Instructions for use

ENTEROVIRUS ELITe MGB® Kit

reagents for RNA reverse transcription and Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS076PLD

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IVD

CHANGE HISTORY

<table>
<tr><th>Rev.</th><th>Notice of change</th><th>Date (dd/mm/yyyy)</th></tr>
<tr><td>07</td><td>Introduction of the Standard Curve Uncertainty, paragraph 11.3
Update of cross-reactivity, paragraph 11.5
Update of inhibition, paragraph 11.8</td><td>26/11/24</td></tr>
<tr><td>06</td><td>Compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements.
NOTE
the following product batches are still placed on the market as per IVDD till to their expiration dates, according to Article 110 of IVDR. If you have these product batches, please contact ELITechGroup staff to request the related previous version of IFUs.
<table>
<tr><th>PRODUCT REF.</th><th>Lot Number</th><th>Expiry date</th></tr>
<tr><td>RTS076PLD</td><td>U0624-154</td><td>31/12/2025</td></tr>
<tr><td>RTS076PLD</td><td>U1123-101</td><td>31/05/2025</td></tr>
<tr><td>RTS076PLD</td><td>U0823-002</td><td>28/02/2025</td></tr>
</table>
Change in PCR-Mix formulation/packaging to improve product usability: change from three components to two components only (RT enzyme and PCR Mix).
Expansion of the use of the product in association with ELITe BeGenius® instrument, with plasma and CSF matrices..
Update of PERFORMANCE CHARACTERISTICS paragraph. New evaluation studies have been performed. The following performances are updated: Clinical sample stability; Limit of detection; Linear Measuring Range; Cross reactivity; Inhibition organisms and substances; Repeatability and Reproducibility.
NOTE
Diagnostic Specificity and Diagnostic Sensitivity results of the new study have been confirmed
New graphics and content setting of the IFU</td><td>18/10/2024</td></tr>
<tr><td>05</td><td>The number of the analytical sessions to be carried out in association with the "ELITe InGenius" system has been modified.</td><td>10/05/2022</td></tr>
<tr><td>04</td><td>The number of reactions that could be performed in association with “ELITe InGenius“ system has been modified.</td><td>26/02/2020</td></tr>
<tr><td>03</td><td>Formal corrections</td><td>07/09/2018</td></tr>
<tr><td>02</td><td>Expansion of the use of the product in association with ELITe InGenius® instrument, with plasma and CSF matrices.
New packaging: plastic box instead of cardboard box</td><td>09/05/2016</td></tr>
<tr><td>00
—
01</td><td>New product development and succeeding changes</td><td>—</td></tr>
</table>

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1 INTENDED USE

The product **ENTEROVIRUS ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids reverse transcription and Real-Time PCR assay for the detection and quantification of the RNA of Human Enterovirus (A, B, C, D), extracted from clinical specimens.

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

The product is intended for use as an aid in the diagnosis and monitoring of Human Enterovirus (EV) infections in patients suspected of having an EV infection or undergoing monitoring of EV infection.

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

2 ASSAY PRINCIPLE

The assay is a quantitative One-Step Reverse Transcription Real-Time PCR detecting Enterovirus RNA isolated from specimens, retro-transcribed and then amplified using a complete reaction mixture that contains primers and probes with ELITe MGB technology.

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

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

3 PRODUCT DESCRIPTION

The **ENTEROVIRUS ELITe MGB Kit** provides the following components:

- **EV PCR Mix,** an optimized and stabilized PCR mixture that contains the specific primers and probes for:
  - **ENTEROVIRUS**, **5' UTR** region, detected in Channel **ENTEROVIRUS**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye.
  - Internal Control (**IC**), specific for a region of the phage MS2 genomic RNA, detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor® 525 (AP525) dye.

The **EV PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase. Each vial contains **600 µL** of solution and is sufficient for **24 tests**, if processing at least 5 samples per session.

- **RT EnzymeMix,** an optimized and stabilized mixture of enzymes for reverse transcription. Each vial contains **20 µL** of solution and is sufficient for **48 tests**, if processing at least 5 samples per session.

The **ENTEROVIRUS ELITe MGB Kit** contains sufficient reagents for **96 tests on ELITe InGenius** and **ELITe BeGenius**, with 20 µL of **EV PCR Mix** and 0.3 µL of **RT EnzymeMix** used per reaction.

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

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
EV PCR Mix ref. RTS076PLD	Mixture of reagents for reverse transcription and Real-Time PCR in tube with **WHITE cap**	**4 x 600 µL**	**-**
RT EnzymeMix ref. RTS003-RT	Reverse transcription enzymes in tube with **cap with BLACK insert**	**2 x 20 µL**	**-**

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.

- Disposable nitrile powder-free gloves or similar material.

- Vortex mixer.

- Bench centrifuge (~5,000 RPM).

- Bench microcentrifuge (~13,000 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).

- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).

- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample, the extraction and inhibition internal control, the amplification positive and negative controls, the DNA standards and the consumables are **not** provided with this product.

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

Table 2

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030) **ELITe InGenius Software** version 1.3.0.19 (or later) **EV ELITe _STD**, Assay Protocol with parameters for Calibrators analysis **EV ELITe _PC**, Assay Protocol with parameters for Positive Control analysis **EV ELITe _NC**, Assay Protocol with parameters for Negative Control analysis **EV ELITe_PL_200_100**, Assay Protocol with parameters for Plasma specimen analysis **EV ELITe_CSF_200_100**, Assay Protocol with parameters for cerebrospinal fluid specimen analysis	**ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS) **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR) **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000) **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only **CPE - Internal Control** (EG SpA, ref. CTRCPE) **ENTEROVIRUS ELITe Standard** (EG SpA, ref. STD076PLD) **ENTEROVIRUS - ELITe Positive Control** (EG SpA, ref. CTR076PLD)
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.2.1. (or later) **EV ELITe _Be_STD**, Assay Protocol with parameters for Calibrators analysis **EV ELITe _ Be_ PC**, Assay Protocol with parameters for Positive Control analysis **EV ELITe _ Be_ NC**, Assay Protocol with parameters for Negative Control analysis **EV ELITe_ Be_ PL_200_100**, Assay Protocol with parameters for Plasma specimen analysis **EV ELITe_ Be_ CSF_200_100**, Assay Protocol with parameters for cerebrospinal fluid specimen analysis	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

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

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

Wear suitable protective clothes and gloves and protect eyes and face.

Never pipette solutions by mouth.

Do not eat, drink, smoke or apply cosmetic products in the work areas.

Carefully wash hands after handling samples and reagents.

Dispose of leftover reagents and waste in compliance with the regulations in force.

Carefully read all the instructions provided before running the assay.

While running the assay, follow the product instructions provided.

Do not use the product after the indicated expiry date.

Only use reagents provided with the product and those recommended by the manufacturer.

Do not use reagents from different batches.

Do not use reagents from other manufacturers.

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

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

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

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles
EV PCR Mix	-20 °C or below (protected from light)	one month	up to five
RT EnzymeMix	-20 °C or below	one month	up to ten times, for up to ten minutes at +2 / +8 °C

8 SPECIMENS AND CONTROLS

8.1 Specimens

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

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ± 10 °C**	**-70 ± 15 °C**
Plasma	EDTA	≤ 24 hours	≤ 3 days	≤ 1 month	≤ 1 month
CSF	-	NR	≤ 24 hours	≤ 1 month	≤ 1 month

NR: not recommended.

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

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

Table 5 Assay Protocols for ENTEROVIRUS ELITe MGB Kit

Specimen	Instrument	Sample transfer	Assay Protocol Name	Report	Characteristics
plasma	**ELITe InGenius**	not required	**EV ELITe_PL_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	not required	**EV ELITe_Be_PL_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
CSF	**ELITe InGenius**	required, in Extraction tube	**EV ELITe_CSF_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	required, in 2 mL Sarstedt Tube	**EV ELITe_Be_CSF_200_100**	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL

When required, 200 µL of sample must be transferred into an Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius).

NOTE

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

The volume of the sample in a primary tube varies according to the type of the tube loaded. Refer to the extraction kit instructions for use or **ELITe InGenius** and **ELITe BeGenius** manual for more information on how to set up and perform the extraction procedure.

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

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

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

8.2 PCR calibrators and controls

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

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

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

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

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the calibration curve and PCR control validation for each lot of PCR reagent.

Calibration curves expire after **60 days**, at which time it is necessary to re-run the calibration.

PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and negative controls.

The Calibrators and PCR controls must be re-run if any of the following events occur:

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

8.3 Quality controls

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

9 ELITe InGenius PROCEDURE

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

Table 6

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

9.1 STEP 1 – Verification of the system readiness

Before starting the session:

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

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

9.2 STEP 2 – Session Setup

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

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Calibration run (PCR Only),

D. Positive Control and Negative Control run (PCR Only).

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

NOTE

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

Before to setup a run:

1. Thaw the needed **EV PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (5 or more tests per session).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 **PCR Mix** from light while thawing because this reagent is photosensitive.

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **48 tests** in optimized conditions (5 or more tests per session). 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 **EV 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.

Table 7

Samples Number (N)	EV 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 in 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** can be used within **7** hours if kept in a refrigerated block (for 2 sessions of 3 hours each and for the time needed to start a third session).The complete reaction mixture **cannot** be stored for re-use.

NOTE

The **complete reaction mixture** is sensitive to the light, do not expose it to direct light.

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

Table 8

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

Table 8 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
15	Close the instrument door.	Close the instrument door.
16	Press “Start”.	Press “Start”.

Table 9

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

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

NOTE

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

NOTE

The **complete reaction mixture** can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3 hours each and for the time needed to start a third session).Mix gently and spin down the content for 5 seconds before starting the next session. The complete reaction mixture **cannot** be stored for re-use.

NOTE

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

NOTE

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

NOTE

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

NOTE

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

NOTE

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

9.3 STEP 3 - Review and approval of results

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

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

NOTE

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

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

1. Validation of Calibration curve,
2. Validation of Positive Control and Negative Control results,
3. Validation of sample results,
4. Sample result reporting.

9.3.1 Validation of Calibration curve

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

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

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

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

9.3.2 Validation of amplification Positive Control and Negative Control results

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

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

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

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

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

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

9.3.3 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the target (Channel **EV**) and the Internal Control (Channel **IC**) with the **EV ELITe_PL_200_100** and **EV ELITe_CSF_200_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in “Results Display” screen.

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

Table 10

1) Calibration Curve	**Status**
EV Q - PCR Standard	APPROVED
2) Positive Control	**Status**
EV - Positive Control	APPROVED
3) Negative Control	**Status**
EV - Negative Control	APPROVED

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

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

Table 11

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

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control RNA was not efficiently detected, which could be due to problems in sample collection, extraction, reverse transcription or PCR steps (e.g. incorrect sampling, 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 (see 14 TROUBLESHOOTING page 32).

Samples reported as “EV:RNA Not detected or below “LoD" copies/mL” are suitable for analysis but EV was not detected. In this case, the sample may be either negative for EV RNA or the EV RNA is present at a concentration below the Limit of Detection of the assay (see 11 PERFORMANCE CHARACTERISTICS page 21).

EV RNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as “EV: RNA Detected, quantity below “LLoQ” copies/mL” (see 11 PERFORMANCE CHARACTERISTICS page 21).

EV RNA positive samples within the Linear Measuring Range (see 11 PERFORMANCE CHARACTERISTICS page 21) are detected and are reported as “EV:RNA Detected, quantity equal to “XXX” copies / mL”.

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

NOTE

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

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

9.3.4 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.

10 ELITe BeGenius PROCEDURE

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

Table 12

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

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

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

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

10.2 STEP 2 – Session Setup

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

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Calibration run (PCR Only),

D. Positive Control and Negative Control run (PCR Only).

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

NOTE

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

Before to setup a run:

1. Thaw the needed **EV PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (5 or more tests per session).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 **PCR Mix** from light while thawing because this reagent is photosensitive.

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **48 tests** in optimized conditions (5 or more tests per session).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 **EV 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.

Table 13

Sample Number (N)	EV 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
13 ≤ N ≤ 18	(N + 3) x 20 µL	(N + 3) x 0.3 µL
19 ≤ N ≤ 23	(N + 4) x 20 µL	(N + 4) x 0.3 µL
N = 24	580 µL	8.7 µL

5. Prepare the **complete reaction mixture** by transferring in 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** can be used within **7** hours if kept in a refrigerated block (for 2 sessions of 3 hours each and for the time needed to start a third session).The complete reaction mixture **cannot** be stored for re-use.

NOTE

The **complete reaction mixture** is sensitive to the light, do not expose it to direct light.

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

Table 14

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If required, transfer 200 µL of sample in a 2mL Sarstedt tube (not provided) previously labeled. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	If needed, **thaw the Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen.

Table 14 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
3	Remove all the Racks from the “Cooler Unit” and place them on the preparation table.	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) of the “Cooler Unit” and place them on the preparation table.
4	Select the “Run mode”: “**Extract + PCR”.**	Select the “Run mode”: “PCR Only”.
5	**Load the samples** into the “Sample Rack”. (**Note;** when secondary tubes “2 mL Tubes” are loaded, use the blue adaptors for the “Sample Rack”).	**Load the samples** into the “Elution Rack”.
6	Insert the “Sample Rack” into the “Cooler Unit” starting from the “Lane 5” (L5). Insert the “Sample ID” (SID) for each “Position” used. (If secondary tubes are loaded, flag “2 mL Tube”. If secondary tubes are not barcoded, type manually the “Sample ID”).	Insert the “Elution Rack” into the “Cooler Unit” starting from “Lane 3” (L3) For each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).
7	Click “Next” to continue.	Click “Next” to continue.
8	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
9	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).
10	Click “Next” to continue.	Click “Next” to continue.
11	When more than 12 samples are processed, repeat the procedure from point 6	When more than 12 samples are processed, repeat the procedure from point 6
12	Load the “Elution tubes” into the “Elution Rack” (Elution tubes can be labelled with barcode to improve traceability).	Not applicable
13	Insert the “Elution Rack” into the “Cooler Unit” starting from “Lane 3” (L3). When more than 12 samples are processed, repeat using “Lane 2” (L2).	Not applicable
14	Click “Next” to continue.	Not applicable
15	**Load CPE** and the **complete reaction mixture** into the “Reagent/Elution Rack”.	**Load the complete reaction mixture** into “Reagent/ Elution Rack”.
16	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). For each PCR Mix and / or CPE enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/ R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). For each PCR Mix enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
17	Click “Next” to continue	Click “Next” to continue.
18	Verify the tips in the “Tip Rack(s)” in the “Inventory Area” and replace Tip Rack(s) if necessary.	Verify the tips in the “Tip Rack(s)” in the “Inventory Area” and replace Tip Rack(s) if necessary.
19	Click “Next” to continue.	Click “Next” to continue.
20	Load the “**PCR Rack**” with “**PCR Cassette**” in the Inventory Area.	Load the “**PCR Rack**” with “**PCR Cassette**” in the Inventory Area.
21	Click “Next” to continue.	Click “Next” to continue.
22	Load the “**Extraction Rack**” with the “ELITe InGenius SP 200” extraction cartridges and required extraction consumables.	Not applicable

Table 14 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
23	Close the instrument door.	Close the instrument door.
24	Press “Start”.	Press “Start”.

Table 15

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

Table 15 (continued)

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
17	Click “Next” to continue.	Click “Next” to continue.
18	Close the instrument door.	Close the instrument door.
19	Press “Start”.	Press “Start”.

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

NOTE

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

NOTE

The **complete reaction mixture** can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3 hours each and for the time needed to start a third session).Mix gently then spin down the content for 5 seconds before starting the next session. The complete reaction mixture **cannot** be stored for re-use.

NOTE

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

NOTE

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

NOTE

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

NOTE

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

NOTE

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

10.3 STEP 3 - Review and approval of results

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

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

NOTE

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

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

1. Validation of Calibration curve,
2. Validation of Positive Control and Negative Control results,
3. Validation of sample results,
4. Sample result reporting.

NOTE

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

11 PERFORMANCE CHARACTERISTICS

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined with Plasma collected in EDTA and Cerebrospinal (CSF) samples in association with ELITe InGenius and ELITe BeGenius instruments.

Plasma Collected in EDTA:

The LoD of the assay was determined with Plasma samples on ELITe InGenius spiked with the reference material of Enterovirus 71 (ZeptoMetrix, US); Probit regression analysis was performed on the results and the LoD defined as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Table 16

Limit of Detection (copies / mL) for Plasma samples and ELITe InGenius		
LoD	95% confidence interval	
	Lower bound	Upper bound
141	101	278

The calculated LoD value was verified by testing negative pool of Plasma collected in EDTA spiked with *Enterovirus* certified reference material at the claimed concentration. The results obtained confirmed the claimed concentration for the target on both ELITe InGenius and ELITe BeGenius instruments.

Cerebrospinal Fluid (CSF):

The LoD of the assay was determined with CSF samples on ELITe InGenius spiked with the reference material of Enterovirus 71 (ZeptoMetrix, US); Probit regression analysis was performed on the results and the LoD defined as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Table 17

Limit of Detection (copies / mL) for CSF samples and ELITe InGenius		
LoD	95% confidence interval	
	Lower bound	Upper bound
490	304	1185

The calculated LoD value was verified by testing negative pool of CSF spiked with Enterovirus certified reference material at the claimed concentration. The results obtained confirmed the claimed concentration for the target on both ELITe InGenius and ELITe BeGenius instruments.

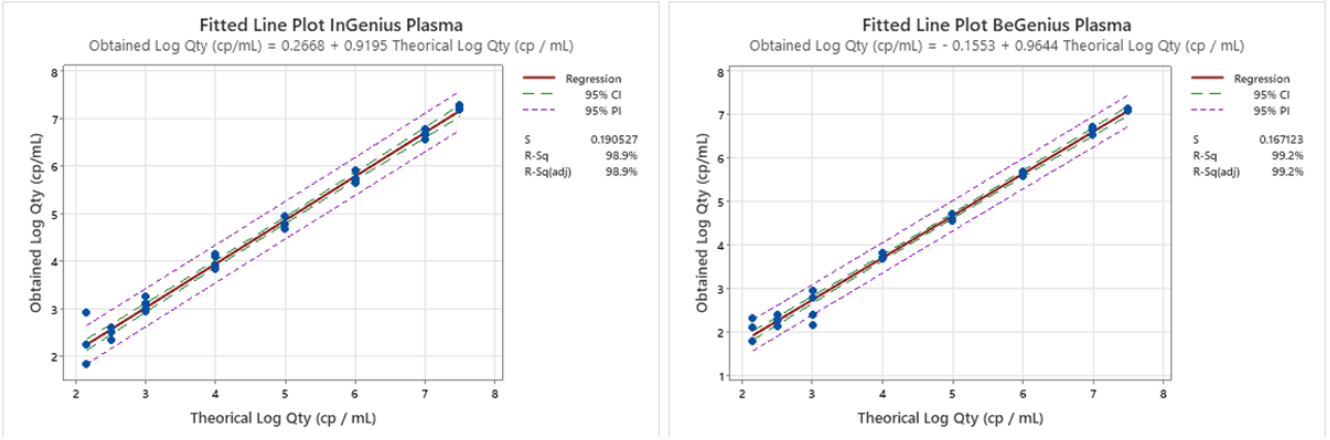
11.2 Linear measuring range

The Linear measuring range of ENTEROVIRUS ELITe MGB Kit was determined with Plasma samples and CSF on ELITe InGenius and ELITe BeGenius.

Plasma collected in EDTA:

The Linear measuring range of ENTEROVIRUS ELITe MGB Kit was determined with Plasma samples on ELITe InGenius and ELITe BeGenius using dilutions of Enterovirus 71 reference material (ZeptoMetrix, US); Linear regression was performed on the results and the Linear Measuring Range was defined.

The results are reported in the following figure.



The final results are summarized in the following table.

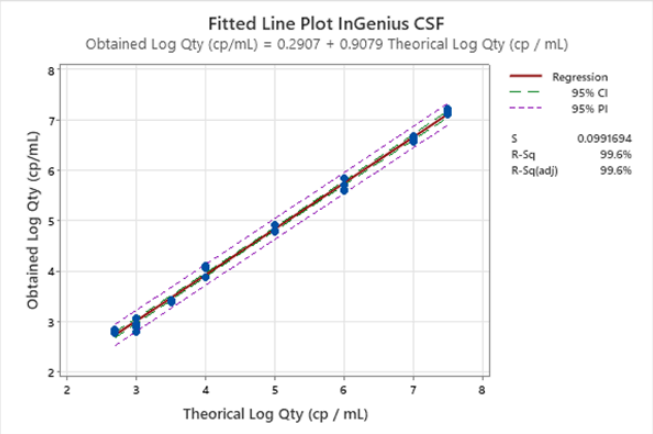
Table 18

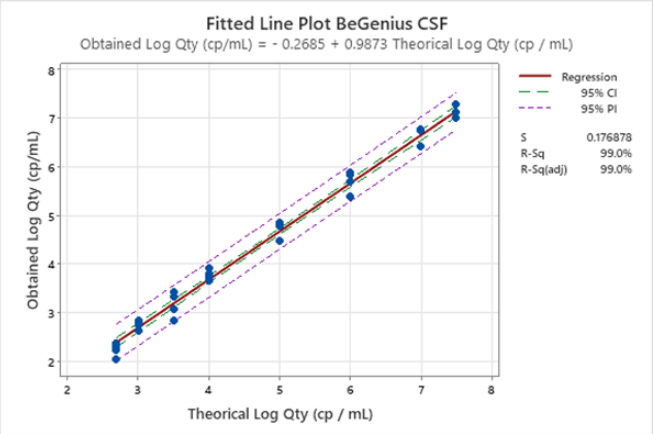
Linear measuring range for EDTA Plasma samples and ELITe InGenius and ELITe BeGenius	
Lower Limit	Upper Limit
141 copies / mL	31,622,777 copies / mL

CSF:

The Linear measuring range of ENTEROVIRUS ELITe MGB Kit was determined with CSF samples on ELITe InGenius and ELITe BeGenius using dilutions of Enterovirus 71 reference material (ZeptoMetrix, US); Linear regression was performed on the results and the Linear Measuring Range was defined.

The results are reported in the following figure.





The final results are summarized in the following table.

Table 19

Linear measuring range for CSF samples and ELITe InGenius and ELITe BeGenius	
Lower Limit	Upper Limit
490 copies / mL	31,622,777 copies / mL

11.3 Standard Curve Uncertainty

The Uncertainty value of the Standard curve was calculated by combining the random errors (SD) of all level quantifications and multiplying for the Coverage factor k = 2 (Expanded Combined Uncertainty) and is equal to 0.1507 Log copies / reaction.

Table 20

Standard curve levels	**Theoretical**	**Measured**	**SD**	**Expanded Combined Uncertainty**
	Log c/rxn	**Log c/rxn**		
EV Q - PCR Standard 10^5	5.0000	5.0214	0.0334	0.1507
EV Q - PCR Standard 10^4	4.0000	3.9727	0.0381	
EV Q - PCR Standard 10^3	3.0000	2.9905	0.0264	
EV Q - PCR Standard 10^2	2.0000	2.0154	0.0491	

11.4 Inclusivity: Efficiency of detection on different genotypes

The inclusivity of the assay, as efficiency of detection for different genotypes of Enterovirus was evaluated by *in silico* analysis of the sequences available in nucleotide databases. The analysis showed sequence conservation and absence of significant mutations. So, efficient detection of the following genotype Enterovirus A (Coxsackievirus A2-A8, A10, A12, A14, A16 e Enterovirus EV71, EV76, EV89-EV92), Enterovirus B (Coxsackievirus B1-B6, A9, Enterovirus EV69, EV73, EV74, EV75, EV77, EV79-EV88, EV97, EV98, EV100, EV101, EV107, Echovirus E1-E21, E24-E27, E29-E33), Enterovirus C (Coxsackievirus A1, A11, A13, A15, A17-A22, A24, Poliovirus PV1-PV3, Enterovirus EV96, EV99, EV102, EV109), Enterovirus D (Enterovirus EV68, EV70, EV94) is expected.

11.5 Potentially interfering markers: Cross-reactivity

The potential Cross-reactivity of the assay with ENTEROVIRUS genomic RNA and other unintended organisms, included Enterovirus phylogenetically close viruses (Rhinovirus and Parechovirus), was evaluated by *in silico* analysis of sequences available in nucleotide databases.

The analysis on Rhinovirus and Parechovirus demonstrated partial specificity. No significant homologies were detected with the genomic sequences of human Parechoviruses A1, A2, A3, A4, A5, A6, A7, A8 while the product shown a possible cross-reactivity with some genotype of Rhinovirus (with an exception for Rhinovirus B14).

The analysis showed no significant homology with others unintended organisms; therefore, no cross-reactivity is expected.

The potential Cross-reactivity with other organisms that might be found in clinical samples of Plasma was also verified by testing a panel of certified reference materials (ATCC and NIBSC) at high titre.

The results are reported in the following table.

Table 21

Sample	Pos. / Rep.	Outcome
HIV1	0 / 6	No cross-reactivity
EBV	0 / 6	No cross-reactivity
HAV	0 / 6	No cross-reactivity
HBV	0 / 6	No cross-reactivity
HHV6	0 / 6	No cross-reactivity
HSV1	0 / 6	No cross-reactivity
HSV2	0 / 6	No cross-reactivity
HEV	0 / 6	No cross-reactivity
RSV	0 / 6	No cross-reactivity
VZV	0 / 6	No cross-reactivity
Flu A	0 / 6	No cross-reactivity
Flu B	0 / 6	No cross-reactivity
CMV	0 / 6	No cross-reactivity
ADV	0 / 6	No cross-reactivity
PVB19	0 / 6	No cross-reactivity
HCV	0 / 6	No cross-reactivity
Aspergillus fumigatus	0 / 6	No cross-reactivity
Staphylococcus aureus	0 / 6	No cross-reactivity
Candida albicans	0 / 6	No cross-reactivity
BKV	0 / 6	No cross-reactivity
Rhinovirus B14	0 / 6	No cross-reactivity
Parechovirus 1	0 / 6	No cross-reactivity

All potentially interfering markers tested showed no cross-reactivity for the EV target amplification using the ENTEROVIRUS ELITe MGB Kit.

11.6 Potentially interfering markers: Inhibition

The potential inhibition caused by other organisms that might be found in clinical samples of Plasma or CSF was verified by testing a panel of certified reference materials.

The results are reported in the following table.

Table 22

Sample	EV Pos. / Rep.	Outcome
HIV1	6 / 6	No interference
EBV	6 / 6	No interference
HAV	6 / 6	No interference
HBV	6 / 6	No interference
HHV6	6 / 6	No interference
HSV1	6 / 6	No interference
HSV2	6 / 6	No interference
HEV	6 / 6	No interference
RSV	6 / 6	No interference
VZV	6 / 6	No interference
Flu A	6 / 6	No interference
Flu B	6 / 6	No interference
CMV	6 / 6	No interference
ADV	6 / 6	No interference
PVB19	6 / 6	No interference
HCV	6 / 6	No interference
Aspergillus fumigatus	6 / 6	No interference
Staphylococcus aureus	5 / 6 *	No interference
Candida albicans	6 / 6	No interference
BKV	6 / 6	No interference
Rhinovirus B14	6 / 6	No interference
Parechovirus 1	6 / 6	No interference

Note: due to an operator error, one sample were not considered in the analyses because resulted invalid. The minimum sample size requested in the test was 5 so the sample was not retested.

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

11.7 Potentially interfering substances: Cross-reactivity

Plasma collected in EDTA:

The Cross-reactivity of the assay with Potentially interfering substances(endogenous and exogenous) that might be found in plasma clinical specimens was evaluated by analysis of a panel of substances at relevant concentration.

The results are reported in the following table.

Table 23

Sample	EV Pos. / Rep.	Outcome
Icteric Plasma	0/6	No cross-reactivity
Lipemic Plasma	0/6	No cross-reactivity
Haemolytic Blood H	0/6	No cross-reactivity
Haemolytic Blood M	0/6	No cross-reactivity
HaemolyticBlood L	0/6	No cross-reactivity
Heparinized Plasma	0/6	No cross-reactivity
EDTA Plasma	0/6	No cross-reactivity
Ampicillin	0/6	No cross-reactivity
Cefedoxime	0/6	No cross-reactivity
Azytromycin	0/5*	No cross-reactivity
Ganciclovir	0/5*	No cross-reactivity
Ribavirin	0/6	No cross-reactivity
Abacavir Sulfate	0/6	No cross-reactivity
Cidofovir	0/6	No cross-reactivity
Cyclosporine A	0/6	No cross-reactivity
Bactrim	0/6	No cross-reactivity
Ciprofloxacin	0/6	No cross-reactivity

Note: due to an operator error, one sample were not considered in the analyses because resulted invalid. The minimum sample size requested in the test was 5 so the sample was not retested.

The test showed that all the substances do not cross-react with EV target amplification using the ENTEROVIRUS ELITe MGB Kit.

CSF:

The Cross-reactivity of the assay with Potentially interfering substance that might be found in CSF clinical specimens was evaluated by analysis of a panel of substances at relevant concentration.

The results are reported in the following table.

Table 24

Sample	EV Pos. / Rep.	Outcome
5% Whole Blood	0/6	No Cross-reactivity

The test showed that Whole Blood do not cross-react with EV target using the ENTEROVIRUS ELITe MGB Kit.

11.8 Potentially interfering substances: Inhibition

Plasma collected in EDTA:

The Inhibition by Potentially interfering substances (endogenous and exogenous) that might be found in plasma clinical specimens was evaluated by analysis of a panel of substances at relevant concentration.

The results are reported in the following table.

Table 25

Sample	EV Pos. / Rep.	Outcome
Icteric Plasma	6/6	No interference
Lipemic Plasma	6/6	No interference
Haemolytic Blood H	6/6	No interference
Haemolytic Blood M	6/6	No interference
HaemolyticBlood L	6/6	No interference
Heparinized Plasma	3/6	Invalid Sample
EDTA Plasma	6/6	No interference
Ampicillin	6/6	No interference
Cefedoxime	6/6	No interference
Azytromycin	6/6	No interference
Ganciclovir	6/6	No interference
Ribavirin	6/6	No interference
Abacavir Sulfate	6/6	No interference
Cidofovir	6/6	No interference
Cyclosporine A	6/6	No interference
Bactrim	6/6	No interference
Ciprofloxacin	6/6	No interference

The test showed that all the substances, with the exception for heparin, do not inhibit the EV target detection and quantification using the ENTEROVIRUS ELITe MGB Kit.

Heparin was confirmed to be capable of inhibiting the assay. However, due to failure of the Internal Control (IC Ct > 35), these sample resulted “Invalid” instead of false “Negative”.

CSF:

The Inhibition by Potentially interfering substance that might be found in CSF clinical specimens was evaluated by analysis of a panel of substances at relevant concentration.

The results are reported in the following table.

Table 26

Sample	EV Pos. / Rep.	Quantification Bias	Outcome
5% Whole Blood	6/6	0.0653	No interference

The test showed that Whole Blood do not inhibit the EV target detection and quantification using the ENTEROVIRUS ELITe MGB Kit.

11.9 Repeatability

The Intra-Session and Inter-Session Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Plasma samples, including one negative sample and two samples spiked by EV certified reference material (Zeptometrix, US).

An example of Intra-Session Repeatability (on one day) results is shown in the tables below.

Table 27

Intra – Session Repeatability ELITe InGenius (Day1)								
Sample	**N**	**ENTEROVIRUS**			**Agreement**	**Internal Control**		
		Mean Ct	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
ENTEROVIRUS ELITe MGB Kit								
Negative	8	-	-	-	100%	29.16	0.23	0.78
3 X LOD	8	35.44	0.20	0.57	100%			
10 X LOD	8	33.88	0.29	0.86	100%			
10 X LOD	8	33.79	0.28	0.83	100%			

Table 28

Intra – Session Repeatability ELITe BeGenius (Day1)								
Sample	**N**	**ENTEROVIRUS**			**Agreement**	**Internal Control**		
		Mean Ct	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
ENTEROVIRUS ELITe MGB Kit U0722-018 (LP1) - Day 1								
Negative	8	-	-	-	100%	30.31	0.71	2.36
3 X LOD	8	37.75	1.44	3.82	100%			
10 X LOD	8	35.46	0.44	1.25	100%			

An example of Inter-Session Repeatability (on two days) results is shown in the tables below.

Table 29

Inter – Session Repeatability ELITe InGenius (Day1 + Day2)								
Sample	**N**	**ENTEROVIRUS**			**Agreement**	**Internal Control**		
		Mean Ct	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
ENTEROVIRUS ELITe MGB Kit								
Negative	16	-	-	-	100%	29.21	0.21	0.73
3 X LOD	16	35.25	0.47	1.34	100%			
10 X LOD	16	33.92	0.43	1.26	100%			

Table 30

Inter – Session Repeatability ELITe BeGenius (Day1 + Day2)								
Sample	**N**	**ENTEROVIRUS**			**Agreement**	**Internal Control**		
		Mean Ct	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
ENTEROVIRUS ELITe MGB Kit U0722-018 (LP1) - Day 1 - 2								
Negative	16	-	-	-	100%	30.45	0.96	3.17
3 X LOD	16	37.46	1.31	3.49	100%			
10 X LOD	16	35.72	0.73	2.04	100%			

In the Repeatability test, the ENTEROVIRUS ELITe MGB Kit detected the target and showed a maximum variability of target Ct values as %CV lower than 5%.

11.10 Reproducibility

The Reproducibility of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Plasma samples, including one negative sample and two samples spiked by EV certified reference material (Zeptometrix, US).

A summary of Inter-Instrument Reproducibility shown in the tables below.

Table 31

ELITe InGenius Inter-Instrument Reproducibility								
Sample	**N**	**ENTEROVIRUS**			**Agreement**	**Internal Control**		
		mean Ct	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	24	-	-	-	100%	29.37	0.36	1.22
3X LoD	24	35.62	0.55	1.55	100%			
10X LoD	24	33.77	0.36	1.05	100%			

Table 32

ELITe BeGenius Inter-Instrument Reproducibility								
Sample	**N**	**ENTEROVIRUS**			**Agreement**	**Internal Control**		
		mean Ct	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	24	-	-	-	100%	30.48	0.49	1.62
3X LoD	24	37.45	1.05	2.81	100%			
10X LoD	24	35.24	0.63	1.78	100%			

A summary of Inter-batch Reproducibility (on three lots) is shown in the tables below:

Table 33

ELITe InGenius Inter-Batch Reproducibility								
		ENTEROVIRUS				Internal Control		
Sample	N	mean Ct	SD	%CV	Agreement	Mean Ct	SD	%CV
Negative	48	-	-	-	100%	29.30	0.32	1.10
3X LoD	48	35.55	0.35	0.99	100%			
10X LoD	48	33.94	0.33	0.98	100%			

Table 34

ELITe BeGenius Inter-Batch Reproducibility								
		ENTEROVIRUS				IC		
Sample	N	mean Ct	SD	%CV	Agreement	Mean Ct	SD	%CV
Negative	48	-	-	-	100%	30.44	0.91	2.99
3X LoD	48	37.54	1.30	3.47	100%			
10X LoD	48	35.47	0.84	2.37	100%			

In the Reproducibility test, the ENTEROVIRUS ELITe MGB Kit correctly detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

11.11 Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative samples, was evaluated in association with ELITe InGenius by analyzing clinical samples of Plasma EDTA and CSF, certified negative for the target. As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Table 35

Negative for EV RNA	N	Positive	Negative	% Diagnostic Specificity
Plasma EDTA	50	0	50	100%
CSF	50	0	50	100%

The Diagnostic Specificity of the ENTEROVIRUS ELITe MGB Kit in association to Plasma EDTA and CSF samples in this test was equal to 100%.

The IC Ct cut-off value is set at 35 for both matrices.

11.12 Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with ELITe InGenius by analyzing clinical samples of Plasma EDTA and CSF spiked with different reference materials. 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 results are summed up in the following table.

Table 36

Spiked for EV RNA	N	Positive	Negative	% Diagnostic Sensitivity
Plasma EDTA	64	64	0	100%
CSF	64	64	0	100%

The Diagnostic Sensitivity of the ENTEROVIRUS ELITe MGB Kit in association to plasma EDTA and CSF samples in this test was equal to 100%.

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File ENTEROVIRUS ELITe MGB® Kit, FTP 076PLD.

12 REFERENCES

W. A. Verstrepen et al. (2001) J Clin Microbiology 39: 4093 - 4096.

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

A. A. Lukhtanov et al. (2007) Nucleic Acids Res. 35: e30.

C. N. Kotton et al. (2018) Transplantation 02: 900 - 931.

13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: Plasma collected in EDTA and CSF.

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

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

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

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

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

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

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

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques such as extraction, reverse transcription, 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 amplification products to prevent 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 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 titer than the product detection limit (see “Performance Characteristics”). In this case the result could be a false negative.

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

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

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

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

14 TROUBLESHOOTING

Table 37

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.
Degradation of complete reaction mixture or of its components.	Do not use the complete reaction mixture for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 ° C for more than 10 minutes. 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 or in the Cooler Unit). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 38

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 Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the complete reaction mixture or of its components.	Prepare again the complete reaction mixture. Use a new aliquot of components.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit.	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 39

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 use the complete reaction mixture for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 ° C for more than 10 minutes. 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 sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 40

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

Table 41

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

Table 42

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

15 SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.

	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE 0123	Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.
UDI	Unique Device Identification
	Contains sufficient for "N" tests.
	Caution, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

16 NOTICE TO THE USERS

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and /or the patient is established. At the moment of the current revision of the IFU, no serious incident or recall with impact on product performance and safety of the device has occurred.

A “Summary of Safety and Performance” will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional. Before the notice of full functionality of Eudamed has been published, the “Summary of Safety and Performance” will be made available to the public upon request by email at emd.support@elitechgroup.com, without undue delay.

17 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 7319022, 7348146, 7381818, 7541454, 7671218, 7718374, 7723038, 7759126, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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

The ELITe MGB® logo, the ELITe InGenius® and ELITe BeGenius® are registered trademarks of ELITechGroup within the European Union.

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

Appendix A ENTEROVIRUS ELITe MGB Kit used in association with Genius series® platforms

CAUTION

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

INTENDED USE

The product **ENTEROVIRUS ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids reverse transcription and Real-Time PCR assay for the detection and quantification of the RNA of Human Enterovirus (A, B, C, D), extracted from clinical specimens.

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

The product is intended for use as an aid in the diagnosis and monitoring of Human Enterovirus (EV) infections in patients suspected of having an EV infection or undergoing monitoring of EV infection.

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

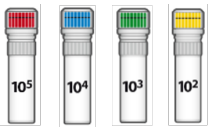
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	EV 5' UTR region	FAM	EV
Internal Control	MS2 phage protein A gene	AP525	IC

Validated matrices

- Plasma collected in EDTA
- Cerebrospinal Fluid (CSF)

Kit content and related products

ENTEROVIRUS ELITe MGB Kit (RTS020PLD)		**ENTEROVIRUS ELITe Standard (STD020PLD)**	**ENTEROVIRUS - ELITe Positive Control (CTR020PLD)**
 X 4		 X 2	 X 2
EV 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 4 levels: 10^5, 10^4, 10^3, 10^2 2 set of 4 tubes of 160 µL 4 freeze-thaw cycles	Ready-to-use Positive Control 2 tubes of 160 µL 8 reactions per kit 4 freeze-thaw cycles
Maximum shelf-life: **18 months** Storage Temperature: **-20 °C**		Maximum shelf-life: **24 months** Storage Temperature: **-20 °C**	Maximum shelf-life: **24 months** Storage Temperature: **-20 °C**

Other products required not provided in the kit

• ELITe InGenius instrument: INT030. • ELITe BeGenius instrument: INT040. • ELITe InGenius SP 200: INT032SP200. • ELITe InGenius SP 200 Consumable Set: INT032CS.	• ELITe InGenius PCR Cassette: INT035PCR. • ELITe InGenius Waste Box: F2102-000. • CPE - Internal Control: CTRCPE • 300 µL Filter Tips Axigen: TF-350-L-R-S. • 1000 µL Filter Tips Tecan: 30180118.

ELITe InGenius and ELITe BeGenius protocol

Table 43

› Extraction Input Volume › CPE volume › Extraction Elution Volume › Sample PCR input volume	200 µL 10 µL 100 µL 10 µL	› PCR Mix volume › Frequency of controls › Frequency of calibration › Unit of quantitative result	20 µL 15 days 60 days copies/mL

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Sensitivity	Specificity
Plasma EDTA	141 copies / mL	**100%** *64 tested samples*	**100%** *50 tested samples*
CSF	490 copies / mL	**100%** *64 tested samples*	**100%** *50 tested samples*

Sample preparation

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

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ± 10 °C**	**-70 ± 15 °C**
Plasma	EDTA	≤ 24 hours	≤ 3 days	≤ 1 month	≤ 1 month
CSF	-	NR	≤ 24 hours	≤ 1 month	≤ 1 month

NR: not recommended

ELITe InGenius Procedures

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

Before analysis

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

4. Prepare the complete reaction mixture

Samples Number (N)	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: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen.	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL".	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID.
4. Select the "Assay Protocol" of interest: EV ELITe_PL_200_100 and EV ELITe_CSF_200_100	**5.** Select the method "Extract + PCR" and the sample position "Extraction Tube".	**6.** Load the complete reaction mixture 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.

NOTE

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

Procedure 2: PCR Only (e.g., eluates, standards, controls)

1. to 4. Follow the Procedure 1 described above (select the Assay Protocols: EV ELITe_PC and EV ELITe_NC or EV ELITe_STD)	**5.** Select the method "PCR Only" and 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

ELITe BeGenius Procedures

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

Before analysis

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

4. Prepare the complete reaction mixture:

Samples Number (N)	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
13 ≤ N ≤ 18	(N + 3) x 20 µL	(N + 3) x 0.3 µL
19 ≤ N ≤ 23	(N + 4) x 20 µL	(N + 4) x 0.3 µL
N = 24	580 µL	8.7 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: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: "200 µL", Eluate: "100 µL"
4. Select the "Assay protocol" of interest (EV ELITe_Be_PL_200_100 or EV ELITe_Be_CSF_200_100 **Note:** If a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the complete reaction mixture and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load "PCR Rack" with "PCR Cassette" and the "Extraction Rack" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE

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

Procedure 2: PCR Only (e.g., eluates, standards, controls)

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

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