostics, United Kingdom), to a viral load of 750 IU / mL. Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **ELITe InGenius** and with ELITechGroup S.p.A. products. The results are reported in the following table.

Samples	N	positive	negative

CMV DNA spiked whole blood collected in EDTA	60	59	1

Diagnostic sensitivity: confirmation of positive samples

Whole Blood and Plasma (sample volume 200 µL):
The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analysing some clinical samples positive for CMV DNA in association with **ELITe InGenius**. As **ELITe BeGenius** showed equivalent analytical performances to **ELITe InGenius**. As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

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

- 60 whole blood samples collected in EDTA that were positive for CMV DNA (tested with a real time amplification CE IVD product).
- 54 plasma samples collected in EDTA from patients that were positive for CMV DNA (tested with a real time amplification CE IVD product).

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 CMV DNA	60	60	0
	Plasma collected in EDTA and positive for CMV DNA	54	54	0

All samples of whole blood and plasma were confirmed positive. The diagnostic sensitivity of the assay, in these tests, was equal to 100% for both whole blood and plasma samples.

Other matrices

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analysing some clinical samples positive for CMV DNA in association with **ELITe InGenius** and the following matrices: plasma (sample volume 1000 µL), cerebrospinal fluid, urine, buccal swab, amniotic fluid, BAL / BA.

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

- 20 cerebrospinal fluid samples negative for CMV DNA, that were spiked for CMV DNA adding “1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom).
- 31 urine samples from patients that were positive for CMV DNA (tested with a real time amplification CE IVD product).
- 50 buccal swab samples negative for CMV DNA, that were spiked for CMV DNA adding “1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom).
- 11 amniotic fluid samples from patients that were positive for CMV DNA (tested with a real time amplification CE IVD product) and on 20 amniotic fluid samples negative for CMV DNA, that were spiked for CMV DNA adding “1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom).
- 49 BAL / BA samples from patients that were positive for CMV DNA (tested with a real time amplification CE IVD product).

The test, starting from 1000 µL of sample, was performed on 60 plasma samples collected in EDTA from patients that were positive for CMV DNA (tested with a real time amplification CE IVD product).

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	CSF spiked for CMV DNA	20	20	0

	Urine positive for CMV DNA	31	31	0
	Buccal swab spiked for CMV DNA	50	50	0
	Amniotic Fluid positive or spiked for CMV DNA	31	31	0
	BAL / BA positive for CMV DNA	49	49	0
1000 µL	Plasma collected in EDTA and positive for CMV DNA	60	58	2

All samples of all matrices, analyzed starting from 200 µL of volume, were confirmed positive. The diagnostic sensitivity of the assay, in these tests, was equal to 100% for each matrix.

All samples of plasma, analyzed starting from 1000 µL of volume, were valid for the analysis, 58 out of 60 plasma samples were confirmed positive, two samples were discrepant negative.
The diagnostic sensitivity of the assay, in this test, was equal to 96.6% for plasma.

Diagnostic specificity: confirmation of negative samples

Whole Blood and Plasma (sample volume 200 µL):

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analysing some clinical samples negative for CMV DNA in association with **ELITe InGenius**. As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic sensitivity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius**

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

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

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 CMV DNA	59	4	55
	Plasma collected in EDTA and negative for CMV DNA	58	1	57

All samples were valid for analysis. The IC Ct cut-off value is set at 35 for both matrices.

Fifty-five (55) out of 59 whole blood samples were confirmed negative for CMV - DNA, four samples were discrepant positive at low titer. This result can be explained since the LoD of the reference method is higher than the LoD of the product under evaluation.
The diagnostic specificity of the assay in association to whole blood in this test was equal to 93.2%.

Fifty-seven (57) out of 58 plasma samples were confirmed negative for CMV - DNA, one sample was discrepant positive at low titer.

The diagnostic specificity of the assay in association to plasma in this test was equal to 98.3%.

Other matrices

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analysing some clinical samples negative for CMV DNA in association with **ELITe InGenius** and the following matrices: plasma (sample volume 1000 µL), cerebrospinal fluid, urine, buccal swab, amniotic fluid, BAL / BA.

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

- 7 CSF samples that were negative for CMV DNA (tested with a CE IVD real time amplification product) and 3 CSF samples that were presumably negative for CMV DNA.
- 8 urine samples that were negative for CMV DNA (tested with a CE IVD real time amplification product) and 46 urine samples that were presumably negative for CMV DNA.
- 52 Buccal swab samples that were presumably negative for CMV DNA.
- 10 amniotic fluid samples that were negative for CMV DNA (tested with a CE IVD real time amplification product) and 22 amniotic fluid samples that were presumably negative for CMV

DNA.

- 49 BAL / BA samples that were negative for CMV DNA (tested with a CE IVD real time amplification product).

The test, starting from 1000 µL of sample was performed on 57 plasma samples collected in EDTA presumably negative for CMV 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	CSF negative or presumably negative for CMV DNA	20	0	20
	Urine negative or presumably negative for CMV DNA	54	0	54
	Buccal swab presumably negative for CMV DNA	52	2	50
	Amniotic Fluid negative or presumably negative for CMV DNA	32	0	32
	BAL / BA negative for CMV DNA	49	0	49
1000 µL	Plasma collected in EDTA and presumably negative for CMV DNA	57	3	54

All samples, analysed starting from 200 µL of volume, were valid for analysis. The IC Ct cut-off value is set at 35 for all matrices.

Fifty (50) out of 52 buccal swab samples were confirmed negative for CMV - DNA, two sample was discrepant positive at low titer.
The diagnostic specificity of the assay in association to buccal swab was equal to 96%.

All amniotic fluid, urine, CSF and BAL / BA samples were confirmed negative for CMV – DNA.
The diagnostic specificity of the assay in association with amniotic fluid, urine and CSF was equal to 100%.
All samples analysed starting from 1000 µL, were valid for analysis.
Fifty-four (54) out of 57 were confirmed negative for CMV - DNA, three (3) samples were discrepant positive at low titer. The diagnostic specificity of the assay in this test was equal to 94.7%.

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

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 the DNA extraction from whole blood (cellular sample) by «**EXTRAblood**» kit, please, follow the instructions for use manual: start from **200 µL** of sample, elute the nucleic acids in **100 µL** of elution buffer.

Note: when you carry out the DNA extraction from whole blood with the **ELITe STAR** 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 400-650 µL, dependent on the tube class used, is always required for each sample. Add **10 µL** / **sample** of **CPE.** The **CPE** must be added to **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from whole blood 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**. 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** 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** to Proteinase-Carrier tube as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from plasma with the **ELITe GALAXY** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses 300 µL of sample and elutes the extract in 200 µL. Samples in primary tubes can be directly loaded on **«ELITe GALAXY»**. A minimum volume 400-650µL, 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's always requested a minimum dead volume of 100 µL. Prepare the solution containing AVE buffer and RNA carrier, according to the instruction manual of the extraction kit. Add **6 µL** of **CPE** to the solution for each requested sample. Load on the instrument, in the "internal control" slot, the tubes containing the solution, as indicated in the instruction for use manual of the kit; indicate the position where eluates will be dispensed and specify the elution volume of **85 µL**. For details on the extraction procedure follow indications in the instruction for use manual of the kit.

Cerebrospinal fluid

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

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

Note: when you carry out the DNA extraction from cerebrospinal fluid with the instrument **«NucliSENS®**

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

Urine

Urine samples for nucleic acid extraction must be collected in preservative-free containers according to laboratory guidelines, transported at room temperature (+18 / +25 °C) and stored at room temperature (+18 / + 25 °C) for a maximum of four hours, otherwise they must be stored at +2 / +8 °C for a maximum of three days. If possible, avoid freezing of first void urine samples. Freezing can cause precipitation of inhibitors and the loss of the DNA titre.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

Note: when you carry out the DNA extraction from urine samples with the instrument **«NucliSENS® easyMAG®»**, please follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip, load the strip on the instrument and run the extraction. After the 10 minute incubation, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica** to the strip content by the multichannel pipet using the program number 3 and proceed with the extraction. Elute the nucleic acids in **100 µL** of elution buffer.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: Buccal Swab, Amniotic Fluid, Bronchoalveolar Lavage (BAL) and Broncho Aspirate (BA), suspensions of leucocytes, suspensions of granulocytes.

Interfering substances

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

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

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

Amplification controls

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

For the negative control, use Molecular biology grade water (not provided with this kit).

For the positive control, use the **«CMV - ELITe Positive Control»** product or the **«CMV 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 "CMV Molecular Q Panel" (code CMVMQP01 by Qnostics Ltd, UK).

PROCEDURE

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

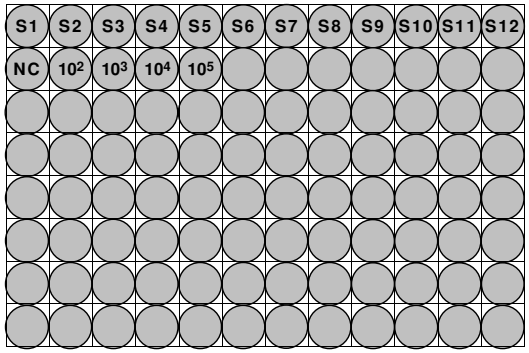
When **7300 Real-Time PCR System** instrument is used.

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 CMV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "CMV";
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "VIC"

(AP525 is analogous to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”;
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

Note: In order to determine the DNA titre in the starting sample, set up a series of reactions with the **Q - PCR Standards** (10^5 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: S1 - **S12**: Samples to be analysed; **NC**: Negative Control of amplification;
10^2: 10^2 standard gEq; **10^3**: 10^3 standard gEq; **10^4**: 10^4 standard gEq; **10^5**: 10^5 standard gEq.

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

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

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

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session and set “Run mode: Fast 7500”;

- set (Detector Manager) the "detector" for the CMV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “CMV”;
- 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 gEq, 10^4 gEq, 10^3 gEq, 10^2 gEq) 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 the dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**.

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

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

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

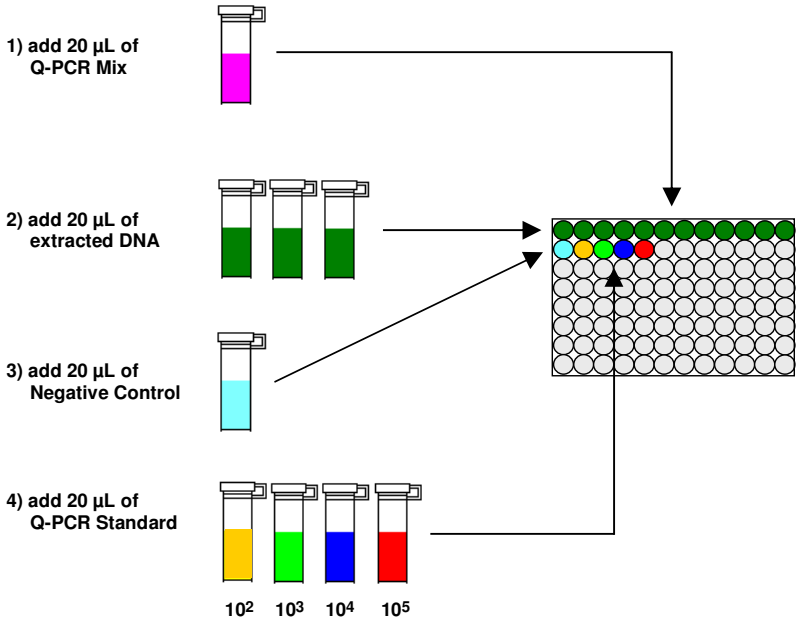
1. Accurately pipet **20 µL** of **CMV Q - PCR Mix** on the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

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 **DNA extract** from the first sample in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the sample by pipetting the **extracted DNA** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other samples of **extracted DNA**.
3. Accurately pipet, by placing into the reaction mixture, **20 µL** of **Molecular biology grade water** (not provided with this product) in the well of **Amplification microplate** of the negative control of amplification, as previously established in the **Work Sheet**. Mix well the negative control by pipetting the **Molecular biology grade water** three times into the reaction mixture. Avoid creating bubbles.
4. Accurately pipet, by placing into the reaction mixture, **20 µL** of **CMV 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 **CMV Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **CMV 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-CMV-EGSpA").

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

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



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

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.

Qualitative analysis of the results

The recorded values of the fluorescence emitted by the specific CMV probe (FAM detector "CMV") 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 CMV DNA, the FAM fluorescence of the CMV specific probe may begin to increase before the cycle 15. In this case the calculation range for the **Baseline** must be adapted from cycle 6 to the cycle in which the FAM fluorescence of the sample begins to increase, as detected by the instrument software (Results > Component).

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

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

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

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

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

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

If the result of the **Q - PCR Standard 10^5** amplification reaction is **Ct > 25** or **Ct Undetermined** or if the **Correlation coefficient** (**R2**) value does not fall within the limits of the acceptability range, the target DNA has not been correctly detected. This means that problems occurred during the amplification or the detection step (incorrect dispensation of the reaction mix or of the standards, degradation of the reaction mix or of the standards, incorrect setting of the standard position, incorrect setting of the thermal cycle), which may lead to incorrect results. The session is not valid and has to be repeated starting from the amplification step.

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

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

In the amplification reaction of each **sample**, the **Ct** value of CMV 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 genome Equivalents in the amplification reaction (see Performance Characteristics).

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	CMV DNA
detector FAM "CMV"	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 CMV and **Ct > 35** or **Ct Undetermined** for the Internal Control, it means that it was impossible to detect efficiently the DNA for the Internal Control. In this case problems have occurred during the amplification step (inefficient or absent amplification) or during the extraction step (degradation of 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 CMV and **Ct ≤ 35** for the Internal Control, it means that the CMV DNA is not detected in the DNA extracted from the sample; but it cannot be excluded that the CMV DNA has a lower titre than the detection limit of the product (see Performance Characteristics). In this case the result could be a false negative.

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

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

Quantitative analysis of the results

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

The CMV **Ct** values in the amplification reactions of each **sample** and the **Standard curve** (Results > Standard Curve) of the amplification session are used to calculate the **Quantity** of target DNA present in the amplification reactions of the samples.

CMV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTK015PLD

This product is able to quantify from about 1,000,000 to about 10 genome Equivalents per amplification reaction (see Performance Characteristics).

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

The results (**Quantity**) of each **sample** (Results > Report) are used to calculate the genome Equivalents **(gEq)** of CMV present in the sample used in the extraction (**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 whole blood samples collected in EDTA and «**EXTRAblood**» extraction kit are used and the result is required **in gEq / mL**, the formula becomes:

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

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

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

When whole blood and plasma samples collected in EDTA and **ELITe GALAXY** are used and the result is required **in gEq / mL**, the formula becomes:

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

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

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

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

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

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

Simplified formula for whole blood and «QIAsymphony® SP/AS»
Nc (gEq / mL) = 24 x Quantity

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

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

Results conversion to International Units

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

Simplified formula for whole blood and «EXTRAblood»
Fc = 0.76 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 19.0 x Quantity

When whole blood samples collected in EDTA and **ELITe STAR** are used and the result is required **in IU / mL**, the formula becomes:

Simplified formula for whole blood and ELITe STAR
Fc = 0.79 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 22.1 x Quantity

When plasma samples collected in EDTA and **ELITe STAR** are used and the result is required **in IU / mL**, the formula becomes:

Simplified formula for plasma and ELITe STAR
Fc = 1.10 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 30.8 x Quantity

When whole blood samples collected in EDTA and **ELITe GALAXY** are used and the result is required **in IU / mL**, the formula becomes:

Simplified formula for whole blood and ELITe GALAXY
Fc = 0.51 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 17.9 x Quantity

When plasma samples collected in EDTA and **ELITe GALAXY** are used and the result is required **in IU / mL**, the formula becomes:

Simplified formula for plasma and ELITe GALAXY
Fc = 0.27 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 9.5 x Quantity

CMV ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTK015PLD

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

Simplified formula for whole blood and «NucliSENS® easyMAG®»
Fc = 0.61 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 30.5 x Quantity

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

Simplified formula for whole blood and «QIAsymphony® SP/AS»
Fc = 0.46 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 11.0 x Quantity

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

Simplified formula for plasma and «QIAsymphony® SP/AS»
Fc = 0.87 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 10.4 x Quantity

Where **Fc** is the conversion factor calculated using the reference calibrated material approved by WHO "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification (NAT) Techniques", NIBSC code 09/162, United Kingdom (see Performance Characteristics paragraph).

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: detection limit

The analytical sensitivity of this assay, as limit of detection, allows detecting the presence of about 11 copies 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 «**EXTRAblood**» was verified with a panel of CMV dilutions within the limiting concentration. The panel was prepared using CMV DNA negative whole blood samples spiked with calibrated and certified reference material OptiQuant CMV DNA (AD169 strain, AcroMetrix Europe B.V., the Netherlands). The viral concentrations ranged from 1 gEq / mL to 3,160 gEq / mL. Each sample of the panel was tested in 24 replicates carrying out the whole analysis procedure, extraction with and amplification, by 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 «EXTRAblood» (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**279 gEq / mL**	198 gEq / mL	466 gEq / mL

Limit of Detection for whole blood samples and «EXTRAblood» (gEq / reaction)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**11.2 gEq / reaction**	7.9 gEq / reaction	18.6 gEq / reaction

The conversion from gEq / mL to gEq / reaction was calculated as shown at page 38.

The analytical sensitivity of this assay used in association to whole blood samples and **ELITe STAR** was verified with a panel of CMV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV 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 12 replicates carrying out the whole analysis procedure, automatic extraction with **ELITe STAR** and amplification, by 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	**263 IU / mL**	128 IU / mL	1,208 IU / 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	**332 gEq / mL**	162 gEq/ mL	1,529 gEq / mL

The analytical sensitivity as gEq/mL for whole blood samples and **ELITe STAR** is calculated by applying the specific conversion factor reported at page 38.

The analytical sensitivity of this assay used in association to plasma samples and **ELITe STAR** was verified with a panel of CMV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV 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 8 replicates carrying out the whole analysis procedure, automatic extraction with **ELITe STAR** and amplification, by 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	**222 IU / mL**	126 IU / mL	1,638 IU / 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	**201gEq / mL**	114 gEq/ mL	1,489 gEq / mL

The analytical sensitivity as gEq/mL for plasma samples and **ELITe STAR** is calculated by applying the specific conversion factor reported at page 38.

The analytical sensitivity of this assay used in association to whole blood samples and **ELITe GALAXY** was verified with a panel of CMV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV 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	**127 IU / mL**	75 IU / mL	435 IU / 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	**249 gEq / mL**	147 gEq / mL	853 gEq / mL

The analytical sensitivity as gEq / mL for whole blood samples and **ELITe GALAXY** is calculated by applying the specific conversion factor reported at page 38.

The analytical sensitivity of this assay used in association to plasma samples and **ELITe GALAXY** was verified with a panel of CMV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV 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	**140 IU / mL**	86 IU / mL	381 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	**519 gEq / mL**	319 gEq / mL	1,411 gEq / mL

The analytical sensitivity as gEq / mL for plasma samples and **ELITe GALAXY** is calculated by applying the specific conversion factor reported at page 38.

Analytical sensitivity: linear measuring range

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

The analytical sensitivity of this assay was determined using a panel of dilutions (1 $\log_{10}$ dilution steps) of a plasmid DNA containing the amplification product, whose initial concentration was measured by spectrophotometer. The dilutions from 2.5 x 10^7 genome Equivalents per reaction to 2.5 x 10^1 genome Equivalents per reaction were tested in 9 replicates carrying out the amplification by the ELITechGroup S.p.A. products. The analysis of the obtained data, performed by linear regression, demonstrated that the assay shows a linear response for all the dilutions (square correlation coefficient greater than 0.99).

The lower limit of the linear measuring range of this assay used in association to whole blood samples and «**EXTRAblood**» was set at about 13 gEq / reaction, because of 316 gEq / mL is the lowest concentration that gives 100% of positiveness in the study of the limit of detection. The lower limit of the linear measuring range is within 1 logarithm from the lowest concentration Q - PCR Standard amplification standard (10^2 gEq / 20 µL).

The upper limit of the linear measuring range of this assay used in association to whole blood samples and «**EXTRAblood**» was set at 10^6 gEq / reaction, within 1 logarithm from the highest concentration Q - PCR Standard amplification standard (10^5 gEq / 20 µL). The results are reported in the following table.

Linear measuring range for whole blood samples and «EXTRAblood»		
	lower limit	upper limit
IU / mL	**240**	**19,000,000**
gEq / mL	**316**	**25,000,000**
gEq / reaction	**12.6**	**1,000,000**

The conversion from gEq / mL to gEq / reaction and vice versa was calculated as shown at page 38. The conversion from gEq / mL to IU / mL and vice versa was calculated as shown at page 39.

The lower limit of the linear measuring range of this assay used in association to whole blood samples and **ELITe GALAXY** was set at about 10 gEq / reaction, because of 350 gEq / mL is the lowest concentration that gives 100% of positiveness in the study of the limit of detection. The lower limit of the linear measuring range is within 1 logarithm from the lowest concentration Q - PCR Standard amplification standard (10^2 gEq / 20 µL).

The upper limit of the linear measuring range of this assay used in association to whole blood samples and **ELITe GALAXY** was set at 10^6 gEq / reaction, within 1 logarithm from the highest concentration Q - PCR Standard amplification standard (10^5 gEq / 20 µL). The results are reported in the following table.

Linear measuring range for whole blood samples and ELITe GALAXY		
	lower limit	upper limit
IU / mL	**178**	**17,800,000**
gEq / mL	**350**	**35,000,000**
gEq / reaction	**10**	**1,000,000**

The conversion from gEq / mL to gEq / reaction and vice versa was calculated as shown at page 38. The conversion from gEq / mL to IU / mL and vice versa was calculated as shown at page 39.

Analytical sensitivity: Precision and Accuracy

The precision of the assay, as the variability of results obtained with several replicates of a sample tested within the same session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of Ct lower than 2%, within the range from 10^6 molecules to 10^1 molecules, in the 20 µL of DNA added to the amplification reaction.

The precision of the assay, as the variability of results obtained with several replicates of a sample tested within the same session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 21% 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.

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reagent for DNA Real Time amplification

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The accuracy of the assay, as the difference between the mean of results obtained with several replicates of a sample tested within the same session and the theoretical concentration of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 20% 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 calculated using data obtained for the study of the linear measuring range.

Analytical sensitivity: reproducibility with certified 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 panel of certified reference material.

The tests were carried out using as calibrated and certified reference material a panel of dilutions of CMV within the limit concentration (AD169 strain, QCMD 2009 Human Cytomegalovirus DNA EQA Panel, Qnostics Ltd, Scotland, UK). Each sample of the panel was tested in 2 replicates carrying out the whole analysis procedure, manual extraction with «**EXTRAblood**» and amplification with ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with certified reference material and «EXTRAblood»				
Sample	Consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} gEq / mL
CMV09-01	4.368	0.465	2 / 2	4.064
CMV09-02	2.995	0.400	2 / 2	2.984
CMV09-03	2.297	0.583	2 / 2	2.038
CMV09-04	5.407	0.442	2 / 2	5.026
CMV09-05	2.996	0.444	2 / 2	2.902
CMV09-06	3.493	0.421	2 / 2	3.231
CMV09-07	4.379	0.412	2 / 2	4.129
CMV09-08	negative	NA	0 / 2	Not detected
CMV09-09	6.374	0.457	2 / 2	5.943
CMV09-10	2.352	0.542	2 / 2	1.996
CMV09-11	2.407	0.513	2 / 2	2.105
CMV09-12	3.645	0.449	2 / 2	3.539

All samples were correctly detected. The quantitative results obtained are within the range defined by the commercial assay Consensus ± 1 SD as required.

Further tests were carried out using as calibrated reference material a panel of dilutions of CMV within the concentration limit (QCMD 2012 Human Cytomegalovirus DNA EQA Panel, Qnostics Ltd, Scotland, 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 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	Consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
CMV12-01	4.409	0.349	2/2	4.580
CMV12-02	3.925	0.335	2/2	4.111
CMV12-03	2.297	0.507	1/2	2.423
CMV12-04	2.021	0.617	1/2	2.320
CMV12-05	3.158	0.613	2/2	3.529
CMV12-06	3.448	0.361	2/2	3.594
CMV12-07	3.490	0.377	2/2	3.321
CMV12-08	Negative	NA	0/2	Not detected
CMV12-09	3.767	0.374	2/2	4.580
CMV12-10	2.826	0.456	1/2	4.111

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

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

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	Consensus Log_{10} virus conc.	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
CMV12-01	4,409	0,349	2/2	4.010
CMV12-02	3,925	0,335	2/2	3.484
CMV12-03	2,297	0,507	1/1	1.811
CMV12-04	2,021	0,617	2/2	1.647
CMV12-05	3,158	0,613	2/2	2.511
CMV12-06	3,448	0,361	2/2	3.106
CMV12-07	3,490	0,377	2/2	3.319
CMV12-08	Negative	NA	0/2	Not detected
CMV12-09	3,767	0,374	2/2	3.486
CMV12-10	2,826	0,456	2/2	2.593

One replicate of CMV12-03 was excluded from the analysis because of system failure during the initial extraction step. In the qualitative analysis, all samples were correctly detected. In the quantitative analysis, 8/9 positive samples were correctly quantified within 0.5 log difference from the expected titer. Samples CMV12-01 and CMV12-07 are paired samples. The difference between CMV12-01 (4.035 Log_{10}) and CMV12-07 (3.296 Log_{10}) resulted equal to 0.691 and falls within the expected interval. The CMV12-07 result was considered valid.

Analytical sensitivity: Conversion factor to International Units

To convert quantitative result from gEq / mL to International Units / mL, the conversion factor to be used with this assay and **whole blood samples** collected in EDTA was defined as:

0.76 International Units / gEq when **«EXTRAblood»** manual extraction kit is used;

0.79 International Units / gEq when **ELITe STAR** automatic extraction system is used;

0.51 International Units / gEq when **ELITe GALAXY** automatic extraction system is used;

0.61 International Units / gEq when «NucliSENS® easyMAG®» automatic extraction system is used;

0.46 International Units / gEq when «QIAsymphony® SP/AS» automatic extraction system is used;

To convert quantitative result from gEq / mL to International Units / mL, the conversion factor to be used with this assay and **plasma samples** collected in EDTA was defined as:

1.10 International Units / gEq when **ELITe STAR** automatic extraction system is used;

0.27 International Units / gEq when **ELITe GALAXY** automatic extraction system is used;

0.87 International Units / gEq when «QIAsymphony® SP/AS» automatic extraction system is used.

Data about each conversion factor are reported below.

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 Human Cytomegalovirus for Nucleic Acid Amplification Techniques", NIBSC code 09/162, 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 calculating a mean conversion factor (Fc) equal to 0.76 International Units (IU) per gEq of CMV detected.

The results are reported in the following table.

Conversion to International Units with whole blood and «EXTRAblood» (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
316,255	5.500	362,383	275,411	5.440
100,000	5.000	155,738	118,361	5.073
31,625	4.500	39,503	30,022	4.477
10,000	4.000	13,623	10,353	4.015

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

The analysis of the data obtained allows calculating a mean conversion factor (Fc) equal to 0.79 International Units (IU) per gEq of CMV detected with whole blood samples.

The results are reported in the following table.

Conversion to International Units with whole blood and ELITe STAR (Fc = 0.79 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	566,090	464,398	5.620
100,000	5.000	135,119	106.744	4.997
31,625	4.500	42,655	33,698	4.488
10,000	4.000	14,486	11,444	4.014
3,162	3.500	3,717	2,936	3.401

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 calculating a mean conversion factor (Fc) equal to 0.51 International Units (IU) per gEq of CMV detected with whole blood samples.

The results are reported in the following table.

Conversion to International Units with whole blood and ELITe GALAXY (Fc = 0.51 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	473,265	240,507	5.370
100,000	5.000	217,626	110,595	5.036
31,625	4.500	55,656	28,284	4.438
10,000	4.000	24,229	12,313	4.076
3,162	3.500	7,809	3,968	3.575

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 0.61 International Units (IU) per gEq of CMV detected.

The results are reported in the following table.

Conversion to International Units with whole blood and «NucliSENS® easyMAG®» (Fc = 0.61 UI / 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	564,835	344,549	5.537
100,000	5.000	178,704	109,009	5.037
31,625	4.500	51,454	31,387	4.497
10,000	4.000	14,141	8,626	3.936

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 0.46 International Units (IU) per gEq of CMV detected, using whole blood samples.

The results are reported in the following table.

Conversion to International Units with whole blood and «QIAsymphony® SP/AS» (Fc = 0.46 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	599,940	275,972	5.435
100,000	5.000	222,073	102,153	5.004
31,625	4.500	70,712	32,527	4.497
10,000	4.000	24,326	11,190	4.038

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 Human Cytomegalovirus for Nucleic Acid Amplification Techniques", NIBSC code 09/162, United Kingdom) in plasma collected in EDTA.

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

The analysis of the data obtained allows calculating a mean conversion factor (Fc) equal to 1.10 International Units (IU) per gEq of CMV detected with plasma samples.

The results are reported in the following table.

Conversion to International Units with plasma and ELITe STAR (Fc = 1.10 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	282,851	311,136	5.481
100,000	5.000	107,043	117,747	5.048
31,625	4.500	30,868	33,955	4.512
10,000	4.000	8,632	9,495	3.972
3,162	3.500	2,814	3,096	3.468

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 calculating a mean conversion factor (Fc) equal to 0.27 International Units (IU) per gEq of CMV detected with plasma samples.

The results are reported in the following table.

Conversion to International Units with plasma and ELITe GALAXY (Fc = 0.27 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
316,228	5.500	1,095,881	301,020	5.413
100,000	5.000	460,141	126,393	5.033
31,623	4.500	117,258	32,209	4.448
10,000	4.000	43,980	12,081	4.053
3,162	3.500	13,713	3,767	3.546

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 0.87 International Units (IU) per gEq of CMV detected, using plasma samples.

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The results are reported in the following table.

Conversion to International Units with plasma and «QIAsymphony® SP/AS» (Fc = 0.87 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	330,340	287,396	5.458
100,000	5.000	101,683	88,464	4.947
31,625	4.500	40,963	35,638	4.551
10,000	4.000	13,148	11,438	4.058

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

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

The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probe in the alignment of the sequences available in the database for the exon 4 of CMV MIEA gene, AD169 and Merlin strains amongst the others, showed conservation and absence of significant mutations.

The diagnostic sensitivity of the assay, as detection and quantification efficiency on different genotypes / subtypes, was evaluated using a plasmid construct containing the amplified region of CMV, strain Merlin.

The detection and quantification efficiency was evaluated using as reference material a plasmid construct containing the amplified region of CMV, strain Merlin (GENEART A.G., Germany). The plasmid DNA was diluted to the concentration of 100,000, 10,000, 1,000, 100 and 10 copies per reaction. Each sample was tested carrying out the amplification by the ELITechGroup S.p.A. products.

The results are reported in the following table.

Detection and quantification efficiency on CMV strain Merlin			
Sample	Theoretical concentration copies / reaction	Positives / Replicates	Mean results copies / reaction
plasmid pMerlin 10^5	100,000	3 / 3	93,699
plasmid pMerlin 10^4	10,000	3 / 3	8,815
plasmid pMerlin 10^3	1,000	3 / 3	898
plasmid pMerlin 10^2	100	3 / 3	100
plasmid pMerlin 10^1	10	9 / 9	11

The plasmid pMerlin was correctly detected and quantified till at the concentration of 10 copies per reaction.

Diagnostic sensitivity: confirmation of positive samples

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

The diagnostic sensitivity was evaluated using as reference material 54 whole blood samples collected in EDTA that were positive for CMV DNA (tested with a CE IVD real time amplification product). Each sample was tested carrying out the whole analysis procedure, manual extraction with «**EXTRAblood**» and amplification, by the ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
CMV DNA positive whole blood collected in EDTA	54	53	0

A sample resulted "non valid" in two independent sessions of analysis. The result "non valid" was possibly caused by a non identified inhibitor in the sample. This sample was not included in the diagnostic sensitivity calculation.

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

The diagnostic sensitivity was evaluated using 60 whole blood samples collected in EDTA that were positive for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure: automatic 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
Whole blood collected in EDTA positive for CMV DNA	60	57	0

Three samples resulted "non valid" in two independent sessions of analysis.
The diagnostic sensitivity of the assay in this test was equal to 100%.

The diagnostic sensitivity was evaluated using 68 plasma samples collected in EDTA that were positive for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure: automatic 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 CMV DNA	68	66	2

Two samples reported a negative result with ELITechGroup S.p.A. products. This discordance may be explained being the CMV titre of the sample close or lower than the detection limit of method used (280 gEq/mL).
The diagnostic sensitivity of the assay in this test was equal to 97.1%.

The diagnostic sensitivity was evaluated using 60 whole blood samples collected in EDTA that were positive for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure, automatic extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.
The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA positive for CMV DNA	60	59	1

One sample reported a negative result with ELITechGroup S.p.A. products. This discordance may be explained with a degradation of sample.
The diagnostic sensitivity of the assay in this test was equal to 98.3%.

The diagnostic sensitivity was evaluated using 51 plasma samples collected in EDTA that were positive for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure, automatic 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 CMV DNA	51	47	4

Four samples reported a negative result with ELITechGroup S.p.A. products. The titres of the discrepant samples (respectively <350 gEq/mL, <350 gEq/mL, 961 gEq/mL and 534 gEq/mL) close than the detection limit of method used.
In this test the diagnostic sensitivity was equal to 92.2 %.

The diagnostic sensitivity was evaluated using as reference material 50 whole blood samples collected in EDTA from normal donors, that were presumably negative for CMV DNA, spiked with a sample of certified calibrated reference material (QCMD 2009 Human Cytomegalovirus DNA EQA Panel, Qnostics Ltd, , UK) to a titre of 1500 copies / mL. Each sample was tested carrying out the whole analysis procedure: extraction with «NucliSENS® easyMAG®» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked with CMV DNA	50	50	0

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

The diagnostic sensitivity was evaluated using as reference material 60 whole blood samples collected in EDTA from normal donors, that were presumably negative for CMV DNA, spiked with a sample of certified calibrated reference material (QCMD 2009 Human Cytomegalovirus DNA EQA Panel, Qnostics Ltd, UK) to a titre of 700 copies / mL. Each sample was tested carrying out the whole analysis procedure: extraction with «QIAsymphony® SP/AS» automatic extraction system and amplification with ELITechGroup S.p.A. products.
The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked with CMV DNA	60	60	0

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

The diagnostic sensitivity was evaluated using as reference material 60 plasma samples collected in EDTA from normal donors, that were presumably negative for CMV DNA, spiked with a sample of certified calibrated reference material (QCMD 2009 Human Cytomegalovirus DNA EQA Panel, Qnostics Ltd, UK) to a titre of 360 copies / mL. Each sample was tested carrying out the whole analysis procedure: extraction with «QIAsymphony® SP/AS» automatic extraction system and amplification with ELITechGroup S.p.A. products.
The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA spiked with CMV DNA	60	60	0

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

The diagnostic sensitivity was evaluated using as reference material 60 cerebrospinal fluid samples, that were negative for CMV DNA, tested positive with a sample of certified calibrated reference material (QCMD 2009 Human Cytomegalovirus DNA EQA Panel, Qnostics Ltd, UK) that were spiked to a titre of 300 copies / mL. Each sample was tested carrying out the whole analysis procedure: extraction with «NucliSENS® easyMAG®» automatic extraction system and amplification with ELITechGroup S.p.A. products.
The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid spiked with CMV DNA	60	60	0

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

The diagnostic sensitivity was evaluated using as reference material 52 clinical samples of urine positive for CMV DNA (tested with a CE IVD real time amplification product). Each sample was used to carry out whole analysis procedure: extraction with «NucliSENS® easyMAG®» automatic extraction system and amplification with ELITechGroup S.p.A. products.
The results are summed up in the following table.

Samples	N	positive	negative
Urine positive for CMV DNA	52	52	0

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

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

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

The analysis of the alignment of the sequences of the primers and of the fluorescent probe with the sequences available in databases for organisms other than CMV, including the HHV6 complete genome, the human herpetic virus that is the most similar to CMV, showed their specificity and the absence of significant homology.

The analytical specificity of the assay, as absence of cross-reactivity with other potential interfering markers, was verified testing some CMV DNA negative clinical samples that were HHV6, EBV or VZV DNA positive. The samples were confirmed negative.

The analytical specificity was verified using as reference material 16 whole blood samples collected in EDTA that were CMV DNA negative and HHV6, EBV or VZV DNA positive (tested with CE IVD real time amplification products). Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are reported in the following table.

Samples	N	positive	negative
HHV6 DNA positive whole blood collected in EDTA	8	0	8
EBV DNA positive whole blood collected in EDTA	7	0	7
VZV DNA positive whole blood collected in EDTA	1	0	1

Diagnostic specificity: confirmation of negative samples

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

The diagnostic specificity was evaluated using as reference material 56 whole blood collected in EDTA samples that were negative for CMV DNA (tested with a CE IVD real time amplification product). Each sample was tested carrying out the whole analysis procedure, manual extraction with «**EXTRAblood**» and amplification, by the ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
CMV DNA negative whole blood collected in EDTA	56	0	56

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

The diagnostic specificity was evaluated using 70 whole blood samples collected in EDTA that were negative for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure: automatic 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
Whole blood collected in EDTA negative for CMV DNA	70	0	63

Seven samples resulted "not valid" in two independent sessions of analysis.

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

The diagnostic specificity was evaluated using 61 plasma samples collected in EDTA that were negative for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure: automatic 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 negative for CMV DNA	61	0	61

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

The diagnostic specificity was evaluated using 66 whole blood samples collected in EDTA that were negative for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure, automatic extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA negative for CMV DNA	66	0	65

A sample resulted "not valid", possibly caused by a not identified inhibitor in the sample. This sample was not included in the diagnostic sensitivity calculation.

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

The diagnostic specificity was evaluated using 64 plasma samples collected in EDTA that were negative for CMV DNA (tested with a real time amplification CE IVD product). Each sample was tested carrying out the whole analysis procedure, automatic 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 CMV DNA	64	0	64

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

The diagnostic specificity was evaluated using as reference material 50 whole blood samples collected in EDTA from normal donors that were presumably negative for CMV DNA. Each sample was tested carrying out the whole analysis procedure: extraction with «NucliSENS® easyMAG®» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA negative for CMV	50	0	50

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

The diagnostic specificity was evaluated using as reference material 60 whole blood samples collected in EDTA from normal donors that were presumably negative for CMV DNA. Each sample was tested carrying out the whole analysis procedure: extraction with «QIAsymphony® SP/AS» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA negative for CMV	60	1	59

One CMV negative blood sample reported a CMV positive result at a very low viral titre (approximately 2 gEq / reaction), with ELITechGroup S.p.A. products. This discordance can be explained by a latent CMV infection, since the virus is widespread in the population.

The diagnostic specificity of the assay in this test was equal to 98.3%.

The diagnostic specificity was evaluated using as reference material 60 plasma samples collected in EDTA from normal donors that were presumably negative for CMV DNA. Each sample was tested carrying out the whole analysis procedure: extraction with «QIAsymphony® SP/AS» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA negative for CMV	60	1	59

One CMV negative plasma sample reported a CMV positive result at a very low viral titre (approximately 2 gEq / reaction), with ELITechGroup S.p.A. products. This discordance can be explained by a latent CMV infection, since the virus is widespread in the population.

The diagnostic specificity of the assay in this test was equal to 98.3%.

The diagnostic specificity was evaluated using as reference material 60 cerebrospinal fluid samples that were negative for CMV DNA (tested with a CE IVD real time amplification product). Each sample was tested carrying out the whole analysis procedure: extraction with «NucliSENS® easyMAG®» automatic extraction system and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Cerebrospinal fluid negative for CMV	60	0	60

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

The diagnostic specificity was evaluated using as the reference material 56 clinical samples of urine negative for CMV DNA (tested with a CE IVD real time amplification product. Each sample was used to carry out the entire analysis procedure, automatic nucleic acid extraction by «NucliSENS® easyMAG®» and amplification with products by ELITechGroup S.p.A. The results are summed up in the following table.

Samples	N	positive	negative
Urine negative for CMV DNA	56	1	55

One CMV negative urine sample reported a CMV positive result with ELITechGroup S.p.A. products. This discordance can be explained by the very low viral titre (4 gEq / reaction), probably lower than the detection limit of the reference method. The diagnostic specificity was equal to 98.2%.

Robustness: absence of cross-contamination

The assay robustness, as absence of cross-contamination, was verified analyzing the results of five sessions in which CMV DNA negative samples were alternated with CMV DNA spiked samples. None of the CMV DNA negative samples resulted positive.

The absence of cross-contamination was verified using a CMV DNA negative whole blood sample spiked with calibrated and certified reference material OptiQuant CMV DNA (AD169 strain, AcroMetrix Europe B.V., the Netherlands) to a viral load of 8,300 gEq / mL and a CMV DNA negative whole blood sample. Five series of 12 samples, alternating a spiked sample with a negative sample, were tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are reported in the following table.

Samples	N	positive	negative
CMV DNA spiked whole blood collected in EDTA	30	30	0
CMV DNA negative whole blood collected in EDTA	30	0	30

Robustness: whole system failure rate

The assay robustness, as the whole system failure rate leading to false negative results, was verified analysing a panel of CMV DNA spiked at low titre samples and resulted lower than 1.7%.

The whole system failure rate was verified using a panel of CMV DNA negative whole blood samples spiked with calibrated and certified reference material OptiQuant CMV DNA (AD169 strain, AcroMetrix Europe B.V., the Netherlands) to a viral load of 900 gEq / mL. Each sample of the panel was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are reported in the following table.

Samples	N	positive	negative
CMV DNA spiked whole blood collected in EDTA	60	60	0

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.

Urine

Urine samples for nucleic acid extraction must be collected in preservative-free containers according to laboratory guidelines, transported at room temperature (+18 / +25 °C) and stored at room temperature (+18 / + 25 °C) for a maximum of four hours, otherwise they must be stored at +2 / +8 °C for a maximum of three days. If possible, avoid freezing of first void urine samples. Freezing can cause precipitation of inhibitors and the loss of the DNA titre.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

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

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: cerebrospinal fluid (CSF), Buccal Swab, Amniotic Fluid, Bronchoalveolar Lavage (BAL) and Broncho Aspirate (BA), suspensions of leucocytes, suspensions of granulocytes.

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 **«CMV - ELITe Positive Control»** product or alternatively **«CMV - ELITe Positive Control RF»** product or the **«CMV ELITe Standard»** product.

Quality controls

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

PROCEDURE

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

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

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

Thermal Cycle		
Stage	**Temperatures**	**Periods**
Decontamination	50°C	2 mins.
Initial denaturation	94°C	2 mins.
Amplification and detection (45 cycles)	94°C	10 sec.
	60°C (fluorescence acquisition)	30 sec.
	72°C	20 sec.
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 CMV sensor with "channel FAM 465-510" and "detector" for the IC internal control sensor with "channel VIC 540-580";

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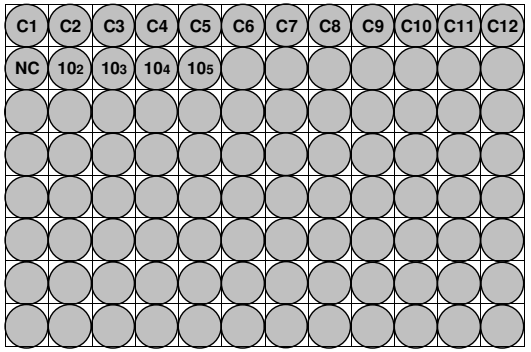
Fill in the **Work Plan** attached at the end of this User Manual, transcribing this information or printing the microplate's layout. This **Work Plan** must be followed carefully when transferring the reaction mixture and samples into the wells.

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 **CMV Q - PCR Mix** required for the session, remembering that the contents of each tube is enough to perform **25 reactions**. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve and thaw the test tubes containing **CMV – Positive Control** or alternatively **CMV - ELITe Positive Control RF** or **CMV 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 **CMV 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:

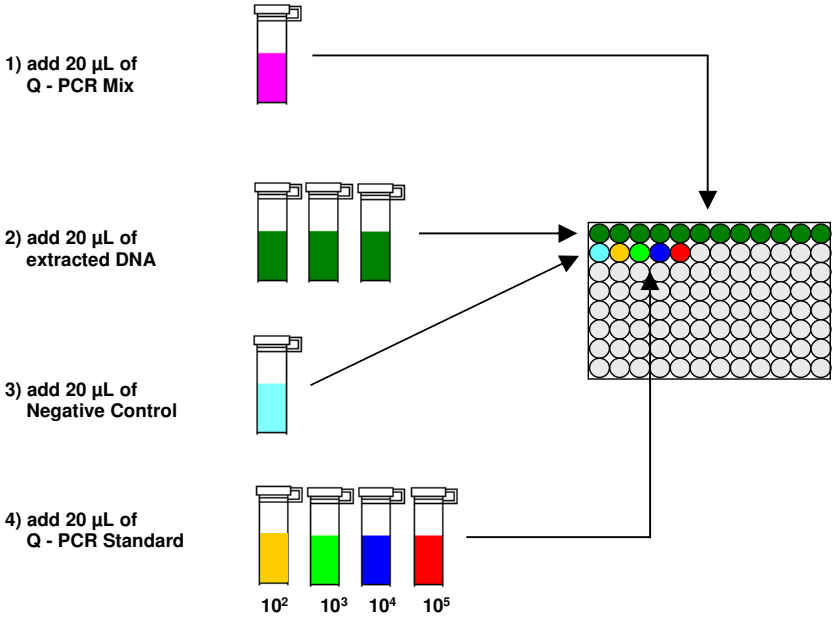
   - When a **qualitative** result is required (detection of CMV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **CMV - Positive Control** or alternatively **CMV - ELITe Positive Control RF** in the corresponding well of **Amplification microplate**, as previously established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

   - When a **quantitative** result is required (quantification of CMV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **CMV 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 **CMV Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **CMV 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-CMV-EGSpA").

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

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



Qualitative results analysis

The emitted fluorescence values recorded by the CMV 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 and Urine** Samples:

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

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

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

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

Standard Curve "CMV" detector	Acceptability range	Amplification / Detection
Correlation Coefficient (R2)	0.99 ≤ R2 ≤ 1.0	CORRECT

If the result of the amplification reaction for the **Q - PCR Standard 10^5** is **Ct > 25** or **Ct Undetermined** or if the value of the **Correlation Coefficient** (**R2**) is not within the limits, 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.

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

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

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

If the result of a sample's amplification reaction is **Ct Undetermined** for CMV and **Ct ≤ 35** for the Internal Control, the CMV DNA was not detected in the DNA extracted from the sample but it cannot be excluded that the CMV 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 CMV 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 CMV 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 CMV 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.

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

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

The results (**Quantity**) relating to each **sample** (Analysis window) are used to calculate the Equivalent Genomes (**gEq**) of CMV 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 gEq 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 gEq / mL**, the formula becomes:

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

Results conversion to International Units

When whole blood samples collected in EDTA and **MagNA Pure 24** are used and the result is required **in IU / mL**, the formula becomes:

Simplified formula for whole blood and MagNA Pure 24
Fc = 0.5 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 12.5 x Quantity

When plasma samples collected in EDTA and **MagNA Pure 24** are used and the result is required **in IU / mL**, the formula becomes:

Simplified formula for plasma and MagNA Pure 24
Fc = 0.4 IU / gEq **Nc (IU / mL) = Nc (gEq / mL) x Fc**
Nc (IU / mL) = 10 x Quantity

When urine samples and **MagNA Pure 24** are used and the result is required **in IU / mL**, the formula becomes:

Simplified formula for urine and MagNA Pure 24
Fc = 1.1 IU / gEq
Nc (IU / mL) = Nc (gEq / mL) x Fc
Nc (IU / mL) = 27.5 x Quantity

Where **Fc** is the conversion factor calculated using the reference calibrated material approved by WHO "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification (NAT) Techniques", NIBSC code 09/162, United Kingdom (see Performance Characteristics paragraph).

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 27 replicates to carry out amplification using ELITechGroup S.p.A. products.

The final results are summarized in the following table.

Samples	**N**	**positives**	**negatives**
10 copies of plasmid DNA + 150,000 copies of Beta-globin	27	26	1

The analytical sensitivity of this assay used in association to whole blood, plasma and urine and **MagNA Pure 24** was verified with a panel of CMV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV 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 the extraction using the automatic 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 for each matrix are reported in the following tables.

Limit of Detection with MagNA Pure 24 (IU / mL)			
Matrix	95% positivity	95% confidence range	
		lower limit	upper limit
whole bood	**135 IU / mL**	84 IU / mL	354 IU / mL
plasma	**88 IU / mL**	54 IU / mL	279 IU / mL
urine	**296 IU / mL**	174 IU / mL	851 IU / mL

The analytical sensitivity as gEq / mL for each matrix is calculated by applying the specific conversion factor reported at page 66.

The analytical sensitivity as gEq / mL is reported below.

Limit of Detection with MagNA Pure 24 (gEq / mL)			
Matrix	95% positivity	95% confidence range	
		lower limit	upper limit
whole bood	**270 gEq / mL**	168 gEq / mL	708 gEq / mL
plasma	**220 gEq / mL**	108 gEq / mL	698 gEq / mL
urine	**269 gEq / mL**	158 gEq / mL	774 gEq / 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 Equivalent Genomes 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 gEq / reaction within one logarithm from the lowest concentration of Q - PCR Standard amplification standard (10^2 gEq / 20 µL).

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

The results are shown in the following table.

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

Conversions from gEq / mL to gEq / reaction and vice versa were calculated as shown on page 60.

The linearity of this assay used in association with different matrices and **MagNA Pure 24** was verified with a panel of CMV dilutions. The panel was prepared by diluting the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in CMV DNA - negative matrix. The panel consisted of five dilution points (1 log10 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 gEq / mL for each matrix is calculated by applying the specific conversion factor reported at page 66.

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**
gEq / mL	**356**	**2,000,000**

Linear measuring range for plasma samples and MagNA Pure 24		
Unit of measure	Lower limit	Upper limit
IU / mL	**100**	**1,000,000**
gEq / mL	**250**	**2,500,000**

Linear measuring range for urine samples and MagNA Pure 24		
Unit of measure	Lower limit	Upper limit
IU / mL	**1000**	**1,000,000**
gEq / mL	**909**	**909,091**

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 maximum Variation Coefficient percentage (CV%) of the values of Ct lower than 1.36% 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 (CV%) of the measured quantities of around 12.5 % 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 2.2% 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 was evaluated using as reference material the calibrated panel «AcroMetrix® CMV_{tc} Panel» (Acrometrix, Life Technologies, US). Each sample of the panel was tested in 2 replicates carrying out the extraction using the automatic extraction 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** 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 IU / mL	Positive / Replicates	Mean results Log IU / mL
CMV DNA 3E6	3,000,000	6.477	2/2	6.299
CMV DNA 3E5	300,000	5.477	2/2	5.280
CMV DNA 3E4	30,000	4.477	2/2	4.298
CMV DNA 3E3	3,000	3.477	2/2	3.364
CMV DNA 3E2	300	2.477	2/2	2.262

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

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Conversion factor to International Units

The conversion factor to be used with this assay to transform the quantitative result from gEq / mL into International Units / mL was determined using a panel of calibrated reference material approved by the WHO ("1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code 09/162, United Kingdom) in the different negative matrices for CMV 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 **0.5** International Units (IU) per gEq of CMV detected with **whole blood** samples.

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

Conversion to International Units with whole blood and «MagNA Pure» (Fc = 0.5 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
111,055	5.046	197,188	98,594	4.991
34,903	4.543	73,891	36,945	4.556
10,970	4.040	23,428	11,714	4.050
3,448	3.538	8,863	4,431	3.605
1,084	3.035	2,963	1,481	3.136
341	2.532	995	497	2.638

The analysis of the data obtained allowed to calculate a mean conversion factor (Fc) equal to **0.4** International Units (IU) per gEq of CMV detected with **plasma** samples.

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

Conversion to International Units with plasma and «MagNA Pure» (Fc = 0.4 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
111,055	5.046	235,469	94,188	4.969
34,903	4.543	89,375	35,750	4.548
10,970	4.040	25,950	10,380	4.008
3,448	3.538	9,683	3,873	3.576
1,084	3.035	3,189	1,276	3.086
341	2.532	910	364	2.526

The analysis of the data obtained allowed to calculate a mean conversion factor (Fc) equal to **1.1** International Units (IU) per gEq of CMV detected with **urine** samples.

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

Conversion to International Units with urine and «MagNA Pure» (Fc = 1.1 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
316,228	5.500	217,719	242,110	5.379
100,000	5.000	91,719	100,891	4.995
31,623	4.500	33,484	36,833	4.557
10,000	4.000	10,550	11,605	4.053
3,162	3.500	3,434	3,777	3.565
1,000	3.000	1,050	1,155	3.054

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity was evaluated using as reference material 51 samples of whole blood collected in EDTA positive for CMV DNA (tested using a CE IVD real-time amplification product), 63 samples of plasma collected in EDTA positive for CMV DNA (tested using a CE IVD real-time amplification product), 6 samples of urine positive for CMV DNA (tested using a CE IVD real-time amplification product) and 45 samples of urine negative for CMV DNA samples which were spiked for CMV DNA adding "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC, United Kingdom, code 09/162).

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 positive for CMV DNA	51	51	0
Plasma collected in EDTA positive for CMV DNA	63	62	1
Urine positive for CMV DNA	6	6	0
Urine spiked for CMV DNA	45	44	1

All samples were valid at first test.

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

Sixty-two (62) samples out of 63 plasma samples were confirmed positive for CMV DNA, while one sample showed a negative discrepant result. The diagnostic sensitivity of the assay associated to plasma samples was 98%

All urine clinical samples were confirmed positive for CMV DNA.

One urine spiked sample showed a negative discrepant result using ELITechGroup S.p.A products. An explanation for this discrepancy can be a preparation error by the operator.

The diagnostic sensitivity of the assay associated to urine samples was 98%.

The total diagnostic sensitivity of the assay was 98.8%.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity was evaluated using as reference material 53 samples of whole blood collected in EDTA presumably negative for CMV DNA, 50 samples of plasma collected in EDTA presumably negative for CMV DNA, 49 samples of urine presumably negative for CMV 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 CMV DNA	53	1	52
Plasma collected in EDTA presumably negative for CMV DNA	50	2	48
Urine presumably negative for CMV DNA	49	0	49

All whole blood samples were valid at first test and fifty-two (52) out of 53 whole blood samples were confirmed negative for CMV DNA, while one sample showed a positive discrepant result. The diagnostic specificity of the assay associated to whole blood samples was 98%.

Forty-nine (49) out of 50 plasma samples were valid at first test, an invalid sample showed a negative result after re-amplification. Forty-eight (48) out of 50 plasma samples were confirmed negative for CMV DNA, while two samples showed positive discrepant results. The diagnostic specificity of the assay associated to plasma samples was 96%.

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

The total diagnostic specificity of the assay was 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 "CMV ELITe MGB®Kit", FTP RTK015PLD.

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REFERENCES

T. E. Fenner et al. (1991) *J Clin Microbiology* 29: 2621 - 2622
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30

PROCEDURE LIMITATIONS

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

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

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

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

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

Use this product only with the validated instruments and associated clinical samples indicated in the section “Samples and Controls”.

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

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

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

When amplification session is manually setup, it is necessary to have separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products to prevent false positive results.

When amplification session is manually setup, 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 CMV DNA is not detected in the DNA extracted from the sample; but it cannot be excluded that the CMV 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 CMV 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 prenatal or emergency diagnosis, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

TROUBLESHOOTING

Target DNA not detected in the Positive Control or Q - PCR Standard reactions or invalid correlation coefficient of the Standard curve	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Take care when dispensing reagents into the microplate wells and comply with the work sheet. Check the volumes of reaction mixture dispensed. Check the volumes of positive control or standard dispensed.
Incorrect session setup on ELITe InGenius and ELITe BeGenius	Check the position of reaction mixture, positive control or standards. Check the volumes of reaction mixture, positive control or standards.
Probe degradation.	Use a new aliquot of reaction mixture.
Positive control or standard degradation.	Use a new aliquot of positive control or standard.
Instrument setting error.	Check the position settings for the 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 control 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 control 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 background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Take care, by pipetting three times, when mixing samples, negative control and standards into the reaction mixture. Avoid creating bubbles.
Baseline setting error.	Set the baseline calculation range within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g. from cycle 6 to cycle 15. Use the automatic baseline calculation by setting the "Auto Baseline" option.

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

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.

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

SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch code.


Use by (last day of month).

IVD *in vitro* diagnostic medical device.

CE 0344 Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device. Certification released by DEKRA Certification B.V., the Netherland.


Contains sufficient for "N" tests.


Attention, consult instructions for use.

CONT Contents.


Keep away from sunlight.


Manufacturer.

CMV ELITe MGB® Kit
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REF **RTK015PLD**

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. Patents Nos. 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800, 9,169,256 and EP patent numbers, 0819133,1068358, 1144429, 1232157, 1261616 1430147, 1781675, 1789587, 1975256, 2714939, as well as applications that are currently pending.

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

CMV ELITe MGB® kit used with Genius series platforms

Ref: RTK015PLD

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 «**CMV ELITe MGB® Kit**» product is a **qualitative** and **quantitative** nucleic acids amplification assay for the detection and quantification of the DNA of Human Cytomegalovirus (CMV) in DNA samples extracted from whole blood collected in EDTA, plasma collected in EDTA, cerebrospinal fluid (CSF), urine, buccal swab, amniotic fluid and bronchoalveolar lavage (BAL) / bronchial aspirate (BA).

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

The assay is CE-IVD validated in combination with **Whole Blood EDTA and Plasma EDTA and** the instruments **ELITe InGenius** and **ELITe BeGenius.**

The assay is CE-IVD validated in combination with **Cerebrospinal Fluid, Urine, Buccal swab, Amniotic fluid, BAL, BA** and the instrument **ELITe InGenius.**

B. Amplified sequence

	Gene	Fluorophore
Target	**CMV MIEA gene (exon 4 region)**	**FAM**
Internal Control	**Human beta Globin gene**	**AP525**

C. Validated matrix

Whole Blood EDTA, Plasma EDTA, Cerebrospinal Fluid, Urine, Buccal swab, Amniotic fluid, BAL, BA

D. Kit component

CMV Q-PCR Mix
4 tubes of 540 µL

› 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

› **CMV - ELITe Positive Control:** CTR015PLD
› **CMV ELITe Standard:** STD015PLD
› **ELITe InGenius Waste Box:** F2102-000
› **300 µL Filter Tips Axygen :** TF-350-L-R-S (for INT030)
› **1000 µL Filter Tips Tecan :** 30180118 (for INT040)

F. ELITe InGenius protocol

› Sample volume	200 µL
› CPE Internal Control volume	10 µL
› Total eluate volume	100 µL
› PCR eluate input volume	20 µL
› CMV Q-PCR Mix volume	20 µL
› Unit of quantitative result	International Unit: IU/mL genome equivalent: gEq/mL (equivalent to copies/mL)
› Frequency of controls	15 days
› Frequency of calibration	60 days

G. ELITe InGenius/ELITe BeGenius Performances

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Whole Blood	**109 IU/mL – 156 gEq/mL**	**100%** 60/60*	**93%** 55/59*
Plasma	**88 IU/mL – 293gEq/mL**	**100%** 54/54*	**98%** 57/58*

**confirmed samples/ tested samples*

Matrix	Linearity (gEq/mL)	Linearity (IU/mL)	CF gEq/mL to IU/mL
Whole Blood	**254 – 1.4x10^8**	**178 – 1 x10^8**	**0.7**
Plasma	**293 – 3.3x10^8**	**88 – 1 x10^8**	**0.3**

H. ELITe InGenius Performances

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Cerebrospinal fluid	**58 IU/mL – 193 gEq/mL**	**100%** 20/20*	**100%** 20/20*
Urine	**151 IU/mL – 216 gEq/mL**	**100%** 31/31*	**100%** 54/54*
Buccal swab	**44 IU/mL – 220 gEq/mL**	**100%** 50/50*	**96%** 50/52*
Amniotic fluid	**57 IU/mL – 285 gEq/mL**	**100%** 31/31*	**100%** 32/32*
BAL / BA	**97 IU/mL – 485 gEq/mL**	**100%** 49/49*	**100%** 49/49*

Matrix	Linearity (gEq/mL)	Linearity (IU/mL)	CF gEq/mL to IU/mL
Cerebrospinal fluid	**335 – 5x10^7**	**101 – 1,5 x10^7**	**0.3**
Urine	**451 – 5x10^7**	**316 – 3,5 x10^7**	**0.7**
Buccal swab	**500 – 5x10^7**	**100 – 1,0 x10^7**	**0.2**
Amniotic fluid	**500 – 5x10^7**	**100 – 1,0 x10^7**	**0.2**
BAL / BA	**890 – 5x10^7**	**178 – 1,0 x10^7**	**0.2**

H. Reference Material

Panel name	Provider	Qualitative results	Quantitative results
Molecular Q Panel: CMVMQP01	Qnostics	Concordance 100% (4/4)*	Titre as expected value ± 0.5 log
Acrometrix: CMVDNA3E	Thermo-Fisher	Concordance 100% (5/5)*	Titre as expected value ± 0.5 log
QCMD 2014: CMVDNA14	Qnostics	Concordance 100% (10/10)*	Titre as expected value ± 1 log**

*confirmed *samples/ tested samples* ****within the range of quantification***

I. ELITe InGenius 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

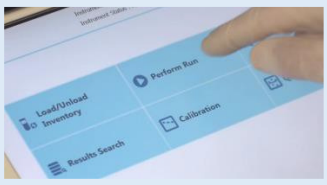
1. Switch on ELITe InGenius
Identification with username and password
Select the mode "Closed"

2. Verify calibrators: CMV Q-PCR standard in the "Calibration menu"
Verify controls: CMV pos. and neg. controls in the "Control menu"
NB: Both have been run, approved and not expired

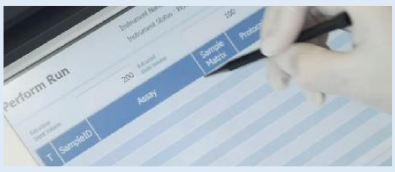
3. Thaw the CMV Q- PCR-Mix and the CPE Internal Control tubes
Vortex gently
Spin down 5 sec

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"

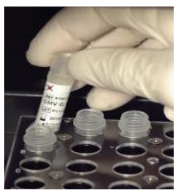


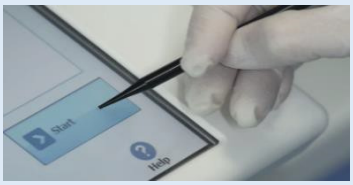
3. Scan the sample barcodes with hand-held barcode reader or type the sample ID

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

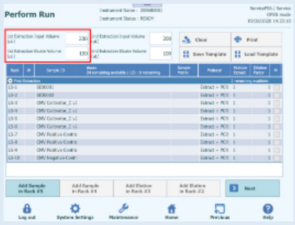
ELITe BeGenius Procedures

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

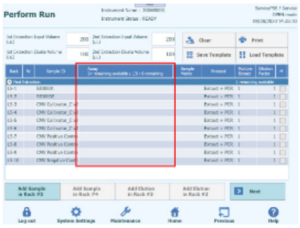
Before analysis

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

Procedure 1 - Complete run: Extraction + PCR

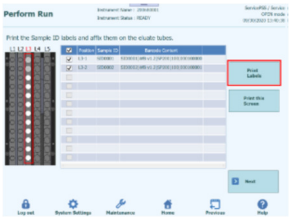
1. Select “Perform Run” on the touch screen 	**2.** Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active 	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL” 

4. Select the “Assay protocol” of interest



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

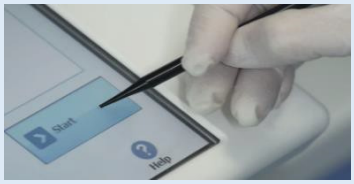
5. Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area



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

CMV ELITe MGB® kit used with ELITe InGenius®

Ref: RTK015PLD

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 CMV ELITe MGB® Kit is a Real-Time PCR assay for the **detection** and **quantification** of the DNA of **Human Cytomegalovirus (CMV)**. The assay is CE-IVD validated in combination with the instrument **ELITe InGenius®**.

B. Amplified sequence

	Gene	Fluorophore
Target	**CMV MIEA gene (exon 4 region)**	**FAM**
Internal Control	**Human beta Globin gene**	**AP525**

C. Validated matrix

Plasma EDTA

D. Kit component

CMV 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: INT035PCR
ELITe InGenius SP200 Consumable Set: INT032CS
CPE – Internal Control: CTRCPE

- **CMV ELITe Positive Control:** CTR015PLD
- **CMV ELITe Standard:** STD015PLD
- **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
CMV Q-PCR Mix volume	20 µL

Unit of quantitative result	International Unit: IU/mLgenome equivalent: gEq/mL *(equivalent to copies/mL)*
Frequency of controls	15 days
Frequency of calibration	60 days

G. Performance

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Plasma	**17 IU/mL** – 57 gEq/mL	**97%** 58/60*	**95%** 54/57* **confirmed samples/ tested samples*
Matrix	**Linearity (gEq/mL)**	**Linearity (IU/mL)**	**CF gEq/mL to IU/mL**
Plasma	**593 – 5x10⁶**	**178 – 1,5 x10⁷**	**0.3**

H. Reference material tested

Panel name	Provider	Qualitative results	Quantitative results
Molecular Q Panel: CMVMQP01	Qnostics	Concordance 100% (4/4)*	Titre as expected value ± 0.5 log
QCMD 2017: CMVDNA17-S	Qnostics	Concordance 100% (10/10)*	Titre as expected value ± 0.5 log**

*confirmed *samples/ tested samples* ****within the range of quantification***

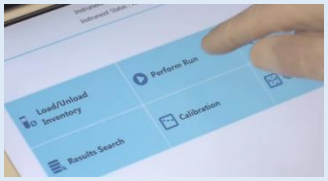
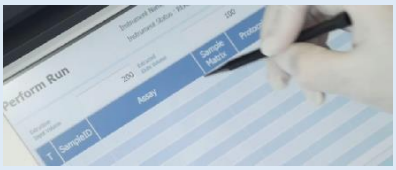
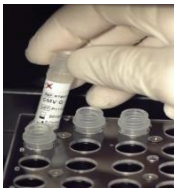
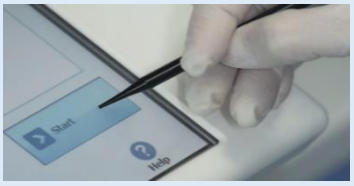
I. Procedures

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: CMV Q-PCR standard in the "Calibration menu" Verify controls: CMV pos. and neg. controls in the "Control menu" *NB:* Both have been run, approved and not expired	**3.** Thaw the CMV 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

CMV ELITe MGB® Kit used with ABI PCR instrument

Ref: RTK015PLD

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 CMV ELITe MGB Kit is a Real-Time PCR assay for the detection and quantification of the DNA **of Human Cytomegalovirus (CMV)**. 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

	Gene	Fluorophore
Target	CMV MIEA gene (Exon 4 region)	FAM
Internal Control	human beta Globin gene	VIC

C. Validated matrix

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

D. Kit Components

CMV 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
› **CPE - Internal Control:** CTRCPE
› **CMV – ELITe Positive Control:** CTR015PLD
› **CMV ELITe Standard:** STD015PLD
› **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	**263 IU/mL** - 332 gEq/mL	**100%** (57/60)*	**100%** (63/70)*
	Plasma	**222 IU/mL** - 201 gEq/mL	**97.1%** (66/68)*	**100%** (61/61)*
ELITe GALAXY - ABI	Whole blood	**127 IU/mL** - 249 gEq/mL	**98.3%** (59/60)*	**100%** (65/66)*
	Plasma	**140 IU/mL** - 519 gEq/mL	**92.2%** (47/51)*	**100%** (64/64)*
easyMAG - ABI	Whole blood	-	**100%** (50/50)*	**100%** (50/50)*
	Cerebrospinal fluid	-	**100%** (60/60)*	**100%** (60/60)*
	Urine	-	**100%** (52/52)*	**98.2%** (55/56)*
QIAsymphony - ABI	Whole blood	-	**100%** (60/60)*	**98.3%** (59/60)*
	Plasma	-	**100%** (60/60)*	**98.3%** (59/60)*

**confirmed samples/tested samples*

System	Linearity (IU/mL)	Conversion factor gEq/reaction to gEq/mL	Conversion factor gEq/mL to IU/mL
ELITe STAR - ABI	221 → 22 x 10^6 (WB), 308 → 30.8 x 10^6 (PL)	28 (WB,PL)	0.79 (WB), 1.10 (PL)
ELITe GALAXY - ABI	178→ 17.8 x 10^6 (WB), 105 → 10 x 10^6 (PL)	35 (WB,PL)	0.51 (WB), 0.27 (PL)
easyMAG - ABI	305 → 30.5 x 10^6 (WB)	50 (WB), 10 (CSF, Urine)	0.61 (WB)
QIAsymphony - ABI	110 → 11 x 10^6 (WB), 104 → 10 x 10^6 (PL)	24 (WB), 12 (PL)	0.46 (WB), 0.87 (PL)

G. Procedure

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

Extraction - Validated systems

Extraction	Validated matrix	Sample volume processed	Min. sample volume	Total eluate volume	CPE Internal Control volume
ELITe Star	WB, Plasma	200 µL	700 µL	100 µL	200 µL for 12 samples
ELITe Galaxy	WB, Plasma	300 µL	400 µL	200 µL	10 µL
EasyMAG	WB	100 µL	-	50 µL	-
	CSF, Urine	500 µL	-	100 µL	5 µL
QIAsymphony	WB	200 µL	300 µL	60 µL	-
	Plasma	500 µL	600 µL	85 µL	6 µL

Amplification - Settings of 7500 Fast Dx and 7300 PCR instruments

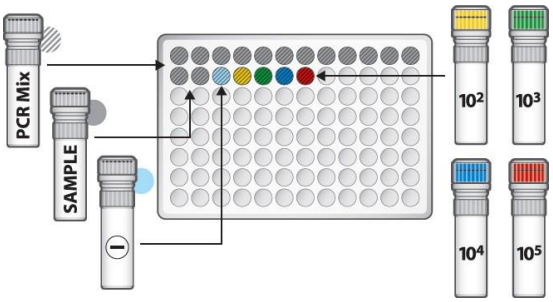
1. Switch on the thermal-cycler
2. Set "CMV" detector with "FAM" and quencher "none"
3. Set "Internal Control" detector with "VIC" and quencher "none"
4. Set passive fluorescence as "Cy5" with 7500 Fast Dx and as "ROX" with 7300 instrument
5. Set up the thermal profil as indicated. Fluorescence acquisition 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 60°C 72°C	10 sec 30 sec 20 sec

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

Amplification - PCR Set-up

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



Amplification - Threshold for qualitative analysis

Instrument	CMV 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

CMV 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 CMV 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 or approximately from 100 to 10^7 gEq/mL.

CMV ELITe MGB® Kit used with Cobas Z 480 PCR instrument

Ref: RTK015PLD

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 CMV ELITe MGB Kit is a Real-Time PCR assay for the detection and quantification of the DNA of **Human Cytomegalovirus (CMV)**. 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

	Gene	Fluorophore
Target	CMV MIEA gene (Exon 4 region)	FAM
Internal Control	human beta Globin gene	VIC

C. Validated matrix

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

D. Kit Components

CMV Q-PCR Mix
4 tubes of 540 µL

X 4

Ready to use complete reaction mixture
Number of tests per kit: 100
Freeze and 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**
› **CMV – ELITe Positive Control:** CTR015PLD
› **CMV – ELITe Positive Control RF:** CTR015PLD-R
› **CMV ELITe Standard:** STD015PLD
› **CPE - Internal Control:** CTRCPE
› **Molecular biology grade water**

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
MagNA Pure 24	Whole blood	**135 IU/mL** 270 gEq/mL	**100%** (51/51)*	**98%** (52/53)*
	Plasma	**88 IU/mL** - 220 gEq/mL	**98%** (62/63)*	**96%** (48/50)*
	Urine	**296 IU/mL** - 269 gEq/mL	**98%** (50/51)*	**100%** (49/49)*

**confirmed samples/tested samples*

System	Matrix	Linearity (IU/mL)	Conversion factor gEq/reaction to gEq/mL	Conversion factor gEq/mL to IU/mL
MagNA Pure 24	Whole blood	**178 IU/mL -> 10^6 IU/mL**	25 (WB, PL, Urine)	0.5
	Plasma	**100 IU/mL -> 10^6 IU/mL**		0.4
	Urine	**10^3 IU/mL -> 10^6 IU/mL**		1.1

G. Procedure

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

Extraction - Validated systems

Extraction	Validated matrix	Sample volume processed	Min. sample volume	Total eluate volume	CPE Internal Control volume
MagNA Pure 24	WB PL, Urine	200 µL	350 µL	100 µL	20 µL Diluted 1:2

Amplification - Settings of Cobas-Z 480 PCR instruments

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

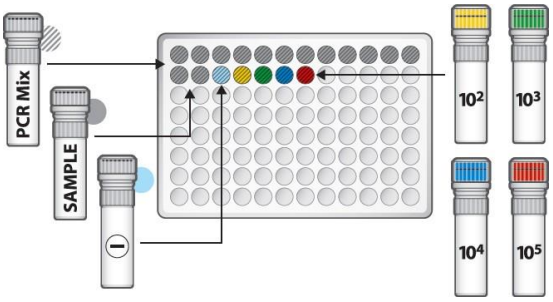
acquisition must be set during hybridation step at 60°C

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

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

Amplification - PCR Set-up

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



Amplification - Threshold for qualitative analysis

Instrument	Matrix	CMV FAM	Internal Control VIC
Cobas – Z 480	WB	0.80	1.5
	Plasma	0.55	1.2
	Urine	0.55	1.2

Interpretation - Qualitative results

CMV 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 CMV 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 or approximately from 250 to 2.5 10^7 gEq/mL.