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NOTICE of CHANGE dated 23/05/2023

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«CMV RNA ELITe MGB® Kit»
Ref. RTS115ING

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

- *Intended use modification*
- *Introduction of the possibility to visualise the melting curve*

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 RNA ELITe MGB® Kit

reagents for RNA reverse transcription and Real-Time PCR

REF RTS115ING

RUO

-20°C

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INTENDED USE

The **CMV RNA ELITe MGB® Kit** is an *in vitro* reagent intended to be used by healthcare professionals as part of a quantitative nucleic acids reverse transcription and amplification assay for the detection and quantification of the **human Cytomegalovirus virion mRNA (CMV RNA)** extracted from non-cellular specimens.

The assay is tested in association with the **ELITe InGenius®** instrument, automated and integrated systems for extraction, reverse transcription, real time PCR and results interpretation, using human specimens of Plasma collected in EDTA.

ASSAY PRINCIPLE

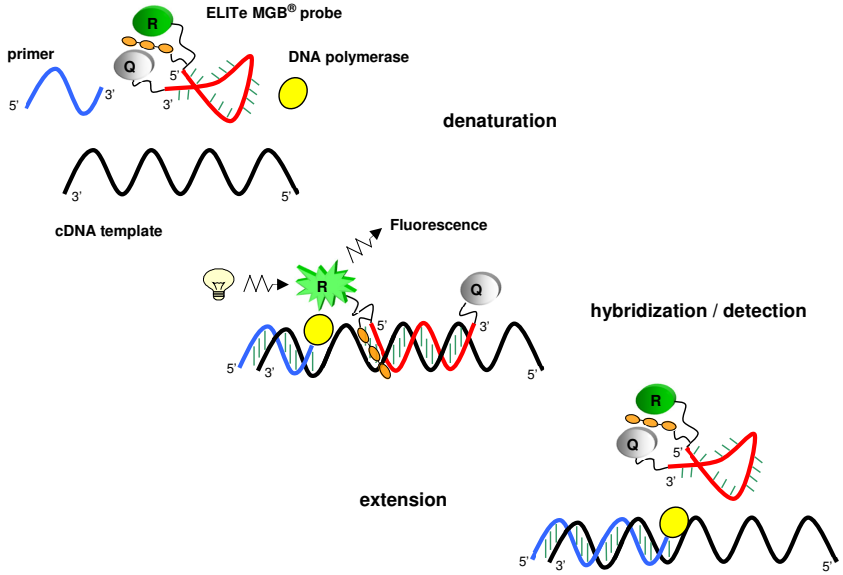
The assay is a quantitative reverse transcription and a Real-Time PCR (one-step method) tested on **ELITe InGenius**, an automated and integrated system for extraction, reverse transcription, amplification and detection of nucleic acids and results interpretation.

Nucleic acids are isolated from plasma collected in EDTA specimens, then **CMV RNA** is reverse transcribed and amplified in a Real-Time PCR by complete **CMV RNA PCR Mix**. Assay reagents contain primers and probe targeting the CMV UL21.5 spliced mRNA. The CMV RNA probe utilizes ELITe MGB® technology and is labelled by FAM fluorophore.

In addition, primers and probe specific for a heterologous Internal Control target (genomic RNA of MS2 phage) are included in the assay reagent. The Internal Control probe also utilizes ELITe MGB® technology and is labelled by AquaPhluor® 525 (AP525) fluorophore. The exogenous Internal Control is added to the lysis buffer and monitors for extraction, reverse transcription and PCR efficiency.

The ELITe MGB probes are activated when hybridize with the related PCR products. ELITe InGenius monitors fluorescence increase and calculates the Ct and quantity based on a stored calibration curve.

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



PRODUCT DESCRIPTION

The **CMV RNA ELITe MGB Kit** contains the following components:

- **CMV RNA PCR Mix**

The **CMV RNA PCR Mix** is an optimized and stabilized PCR mixture, **aliquoted into four vials** (WHITE cap). Each vial contains **600 µL** of solution and is sufficient for **24 tests on the ELITe InGenius**, if processing at least 5 samples per session.

Primers and the probe for **CMV RNA** are specific for the CMV UL21.5 spliced mRNA. The CMV RNA probe is stabilized by MGB®, quenched by Eclipse Dark Quencher®, and labelled by FAM fluorophore for detection in Channel 1 (**CMV RNA**) of the **ELITe InGenius**.

Primers and the probe for Internal Control (**IC**) are specific for the phage MS2 genomic RNA. The Internal Control probe is stabilized by MGB®, by Eclipse Dark Quencher®, and labelled by AP525 fluorophore for detection in Channel 2 (**IC**) of the **ELITe InGenius**.

The **CMV RNA PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

- **RT EnzymeMix**

The **RT EnzymeMix** is an optimized and stabilized mixture of enzymes for reverse transcription, **aliquoted into two vials** (cap with BLACK insert). Each vial contains **20 µL** of solution and is sufficient for **48 tests on the ELITe InGenius**, if processing at least 5 samples per session.

The **CMV RNA ELITe MGB Kit** contains sufficient reagents for **96 tests on ELITe InGenius**, including controls and standards, with 20 µL of **CMV RNA PCR Mix** and 0.3 µL **RT EnzymeMix** used per reaction.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
CMV RNA PCR Mix **Ref. RTS115ING**	mixture of reagents for reverse transcription and Real-Time PCR, **WHITE** cap	**4 x 600 µL**	-
RT EnzymeMix **Ref. RTS003-RT**	Reverse transcription enzymes, cap with **BLACK insert**	**2 x 20 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Sarstedt 2.0 mL skirted tube with screwcap (Sarstedt Ref. 72.694.005).
- Molecular biology grade water.

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OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample RNA, the internal control template, the amplification positive control, the DNA standards and the consumables are **not** included in this product.

For automated extraction of nucleic acid, reverse transcription, Real-Time PCR and result interpretation of samples, the **ELITe InGenius** instrument (ELITechGroup S.p.A., EG SpA, ref. INT030) and the following specific Assay Protocols (EG SpA), are required:

- parameters for calibration Standards **CMVRNA ELITe_Open_STD,**
- parameters for Positive Control **CMVRNA ELITe_Open_PC**,
- parameters for Negative Control **CMVRNA ELITe_Open_NC**,
- parameters for Plasma samples **CMVRNA ELITe_PL_Op_600_50.**

The following generic products are required instrument for **ELITe InGenius**:

- extraction cartridges **ELITe InGenius® SP 1000** (EG SpA, ref. INT033SP1000),
- extraction consumables **ELITe InGenius® SP 200 Consumable Set** (EG SpA, ref. INT032CS),
- amplification cassette **ELITe InGenius® PCR Cassette** (EG SpA, ref. INT035PCR),
- tips **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S),
- waste boxes **ELITe InGenius® Waste Box** (EG SpA, ref. F2102-000).

The generic product **CPE - Internal Control** (EG SpA, ref. CTRCPE) is required as template of internal control. The product contains a stabilised solution containing plasmid DNAs and genomic RNA of MS2 phage.

The product **CMV RNA ELITe Standard** (EG SpA, ref. STD115ING) is required to calculate the system standard curve (instrument and product batch). The product contains four stabilised dilutions of plasmid DNA at known concentration to obtain the standard curve.

Note: As this product targets CMV virion mRNA and not CMV genomic DNA, the assay results are not traceable to the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques" (NIBSC code: 09/162) that is designed for genomic DNA quantification.

The product **CMV RNA - ELITe Positive Control** (EG SpA, ref. CTR115ING) is required for the system validation (instrument and product batch). The product provides a stabilised solution of plasmid DNA at known concentration.

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. 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 (bleach) or autoclaved for one hour at 121 °C before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be 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 regulations in force.
Carefully read all instructions provided before running the assay.
While running the assay, follow the product instructions provided.
Do not use the product after the indicated expiry date.
Only use reagents provided with the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

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

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

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

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

The extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination.

The PCR Cassettes must be handled carefully and never opened in order to avoid PCR product diffusion into the environment and sample and reagent contamination.

Warnings and precautions specific for the components

CMV RNA PCR Mix

The **CMV RNA PCR Mix** must be stored at temperature of -20 °C or below and protected from light.
The **CMV RNA PCR Mix** must be used within one month from the first opening.
The **CMV RNA PCR Mix** can be frozen and thawed up to **five times**: further freeze / thaw cycles may cause a loss of product performances.

RT EnzymeMix

The **RT EnzymeMix** must be stored at temperature of -20 °C or below.
The **RT EnzymeMix** must be used within one month from the first opening.
The **RT EnzymeMix** can be handled at temperatures below +10 °C up to **ten times for ten minutes each time**: further uses may cause a loss of product performances.

SAMPLES AND CONTROLS

This product must be used with samples of **Plasma collected in EDTA**.

Plasma samples for nucleic acid extraction must be collected in EDTA, transported, and stored at room temperature (~+25 °C) or at +2 / +8 °C for a maximum of 24 hours. Alternatively, they must be frozen and stored at ~-20 °C for 1 month.

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

Note: Whole blood samples for Plasma preparation must be collected in EDTA, transported, and stored at room temperature (~+25 °C) or at +2 / +8 °C for a maximum of 24 hours before centrifugation.

Note: The nucleic acid extraction from EDTA Plasma is performed on the **ELITe InGenius** with **ELITe InGenius Software** version 1.3 (or later equivalent versions) using the Assay Protocol **CMVRNA ELITe_PL_Op_600_50**. This protocol processes 600 µL of sample starting from secondary tube, adds 10 µL of **CPE** (Internal Control) to each extraction at and elutes the nucleic acids in 50 µL. Primary tube cannot be used in association with the Assay Protocol.

Purified nucleic acids can be stored at +2 / +8 °C for 16 hours or at ~-20 °C for 1 month.

Interfering substances

Available data concerning inhibition caused by drugs and other substances are reported in Potential Interfering substances in the Performance characteristics section.

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

Calibration curve and Amplification controls

Before analysing any sample, it is necessary to generate the calibration curve and the amplification controls for each lot of PCR reagent:

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- for the Calibration curve, use the four levels of the **CMV RNA ELITe Standard** (not provided with this kit) and **CMVRNA ELITe_Open_STD** Assay Protocol,

- for the Positive Control, use the **CMV RNA - ELITe Positive Control** (not provided with this kit) and **CMVRNA ELITe_Open_PC** Assay Protocol,

- for the Negative Control, use molecular biology grade water (not provided with this kit) and **CMVRNA ELITe_Open_NC** Assay Protocol.

Note: ELITe InGenius requires an approved and valid calibration curve and of amplification controls for each lot of PCR reagent.
Calibration curves stored in the database expire after **60 days**, at which time it is necessary to re-run the **CMV RNA ELITe Standard** with the appropriate PCR reagent lot.
Amplification control results stored in the database expire after **15 days**, at which time it is necessary to re-run the Positive and Negative Controls with the appropriate PCR reagent lot.
Furthermore, calibration curve and amplification controls must be re-run when:
- a new lot of amplification reagents is started,
- results of Quality control (see following paragraph) are out of specification,
- any major maintenance or service is performed on the **ELITe InGenius** instrument.

Quality controls

Validation of the extraction and PCR procedure is recommended. Archived samples or reference materials can be used. When available, external controls shall be used in accordance with local, state, and federal accrediting organizations, as applicable.

PROCEDURE

Using the **CMV RNA ELITe MGB Kit** with the system **ELITe InGenius** consists of three steps:
- Verification of the system readiness,
- Session setup,
- Review and export of results.

Verification of the system readiness

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

- switch on the **ELITe InGenius** and select the login in "**OPEN**" mode,

- verify the Calibration curve (**CMV RNA Q-PCR Standards**) is approved and valid (Status) for the PCR reagent lot (**CMV RNA PCR Mix**) to be used. If no valid Calibration curve is available for the PCR reagent lot, perform calibration as described in the following sections,

- verify the amplification controls (**CMV RNA Positive Control, CMV RNA Negative Control**) are approved and valid (Status) for the PCR reagent lot (**CMV RNA PCR Mix**) to be used. If no valid amplification Controls are available for the PCR reagent lot, run the amplification Controls as described in the following sections,

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

The Assay Protocol available for sample testing with the product **CMV RNA ELITe MGB Kit** is described in the table below.

Assay Protocol for CMV RNA ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
CMVRNA ELITe_PL_Op_600_50	Plasma	Positive, copies/mL, Negative	Extraction Input Volume: 600 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Dilution Factor: 1.7 Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Session Setup

The product **CMV RNA ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

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

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

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

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

1. Thaw the needed **CMV RNA PCR Mix** vials (WHITE cap) for 30 minutes at room temperature (~+25 °C). Each vial is sufficient for **24 tests**. Mix by vortexing at low speed for 10 seconds three times then spin down the contents for 5 seconds and keep on ice or cool block.

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

2. Take the needed **RT EnzymeMix** vials (cap with BLACK insert). Each tube is sufficient for **48 tests**. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

Note: The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

3. Prepare one 2 mL tube (Sarstedt, Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and label it with a permanent marker.
4. Calculate the needed volumes of **CMV RNA PCR Mix** and **RT EnzymeMix** for preparing the **complete reaction mixture** on the basis of the number of samples (N) to be analyzed, as described in the table below.

Sample Number (N)	CMV RNA PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

5. Prepare the **complete reaction mixture** by transferring into the labeled 2 mL tube the calculated volumes of the two components. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

Note: The **complete reaction mixture** should be used within **7** hours and **cannot** be stored for re-use.

Note: Protect the **complete reaction mixture** from light because this reagent is photosensitive.

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

A. Integrated run

To setup an integrated run with sample extraction and amplification, follow the steps below while referring to the GUI:

1. Thaw samples at room temperature (~+25 °C) and handle according to laboratory guidelines and "Samples and Controls" section. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

Note: For this assay 600 µL of sample are needed and must be transferred in labelled "Extraction tubes" (not included in the kit).

2. Thaw the needed **CPE** vials for 30 minutes at room temperature (~+25 °C). Each tube is sufficient for 12 extractions. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.
3. Select "Perform Run" from the "Home" screen.
4. Ensure that the "Extraction Input Volume" is 1000 µL and the Extracted Elute Volume is 50 µL, even if 600 µL of sample will be used.
5. For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.
6. Select the Assay Protocol to be used in the "Assay" column (e.g., CMVRNA ELITe_PL_Op_600_50).

7. Ensure that the "Protocol" displayed is "Extract + PCR".
8. Select the sample loading position as "Extraction Tube" in the "Sample Position" column.
9. Ensure that the "Dilution Factor" is "1.7". Click "Next" to continue.

Note: For this assay, 600 µL of sample must be transferred in a labeled "Extraction Tube". Exceeding volume will be left in the "Extraction Tube" by the **ELITe InGenius**.

10. Load the **complete reaction mixture** and the **CPE** on the designated "Inventory Block" referring to the "Load List" and enter the **CMV RNA PCR Mix** and **CPE** lot number and expiry date. Click "Next" button to continue.
11. Verify the tips in the "Tip Racks" in the "Inventory Area" and replace "Tip Rack" if necessary. Click "Next" button to continue.
12. Load the "**PCR Cassettes**", the "**ELITe InGenius SP 1000**" extraction cartridges, and all required consumables and samples to be extracted, following the GUI instruction. Click "Next" to continue.
13. Close the instrument door.
14. Press "Start" to start the run.

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

Note: At the end of the run, the remaining extracted sample in the "Elution tube" must be removed from the instrument, capped, labeled, and stored at -20 °C for one month. Avoid spilling the extracted sample.

Note: At the end of the run, the PCR Cassettes, the extraction cartridges, and the consumables must be disposed of following governmental and environmental regulations. Avoid spilling the reaction products.

Note: The **complete reaction mixture** can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently, then spin down the contents for 5 seconds before starting next session.

B. Amplification run

To set up the amplification run starting from extracted nucleic acids, follows the steps below while referring to the GUI:

1. Thaw the "Elution tubes" containing the extracted nucleic acids at room temperature (~+25 °C). Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2. Select "Perform Run" from the "Home" screen.
3. Ensure that the "Extraction Input Volume" is 1000 µL and the "Extracted Elute Volume" is 50 µL, even if extraction is not being performed.
4. For each sample, assign a Track and enter the SID by typing or by scanning the sample barcode.
5. Select the Assay Protocol to be used in the "Assay" column (e.g., CMVRNA ELITe_PL_Op_600_50).
6. Select "PCR Only" in the "Protocol" column.
7. Ensure that the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
8. Ensure that the "Dilution Factor" is "1.7". Click "Next" to continue.
9. Load the **complete reaction mixture** on the "Inventory Block" referring to the "Load List" and enter the **CMV RNA PCR Mix** lot number and expiry date. Click "Next" to continue.
10. Verify the tips in the "Tip Racks" in the "Inventory Area" and replace "Tip Rack" if necessary. Click "Next" to continue the setup.
11. Load the "**PCR Cassettes**" and the "Elution tubes" with extracted nucleic acids following the GUI instruction. Click "Next" to continue.
12. Close the instrument door.
13. Press "Start" to start the run.

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

Note: At the end of the run the remaining extracted sample in the "Elution tube" must be removed from the

instrument, capped, and stored at -20 °C for one month. Avoid the spilling of the extracted sample.

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

Note: The **complete reaction mixture** can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently, then spin down the contents for 5 seconds before starting next session.

C. Calibration run

To setup the Calibration run with Q-PCR Standard, follow the steps below while referring to the GUI:

1. Thaw the needed **CMV RNA Q-PCR Standard** vials (Cal1: CMV RNA Q-PCR Standards 10^2, Cal2: CMV RNA Q-PCR Standards 10^3, Cal3: CMV RNA Q-PCR Standards 10^4, Cal4: CMV RNA Q-PCR Standards 10^5) for 30 minutes at room temperature (~+25°C). Each tube is sufficient for 4 reactions. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.
2. Select “Perform Run” from the “Home” screen.
3. Ensure that the “Extraction Input Volume” is 1000 µL and the “Extracted Elute Volume” is 50 µL, even if extraction is not being performed.
4. For the **CMV RNA Q-PCR Standards**, assign the Track, select the Assay Protocol “CMVRNA ELITe_Open_STD” in the “Assay” column and enter the reagent lot number and expiry date.
5. Ensure “PCR Only” in the “Protocol” column.
6. Ensure that the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”.
7. Ensure that the “Dilution Factor” is “1.7”. Click “Next” to continue.
8. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **CMV RNA PCR Mix**. Click “Next” to continue the setup.
9. Verify the tips in the “Tip Racks” in the “Inventory Area” and replace “Tip Rack” if necessary. Click “Next” to continue.
10. Load the “**PCR Cassettes**” and the **CMV RNA Q-PCR Standard** vials following the GUI instruction. Click “Next” to continue.
11. Close the instrument door.
12. Press “Start” to start the run.

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

Note: At the end of the run, the remaining **CMV RNA Q-PCR Standards** must be removed from the instrument, capped and stored at -20 °C.

Note: The **CMV RNA Q-PCR Standards** can be used for 4 separate “PCR Only” sessions of 2 hours each.

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

Note: The **complete reaction mixture** can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently, then spin down the content for 5 seconds before starting next session.

D. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, follow the steps while below referring to the GUI:

1. Thaw the **CMV RNA Positive Control** vial for 30 minutes at room temperature (~+25°C). Each vial is sufficient for 4 reactions. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.
2. As **Negative Control**, transfer at least 50 µL of molecular biology grade water to an “Elution tube”, provided with the “**ELITe InGenius SP 200 Consumable Set**”.

3. Select “Perform Run” from the “Home” screen.
4. Ensure that the “Extraction Input Volume” is 1000 µL and the “Extracted Elute Volume” is 50 µL, even if extraction is not being performed,
5. For the Positive Control, assign the Track, select the Assay Protocol “CMVRNA ELITe_Open_PC” in the “Assay” column and enter the **CMV RNA Positive Control** lot number and expiry date.
6. For the Negative Control, assign the Track, select the Assay Protocol “CMVRNA ELITe_Open_NC” in the “Assay” column and enter the molecular biology grade water lot number and expiry date.
7. Ensure “PCR Only” in the “Protocol” column.
8. Ensure that the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”.
9. Ensure that the “Dilution Factor” is “1.7”. Click “Next” to continue.
10. Load the **complete reaction mixture** on the “Inventory Block” referring to the “Load List” and enter the **CMV RNA PCR Mix** lot number and expiry date. Click “Next” to continue.
11. Verify the tips in the “Tip Racks” in the “Inventory Area” and replace “Tip Racks” if necessary. Click “Next” to continue.
12. Load the “**PCR Cassettes**”, the **CMV RNA Positive Control** and **Negative Control** vials following the GUI instruction. Click “Next” to continue.
13. Close the instrument door.
14. Press “Start” to start the run.

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

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

Note: The **CMV RNA Positive Control** can be used for 4 separate “Extract + PCR” sessions of 3 hours each.

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

Note: The **complete reaction mixture** can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently, then spin down the contents for 5 seconds before starting next session.

Review and approval of results

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

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

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

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

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

A. Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the CMV RNA probe (Channel “CMV RNA”) of the Calibrator reactions with the **CMV RNA ELITe_Open_STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

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

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

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

B. Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the CMV RNA probe (Channel "CMV RNA") of the Positive Control and Negative Control reactions with the **CMV RNA ELITe_Open_PC** and **CMV RNA ELITe_Open_NC** Assay Protocols parameters. The resulting target Ct values are converted to concentration and used to validate the system (reagents lot and instrument).

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

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

Before analysing any sample, it is mandatory to verify that Positive Control and Negative Control results are approved and valid for the PCR reagent lot. The Status of Positive Control and Negative Control results for each lot of PCR reagent is shown in the "Controls" module. If the results of Positive Control and/or Negative Control are missing or expired, run the control(s) as described above.

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

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

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

C. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the CMV RNA probe (Channel "CMV RNA") and the Internal Control probe (Channel "IC") with the CMVRNA ELITe_PL_Op_600_50 Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Result Display" module.
The sample results can be approved when the three conditions in the table below are true.

1) Calibration Curve	Status
CMV RNA Q-PCR Standards	APPROVED
2) Positive Control	**Status**
CMV RNA Positive Control	APPROVED
3) Negative Control	**Status**
CMV RNA Negative Control	APPROVED

The sample results are automatically interpreted by the **ELITe InGenius software** using Assay Protocol parameters. The possible result messages are listed in the table below.

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

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Note: The quantification of CMV RNA is only expressed in copies / mL and cannot be traced to the WHO International Unit as this standard is referred to CMV genomic DNA.

Samples reported as "Invalid - Retest Sample" are not suitable for result interpretation. In this case, the Internal Control RNA was not efficiently detected, which could be due to problems in the reverse transcription, PCR or extraction step (degradation or loss of RNA during the extraction or inhibitors in the eluate), which may cause incorrect results.

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

Samples reported as "CMV RNA: RNA Not Detected or below LoD" are suitable for analysis but CMV RNA was not detected. In this case, the sample may be either negative for CMV RNA or the CMV RNA is present at a concentration below the limit of detection of the assay (see "Performance characteristics").

CMV RNA positive samples at a concentration below the LoD, if detected, are reported as "CMV RNA: RNA Detected, quantity below LLoQ copies/mL" (see "Performance characteristics").

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

D. Sample result reporting

The sample results are stored in the database and reports can be exported as "Sample Report" and "Track Report".
The "Sample Report" shows the results details by selected sample (SID).
The "Track Report" shows the results details by selected Track.
The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) of CMV RNA ELITe MGB Kit was determined with Plasma samples on ELITe InGenius.

The LoD was determined by testing a panel of CMV RNA negative Plasma samples spiked with CMV RNA reference material (CMV culture supernatant, University of Turin) at known titres. Six dilution levels were prepared starting from 0.56 PFU/mL to 0.032 PFU/mL. Each dilution level was processed in 24 replicates on ELITe InGenius in "Extract + PCR" mode. Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.
The results are reported in the following table.

Limit of Detection for Plasma samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
CMV RNA	0.19 PFU/mL	0.15 PFU/mL	0.29 PFU/mL
	30 copies/mL	N.A.	N.A.

The calculated LoD value was verified by testing 30 replicates of Plasma samples spiked with CMV RNA reference material (CMV culture supernatant, University of Turin) at the claimed concentration. The LoD is confirmed if at least 27 out of 30 replicates give a positive result as per CLSI standard EP17-A.
The results are reported in the following table.

Limit of Detection for Plasma samples and ELITe InGenius				
Sample	Titer	N	Positive	Negative
CMV RNA spiked Plasma	0.19 PFU/mL	30	29	1

The LoD value for CMV RNA target was confirmed at 0.19 PFU/mL corresponding to 30 copies/mL for Plasma sample.

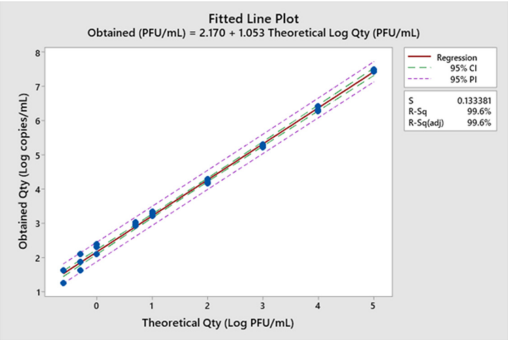
Linear measuring range

The Linear measuring range of CMV RNA ELITe MGB Kit was determined with Plasma samples on ELITe InGenius.

The Linear measuring range was determined using a panel of dilutions of CMV RNA reference material (CMV culture supernatant, University of Turin) in negative Plasma samples. The panel consisted of nine dilution points from about 10^5 PFU/mL to 0.25 PFU/mL. Each sample of the panel was tested in triplicate on ELITe InGenius in "Extract + PCR" mode.

The data were analysed by linear regression and polynomial analysis, and results demonstrated that the assay shows a linear response for all the dilutions with a Squared Correlation Coefficient (R2) equal to 0.996.

The results are reported in the following figure.



The Lower Limit of Quantification (LLoQ) was determined to be at the LoD, 0.19 PFU/mL, where target measurement showed a precision within ±0.5 Log as Standard Deviation.

The Upper Limit of Quantification (ULoQ) was determined to be 100,000 PFU / mL, where target measurement showed a precision within ±0.5 Log as Standard Deviation.

The final results are summarized in the following table.

Linear measuring range for EDTA Plasma samples and ELITe InGenius	
Lower Limit	Upper Limit
0.19 PFU / mL	100,000 PFU / mL
30 copies / mL	29,392,000 copies / mL

Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

The efficiency of detection for different genotypes of CMV was evaluated by *in silico* analysis of the sequences available in nucleotide databases.

The analysis of the regions specific for the hybridization of primers and probe in the UL21.5 spliced mRNA showed sequence conservation and absence of significant mutations in the available CMV sequences in the nucleotide database. So, an efficient detection and quantification for the different CMV genotypes / strains is expected.

The Inclusivity of the assay, assessed by detection and quantification efficiency on different strains, was verified by testing the different reference material (ATCC and University of Turin) including the main CMV strains.

Each sample of the panel was diluted at low concentration in negative Plasma samples and tested on ELITe InGenius in "Extract + PCR" mode.

The results are reported in the following table.

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Sample ID	Pos. / Rep.	CMV RNA Mean Ct	CMV RNA Mean copies/mL
AD169	3 / 3	33.79	153
Towne	3 / 3	35.04	57
Davis	3 / 3	33.36	184
Merlin	3 / 3	33.81	142

All samples were correctly detected by the CMV RNA ELITe MGB Kit on ELITe InGenius.

Potentially interfering markers: cross-reactivity

The Potential cross-reactivity of the assay with CMV genomic DNA and other unintended organisms was evaluated by *in silico* analysis of sequences available in nucleotide databases.

The primer and probe sequences were assessed for homology with the sequences of CMV genomic DNA and other organisms available in nucleotide databases. The results showed significant mismatches with CMV genomic DNA, therefore no significant cross - reactivity is expected. The results showed no significant homology with other unintended organisms, therefore, no cross- reactivity is expected.

The absence of cross-reactivity with other organisms that can be found in samples of Plasma samples was also verified by testing a panel of certified reference materials.

Samples of genomic DNA or RNA from different potentially interfering markers (ATCC, NIBSC, ZeptoMetrix) were analyzed at high concentration (at least 10^5 copies/reaction) in triplicate on ELITe InGenius in "PCR Only" mode. The genomic DNA or RNA of each organism was also added with 80,000 Internal Control copies per reaction in order to mimic the extracted sample.

The results are reported in the following table.

Sample	CMV RNA Pos. / Rep.	IC Mean Ct	Outcome
HIV1	0 / 3	29.58	No cross-reactivity
EBV	0 / 3	29.48	No cross-reactivity
HAV	0 / 3	29.72	No cross-reactivity
HBV	0 / 3	29.45	No cross-reactivity
HHV6	0 / 3	29.35	No cross-reactivity
HSV1	0 / 3	29.56	No cross-reactivity
HSV2	0 / 3	29.49	No cross-reactivity
HEV	0 / 3	29.64	No cross-reactivity
RSV	0 / 3	29.65	No cross-reactivity
VZV	0 / 3	29.60	No cross-reactivity
Influenza A virus (H1N1)	0 / 3	29.85	No cross-reactivity
Influenza B virus (Florida)	0 / 3	29.71	No cross-reactivity
Dengue Virus Type 3	0 / 3	29.62	No cross-reactivity
Adenovirus 2	0 / 3	29.89	No cross-reactivity
West Nile Virus	0 / 3	29.76	No cross-reactivity
Echovirus 4	0 / 3	29.94	No cross-reactivity
Parvovirus B19	0 / 3	29.54	No cross-reactivity
HCV	0 / 3	29.62	No cross-reactivity
Aspergillus fumigatus	0 / 3	30.19	No cross-reactivity
Staphylococcus aureus	0 / 3	29.69	No cross-reactivity
Candida albicans	0 / 3	29.89	No cross-reactivity

All potentially interfering markers tested showed no cross-reactivity for the CMV RNA target using the CMV RNA ELITe MGB Kit.

Potentially interfering markers: Inhibition

The Absence of interference caused by other organisms' presence in Plasma samples was verified by testing a panel of certified reference materials.

Samples of genomic DNA or RNA from potentially interfering markers (ATCC, NIBSC, ZeptoMetrix) at high concentration (at least 10^5 copies/reaction) were spiked with CMV RNA extracted from reference material (CMV culture supernatant, University of Turin) at low concentration (about 20 copies/reaction). Each sample was also added with 80,000 Internal Control copies per reaction in order to mimic the extracted sample. The samples were analyzed in triplicate on ELITe InGenius in "PCR Only" mode. The analysis of mean target Logarithmic Quantity values (reference and test) was performed and the calculated quantity values were within the interval of ±0.5 Log (C. N. Kotton et al., 2018).

The results are reported in the following table.

Sample	CMV RNA Pos. / Rep.	Quantification Bias Ref. - test	Outcome
HIV1	3 / 3	-0.1807	No interference
EBV	3 / 3	-0.1196	No interference
HAV	3 / 3	-0.0964	No interference
HBV	3 / 3	-0.2270	No interference
HHV6	3 / 3	-0.2004	No interference
HSV1	3 / 3	-0.1529	No interference
HSV2	3 / 3	-0.0964	No interference
HEV	3 / 3	-0.1529	No interference
RSV	3 / 3	-0.1784	No interference
VZV	3 / 3	-0.2270	No interference
Influenza A virus (H1N1)	3 / 3	-0.1060	No interference
Influenza B virus (Florida)	3 / 3	-0.2014	No interference
Dengue Virus Type 3	3 / 3	-0.2472	No interference
Adenovirus 2	3 / 3	-0.1682	No interference
West Nile Virus	3 / 3	-0.2382	No interference
Echovirus 4	3 / 3	-0.0253	No interference
Parvovirus B19	3 / 3	-0.1927	No interference
HCV	3 / 3	-0.1666	No interference
Aspergillus fumigatus	3 / 3	-0.2357	No interference
Staphylococcus aureus	3 / 3	-0.1782	No interference
Candida albicans	3 / 3	-0.0423	No interference

All potentially interfering organisms tested showed no inhibition of the CMV RNA target amplification using the CMV RNA ELITe MGB Kit.

Potentially interfering substances: cross-reactivity

The cross-reactivity of Potentially interfering substances was evaluated by analyzing the "AcroMetrix® Inhibition Panel" (Thermo Fisher Scientific Inc.) of plasma samples containing endogenous substances, resulting from haemolysis, icterus and lipemia, and exogenous substances, EDTA and Heparin anticoagulants.

In addition, 9 other potentially interfering substances, drugs, were tested at relevant concentration in plasma samples: Azithromycin, Cyclosporine A, Valganciclovir, Cidofovir, Abacavir, Ganciclovir, Foscarnet, Ribavirin and Letermovir.

The samples were processed in triplicate on ELITe InGenius in “Extract + PCR” mode.
The results are reported in the following table.

Sample	CMV RNA Pos. / Rep.	IC Mean Ct	Outcome
Icteric Plasma	0 / 3	28.64	No cross-reactivity
Lipemic Plasma	0 / 3	29.11	No cross-reactivity
Haemolytic Blood High	0 / 3	29.52	No cross-reactivity
Haemolytic Blood Medium	0 / 3	29.67	No cross-reactivity
HaemolyticBlood Low	0 / 3	29.45	No cross-reactivity
Heparinized Plasma	0 / 3	35.54	Invalid Sample
EDTA Plasma	0 / 3	29.16	No cross-reactivity
Azithromycin	0 / 3	29.04	No cross-reactivity
Cyclosporine A	0 / 3	28.96	No cross-reactivity
Valganciclovir	0 / 3	29.37	No cross-reactivity
Cidofovir	0 / 3	29.26	No cross-reactivity
Abacavir	0 / 3	29.08	No cross-reactivity
Ganciclovir	0 / 3	29.26	No cross-reactivity
Foscarnet	0 / 3	29.11	No cross-reactivity
Ribavirin	0 / 3	28.96	No cross-reactivity
Letermovir	0 / 3	29.05	No cross-reactivity

The test showed that EDTA, Hemoglobin, Triglycerides, Bilirubin, Azithromycin, Cyclosporine A, Valganciclovir, Cidofovir, Abacavir, Ganciclovir, Foscarnet, Ribavirin and Letermovir do not cross-react with the CMV RNA amplification.

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Heparin was confirmed to be capable of inhibiting the amplification of CMV RNA; however, due to the Internal Control Ct cut-off (IC Ct < 32), the sample results were "not valid".

Potentially interfering substances: inhibition

The inhibition of Potentially interfering substances was evaluated by analyzing the "AcroMetrix® Inhibition Panel" (Thermo Fisher Scientific Inc.) of plasma samples containing endogenous substances, resulting from haemolysis, icterus and lipemia, and exogenous substances, EDTA and Heparin anticoagulants.

In addition, 9 other potentially interfering substances, drugs, were tested at relevant concentration in plasma samples: Azithromycin, Cyclosporine A, Valganciclovir, Cidofovir, Abacavir, Ganciclovir, Foscarnet, Ribavirin and Letermovir.

The plasma samples spiked with CMV RNA reference material (CMV culture supernatant, University of Turin) at a concentration of 3x LoD (about 0.60PFU/mL corresponding to ~90 copies/mL). The samples were processed in triplicate on ELITe InGenius in “Extract + PCR” mode. The analysis of mean target Logarithmic Quantity values was performed and the calculated quantity values were within the interval of ±0.5 Log (C. N. Kotton et al., 2018).
The results are reported in the following table.

Sample	CMV RNA Pos. / Rep.	Quantification Bias Ref. - test	Outcome
Icteric Plasma	3 / 3	0.0778	No interference
Lipemic Plasma	3 / 3	-0.1038	No interference
Haemolytic Blood High	3 / 3	0.0820	No interference
Haemolytic Blood Medium	3 / 3	0.1225	No interference
HaemolyticBlood Low	3 / 3	0.0858	No interference
Heparinized Plasma	0 / 3	N.A.	Invalid Sample
EDTA Plasma	3 / 3	0.0150	No interference
Azithromycin	3 / 3	0.0293	No interference
Cyclosporine A	3 / 3	-0.0581	No interference
Valganciclovir	3 / 3	-0.0956	No interference
Cidofovir	3 / 3	0.0796	No interference
Abacavir	3 / 3	-0.0810	No interference
Ganciclovir	3 / 3	-0.0800	No interference
Foscarnet	3 / 3	0.0565	No interference
Ribavirin	3 / 3	-0.0441	No interference
Letermovir	3 / 3	0.0311	No interference

The test showed that EDTA, Hemoglobin, Triglycerides, Bilirubin, Azithromycin, Cyclosporine A, Valganciclovir, Cidofovir, Abacavir, Ganciclovir, Foscarnet, Ribavirin and Letermovir do not inhibit the CMV RNA amplification. The CMV RNA Logarithmic Quantity values were within the interval of ±0.5 Log.

Heparin was confirmed to be capable of inhibiting the amplification of CMV RNA. However, due to the Internal Control Ct cut-off (IC Ct < 32), the sample results were "not valid" instead of "false negative".

Absence of cross-contamination

The Absence of cross-contamination was tested by analyzing the results of five sessions in which plasma samples negative for CMV RNA were alternated with plasma samples spiked with CMV RNA reference material (CMV culture supernatant, University of Turin) at a concentration of ~$1x10^6$ copies/mL.

Five sets of samples, alternating six positive samples with six negative samples, were tested on ELITe InGenius in “Extract + PCR” mode.
The results are reported in the following table.

Samples	N	Negative	Positive
CMV RNA spiked Plasma at ~$1x10^6$ copies/mL	30	0	30
CMV RNA negative Plasma	30	30	0

None of the tested CMV RNA negative samples gave false positive results. In this test cross-contamination was neither detected within sessions nor between sessions.

Whole system failure rate

The Whole system failure rate was verified by analysing a panel of samples spiked for CMV RNA at low titre and determining the frequency of “false negative” results.

50 individual EDTA Plasma samples, tested negative for CMV DNA, were spiked with CMV RNA reference material (CMV culture supernatant, University of Turin) at a concentration of 3x LoD (about 0.60

PFU / mL corresponding to ~90 copies / mL). The samples were tested on ELITe InGenius in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	Negative	Positive	CMV RNA Mean copies/mL
CMV RNA Spiked Plasma	50	0	50	76.82

None of the tested CMV RNA positive samples gave false negative results. In this test the whole system failure rate was equal to 0%.

Repeatability

The Repeatability of results obtained with the CMV RNA ELITe MGB Kit on ELITe InGenius was assessed by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with CMV RNA reference material (CMV culture supernatant, University of Turin) at concentrations of 3x LoD (about 0.60 PFU / mL corresponding to ~90 copies / mL) and of 10x LoD (about 2 PFU / mL corresponding to ~300 copies / mL).

The Repeatability was determined by analysing of panel members in four replicates, in two runs per day, one lot of product per day, on two different days. Testing was performed with a total of three lots of product, all using the same instrument, by the same operator. Samples were processed in randomized positions on ELITe InGenius in "Extract + PCR" mode.

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

A summary of results obtained with the third Pilot Lot is shown in the tables below.

Intra – Session Repeatability								
Sample	CMV RNA				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	27.82	0.23	0.82
3x LoD	8 / 8	33.19	0.43	1.30				
10x LoD	8 / 8	31.69	0.09	0.27				

Inter – Session Repeatability								
Sample	CMV RNA				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	27.93	0.30	1.09
3x LoD	16 / 16	33.19	0.34	1.03				
10x LoD	16 / 16	31.62	0.18	0.56				

In the Repeatability test, the assay detected the CMV RNA target as expected and showed Ct values with %CV below 5% for CMV RNA and Internal Control.

Reproducibility

The Reproducibility of results obtained by the CMV RNA ELITe MGB Kit on ELITe InGenius was assessed by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with CMV RNA reference material (CMV culture supernatant, University of Turin) at concentrations of 3x LoD (about 0.60 PFU / mL corresponding to ~90 copies / mL) and of 10x LoD (about 2 PFU / mL corresponding to ~300 copies / mL).

The Inter – Batch Reproducibility was determined by analysing of panel members in four replicates, in two runs per day, one lot of product per day, on two different days. Testing was performed with a total of three lots of product, all using the same instrument, by the same operator. Samples were processed in randomized positions on ELITe InGenius in "Extract + PCR" mode.

The Ct values of the CMV RNA target and 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 – Batch Reproducibility								
Sample	CMV RNA				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 48	N.A.	N.A.	N.A.	144 / 144	28.14	0.32	1.13
3 x LoD	48 / 48	33.18	0.33	0.99				
10 x LoD	48 / 48	31.47	0.31	1.00				

The Inter – Instrument Reproducibility was determined by analysing panel members in four replicates, in one run per day, in two days per instrument. Three different lots of product were used with three different instruments by three different operators. Samples were processed in randomized positions on ELITe InGenius 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								
Sample	CMV RNA				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 24	N.A.	N.A.	N.A.	72 / 72	28.85	0.28	0.95
3x LoD	24 / 24	34.53	0.56	1.63				
10x LoD	24 / 24	32.28	0.31	0.97				

In the Reproducibility test, the assay detected the CMV RNA target as expected and showed Ct values with %CV below 5% for CMV RNA and Internal Control.

Confirmation of negative samples

The Specificity of the assay, assessed by Negative Percent Agreement of different methods, was evaluated by analyzing plasma samples from CMV DNA negative whole blood samples tested by the external laboratory with a CE IVD marked reference method (EG SpA).

Each sample was subjected to the entire procedure including extraction, amplification, detection, and result interpretation by the ELITechGroup S.p.A. products. The results obtained by the CMV RNA ELITe MBG Kit were used to calculate the Negative Percent Agreement with the reference method.

The results, after discrepant analysis, are summarized in the following table.

Samples	N	Positive	Negative	Invalid	Specificity
Plasma from CMV DNA negative whole blood	40	0	40	0	100%

In this test, all the 40 samples were confirmed negative. The Specificity of the CMV RNA ELITe MBG Kit was equal to 100%.

The Internal Control Ct Cut-off value is set at 32.

Method agreement

The agreement with a reference method was evaluated by analysing 40 plasma samples from CMV DNA positive whole blood of 12 patients undergoing antiviral therapy with inhibitor of the CMV terminase complex (Letermovir). The samples' CMV DNA levels were tested by the external center with a CE IVD marked reference method (EG SpA).

As per external laboratory validated procedure, these samples were also tested for CMV DNAemia on plasma after DNase treatment (I. Cassaniti et al., 2021). Just 8 samples were found CMV DNA positive, while 31 samples resulted negative and 1 sample was invalid. The positive samples have encapsidated CMV DNA protected from DNase digestion.

These samples were also tested using CMV RNA ELITe MBG Kit on plasma. Just 12 samples were found CMV RNA positive, while 26 samples resulted negative and 2 samples were invalid. The positive samples have virion CMV mRNA.

The discrepant results were resolved using the clinical outcome of the patients and other 6 samples were excluded.

The agreement between CMV RNA ELITe MBG Kit on plasma and CMV DNAemia on plasma after DNase treatment (reference) was analysed using the Cohen's Kappa value. The comparison after the discrepant result resolution is summarized in the following table.

	CMV DNAemia on DNase treated plasma		
CMV RNA ELITe MGB Kit	Positive	Negative	Total
Positive	6	1	7
Negative	1	24	25
Total	7	25	32

In this analysis, the CMV RNA ELITe MGB Kit showed a very good agreement with the CMV DNAemia

on DNase treated plasma method analysis generated a Cohen's Kappa value equal to 0.820.

A further evaluation of significance of results of CMV RNA ELITe MGB Kit versus CMV DNAemia on DNase treated plasma method is shown in the table below.

	Result
Sensitivity	85.7%
Specificity	96.0%
AUC	93.8%

Again, the CMV RNA ELITe MGB Kit showed a very good agreement with the CMV DNase plasma treatment method with an Area Under Curve (AUC) equal to 93.8%.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File "CMV RNA ELITe MGB Kit", FTP115ING.

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PROCEDURE LIMITATIONS

Use this product only with the following samples: plasma collected in EDTA.

Plasma collected in EDTA may be separated from whole blood stored at +2 / +25 °C for no longer than 24 hours.

Do not use this product with samples of plasma collected in heparin: heparin inhibits the reverse transcription and PCR reaction of nucleic acids and causes invalid results.

Currently, there are no data available concerning product performances with the following samples: Whole Blood, Cerebrospinal Fluid, Urine, Buccal Swab, Amniotic Fluid, Broncho-Alveolar Lavage.

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

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

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

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

CMV RNA ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTS115ING

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

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

It is necessary to have separate areas for the preparation of the complete reaction mixture and the extraction / amplification / detection of nucleic acids to prevent false positive results.

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

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

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

TROUBLESHOOTING

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture, Q-PCR Standards and Positive Control. Check the volumes of complete reaction mixture, Q-PCR Standards and Positive Control.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its components.	Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Prepare again the complete reaction mixture. Use a new aliquot of components.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 4 independent sessions (2 hours each in the Extraction Area). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area). Use new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

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

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture, internal control and sample. Check the volumes of complete reaction mixture, internal control and sample.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its components.	Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Prepare again the complete reaction mixture. Use a new aliquot of components.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the primary sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the standards or positive control.	Check for 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 with a possible mutation. The target in the sample should be sequenced to confirm mutation.

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

CMV RNA ELITe MGB® Kit
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SYMBOLS

REF Catalogue Number.

Upper limit of temperature.

LOT Batch code.

Use by (last day of month).

RUO Research use only.

Σ Contains sufficient for "N" tests.

Attention, consult instructions for use.

CONT Contents.

Keep away from sunlight.

Manufacturer.

NOTICE TO PURCHASER: LIMITED LICENSE

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

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

The “Human cytomegalovirus Detection and Quantification Method based on virionic RNA” is covered by Italian patent 102020000007357 and pending applications.

ELITe InGenius® technology is covered by patents and pending applications.

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

CMV RNA ELITe MGB® Kit
used in association with ELITe InGenius®
Ref: RTS115ING

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

Intended use

The CMV RNA ELITe MGB® Kit product is an *in vitro* reagent intended to be used as part of a quantitative nucleic acids reverse transcription and amplification assay for the detection and quantification of the human Cytomegalovirus virion mRNA (CMV RNA) extracted from non-cellular specimens.

The assay is tested in association with the ELITe InGenius® instrument, automated and integrated systems for extraction, reverse transcription, real time PCR and results interpretation, using human specimens of Plasma collected in EDTA.

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	CMV UL21.5 spliced mRNA	FAM	CMV RNA
Internal Control	MS2 phage	AP525	IC

Matrix

› **Plasma EDTA**

Kit content

CMV RNA ELITe MGB Kit (RTS115ING)		CMV RNA ELITe Standard (STD115ING)	CMV RNA - ELITe Positive Control (CTR115ING)
 X 4	 X 2	 X 2	 X 3
CMV RNA PCR Mix 4 tubes of 600 µL 96 reactions per kit 5 freeze-thaw cycles	RT EnzymeMix 2 tubes of 20 µL 96 reactions per kit 10 freeze-thaw cycles	Ready-to-use Calibrators: 10^5, 10^4, 10^3, 10^2 8 sessions per kit 2 set of 4 tubes of 160 µL 4 freeze-thaw cycles	Ready-to-use PC 3 tubes of 160 µL 12 sessions per kit 4 freeze-thaw cycles

Maximum shelf-life: **12 months**

Material required not provided in the kit

› ELITe InGenius instrument: INT030
› ELITe InGenius SP 1000 Extraction Cartridge: INT033SP1000
› ELITe InGenius PCR Cassette: INT035PCR
› ELITe InGenius SP200 Consumable Set: INT032CS
› ELITe InGenius Waste Box: F2102-000
› Filter Tips 300: TF-350-L-R-S

ELITe InGenius protocol

› Sample volume	600 µL	› Unit of quantitative result	copies/mL
› CPE volume	10 µL	› Frequency of controls	15 days
› Total elution volume	50 µL	› Frequency of calibration	60 days
› PCR input volume	20 µL		
› CMV RNA PCR Mix volume	20 µL		

Performance

Matrix	Limit of Detection	Method Agreement	Specificity
Plasma	30 copies / mL (0.19 PFU/mL)	**AUC = 93.8%** *32 certified samples* reference method: "CMV DNAemia on DNase treated plasma"	**100%** *40 confirmed samples / 40 tested samples* reference method: "CMV DNAemia on WB"

Sample preparation

Plasma samples collected in EDTA must be identified according to laboratory guidelines, transported and stored at room temperature (~+25 °C) or at +2 / +8 °C for a maximum of 24 hours. Otherwise, they must be frozen and stored at ~-20 °C for a maximum of one month. For longer periods, storage at ~-70 °C is recommended. Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

Procedures

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, reverse transcription, 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.
Log in with username and password.
Select the mode "Open".

2. Verify calibrators: **CMV RNA Q-PCR Standard** in the "Calibration" menu.
Verify controls: **CMV RNA Positive Control** and **CMV RNA Negative Control** in the "Controls" menu.
Note: Both must have been run, approved and not expired.

3. Thaw the **CMV RNA PCR Mix** and the **CTR CPE** tubes.
Vortex gently.
Spin down 5 sec.
Keep the **RT EnzymeMix** in ice.

4. Prepare the complete reaction mixture

Sample Number (N)	CMV RNA PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

5. Vortex gently
Spin down 5 sec
Keep the complete reaction mixture in ice. Do not expose to direct light.

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen

2. Verify the extraction volumes: Input: "1000 µL", elution: "50 µL"

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

4. Select the "Assay protocol" of interest: CMV RNA ELITe_PL_Op_600_50

5. Select the method "Extract + PCR" and the sample position: Extraction Tube

6. Load the complete reaction mixture and the Internal Control in the inventory block

7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube 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 method "PCR only" and set the sample position "Elution Tube"

6. Load the complete reaction mixture in the inventory block

7. Load: PCR cassette rack and the Elution tube rack with the extracted nucleic acid

8. Close the door
Start the run

9. View, approve and store the results

Procedure 3 - Extraction only

1 to 4: Follow the Complete Run procedure described above

5. Select the method "Extraction Only" and set the sample position: Extraction Tube

6. Load the Internal Control in the inventory block

7. Load: Extraction cartridge, Elution tube, Tip cassette, Extraction Tube racks

8. Close the door
Start the run

9. Archive the eluate sample