Instructions for use

GI Viral PLUS ELITe MGB® Kit

reagents for RNA reverse transcription and Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS501ING

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IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
01	New cap color of the PCR Mix component tubes. Update of the paragraphs: “Other product required”, “Materials required but not provided”, ”Symbols”, “Notice to the users” and “Notice to purchaser” New graphics and content setting of the IFU.	28/10/25
00	new product development	15/03/24

NOTE
The revision of this IFU is also compatible with the previous versions of the kit

TABLE OF CONTENT

1 INTENDED USE

The product **GI Viral PLUS ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids reverse transcription and Real-Time PCR assay for the **detection and identification** of the genomic DNA of Adenovirus (ADV), and the genomic RNA of Norovirus (NV), Rotavirus (RV), Astrovirus (ASV) and Sapovirus (SV), extracted from clinical specimens.

The assay is able to detect the DNA of Adenovirus belonging to serotypes F40 and F41 (typed by melting analysis), the RNA of Norovirus belonging to genogroups GI and GII (typed by melting analysis), Rotavirus belonging to group A, human Astrovirus and human Sapovirus.

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 stool specimens.

The product is intended for use as an aid in the diagnosis of gastrointestinal viral infections in patients suspected of having Adenovirus, Norovirus, Rotavirus, Astrovirus or Sapovirus infection.

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

2 ASSAY PRINCIPLE

The assay is a qualitative multiplex One-Step Reverse Transcription Real-Time PCR detecting the DNA of Adenovirus and RNA of Norovirus, Rotavirus, Astrovirus and Sapovirus 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).

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 **GI Viral PLUS ELITe MGB Kit** provides the following components:

- **GI-V PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:
- Adenovirus F40 and F41 Hexon protein gene, detected in Channel **ADV**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor® 639 (AP639) dye,
- Norovirus GI and GII Polyprotein RdRp gene, detected in Channel **NV**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by FAM dye,
- Rotavirus group A NSP3 gene, detected in Channel **RV**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 593 (AP593) dye,
- Astrovirus Capsid protein gene, detected in Channel **ASV**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 690 (AP690) dye,
- Sapovirus GI/GII/GIV and Sapovirus GV Polyprotein gene, detected in Channel **SV**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 559 (AP559) 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 **GI-V PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

- **RT EnzymeMix**, an optimized and stabilized mixture of enzymes for reverse transcription.

The **GI Viral PLUS ELITe MGB Kit** contains sufficient reagents for **96 tests** on the **ELITe InGenius** and **ELITe BeGenius,** with 20 µL of **GI-V PCR Mix** and 0,3 µL **RT EnzymeMix** used per reaction.

The **GI Viral PLUS 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
GI-V PCR Mix ref. RTS501ING	Mixture of reagents for reverse transcription and Real-Time PCR in tube with **NATURAL 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).
- Thermomixer.
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (volume range: 0.5-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, ref. 72.694.005).
- 0.5 mL sterile screw capped tubes (Sarstedt, ref. 72.730.005)
- 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 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) **GI Viral PLUS ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **GI Viral PLUS ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **GI Viral PLUS ELITe_ST_200_100**, Assay Protocol with parameters for Stool specimen analysis.	**GI Viral PLUS - ELITe Positive Control** (EG SpA, ref. CTR501ING) **CPE - Internal Control** (EG SpA, ref. CTRCPE), **ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use) **InhibitEX Buffer** (QIAGEN GmBH, Germany, ref. 19593) or an equivalent device. **Minitip Flocked Swab®** (COPAN Italia S.p.A., Italy, ref. 501CS01) or an equivalent device. **FecalSwab™** (COPAN Italia S.p.A., Italy, ref. 470CE,) or an equivalent device with Cary Blair medium.
ELITe BeGenius (EG SpA, ref. INT040) **ELITe BeGenius Software** version 2.3.0 (or later) **GI Viral PLUS ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis. **GI Viral PLUS ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis. **GI Viral PLUS ELITe_Be_ST_200_100**, Assay Protocol with parameters for Stool 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 to prevent dispersion into the environment and to avoid contamination of the instrument's working area.

The PCR Cassette must be handled carefully and never opened to prevent PCR product diffusion and carryover contamination.

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles
GI-V 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-**
Stool	collected without preservatives	≤ 24 hours	≤ 48 hours	≤ 1 month	≤ 2 months
	collected in FecalSwab	≤ 48 hours	≤ 5 days	≤ 1 month	≤ 2 months

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.

Follow the instructions described below for specimen's pre-treatment.

Pre-treatment procedure starting from native stool collected without preservatives:

1. transfer 1 mL of InhibitEX Buffer in a 2 mL Sarstedt tube,

2. collect the stool sample with a Minitip Flocked Swab with 80mm Break (Copan), pick up the sample from different stool portions and discard the excess by leaning against the container wall,
3. insert the swab into the 2 mL Sarstedt tube containing the InhibitEX Buffer and rotate it at least 10 times, leaning against the wall,
4. discard the swab and close the tube cap,
5. mix by vortexing for ~60 sec,
6. incubate in a thermomixer at ~+80 °C and ~800 RPM for 10 min,
7. spin at 10,000x RCF for 15 sec,
8. carefully transfer 200 µL of the clarified stool supernatant into an Extraction tube (for ELITe InGenius instrument) or into a 2 mL Sarstedt tube (for ELITe BeGenius instrument) being careful not to disturb the pelleted fecal material.

Pre-treatment procedure starting from stool collected in FecalSwab:

1. transfer 500 µL of InhibitEX Buffer in a 2 mL Sarstedt tube,
2. transfer 500 µL of sample suspension from the FecalSwab into the 2 mL Sarstedt tube containing the InhibitEX buffer,
3. cap the tube securely and mix by vortexing for ~60 sec,
4. incubate in a thermomixer at ~+80 °C and ~800 RPM for 10 min,
5. spin at 10,000x RCF for 15 sec,
6. carefully transfer 200 µL of the clarified stool supernatant into an Extraction tube (for ELITe InGenius instrument) or into a 2 mL Sarstedt tube (for ELITe BeGenius instrument) being careful not to disturb the pelleted fecal material.

To perform samples testing on the **ELITe InGenius** and **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 GI Viral PLUS ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
Native Stool or Stool collected in FecalSwab	ELITe InGenius	**GI Viral PLUS ELITe_ST_ 200_100**	Positive / Negative	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	**GI Viral PLUS ELITe_Be_ ST_200_100**		

For all protocols, 200 µL of sample must be transferred into 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.

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.

8.2 PCR controls

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

- For the Positive Control, use the product **GI Viral PLUS - ELITe Positive Control** (not provided with this kit) with the **GI Viral PLUS ELITe_PC** or **GI Viral PLUS ELITe_Be_PC** Assay Protocols.

- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **GI Viral PLUS ELITe_NC** or **GI Viral PLUS ELITe_Be_NC** Assay Protocols.

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the PCR control validation for each lot of PCR reagent. PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and negative controls. The 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 **GI Viral PLUS 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) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) 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 “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 **GI Viral PLUS ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. 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 **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session).Mix 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**. 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 **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)	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 has to be freshly prepared for each work session and **cannot** be stored for reuse.

NOTE

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

To set up one of the three 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)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature. Pre-treat the samples according to the procedure described in the "Specimens and Controls" section. For this assay, 200 µL of pre-treated sample must be transferred in an Extraction tube previously labelled.	**Thaw Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**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. Each tube is sufficient for 4 reactions.
2	**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.	Not applicable.	**Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with ELITe InGenius SP 200 Consumable Set.
3	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
4	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.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
5	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.	Not applicable.
6	**Select the Assay Protocol** in the "Assay" column (see "Specimens and Controls").	**Select the Assay Protocol** in the "Assay" column (see "Specimens and Controls").	**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.
7	Ensure the "Protocol" displayed is: "Extract + PCR".	Select "PCR Only" in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
8	Select the sample loading position as "Extraction Tube" in the "Sample Position" column.	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
9	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
10	**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.	**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.
11	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
12	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.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
13	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
14	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**Load** PCR Cassette and Elution tube with samples extracted	**Load** PCR Cassette, Positive Control and Negative Control tubes.
15	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.

Table 8 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
16	Close the instrument door.	Close the instrument door.	Close the instrument door.
17	Press “Start”.	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 has to be freshly prepared for each work session and **cannot** be stored for re-use.

NOTE

At the end of the run, the remaining **Positive Controll** 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 **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 **GI Viral PLUS ELITe MGB Kit** through the following procedure:

A. Validation of Positive Control and Negative Control results,

B. Validation of sample results,

C. Sample result reporting.

9.3.1 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius Software** interprets the PCR results for the targets of the Positive Control and Negative Control reaction with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct and Tm values are 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 results of the Positive Control and Negative Control amplification are used by the **ELITe InGenius software** to set up the Control Charts monitoring the amplification step performances. 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.

9.3.2 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (Channels **NV**, **SV, ADV, RV** and **ASV**) and the Internal Control (Channel **IC**) with the **GI Viral PLUS ELITe _ST_200_100** Assay Protocol parameters.

Results are shown in “Results Display” screen.

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

Table 9

1) Positive Control	**Status**
GI-V Positive Control	APPROVED
2) Negative Control	**Status**
GI-V 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 DNA and RNAs are either detected or not detected.

Table 10

Result of sample run	**Interpretation**
NV:RNA Detected Genogroup I	**Norovirus RNA was detected** in the sample and typed as Genogroup I.
NV:RNA Detected Genogroup II	**Norovirus RNA was detected** in the sample and typed as Genogroup II.
NV:RNA Detected Typing not determined	**Norovirus RNA was detected** in the sample, but the analysis for genogroup typing was not feasible. The test should be repeated.
NV:RNA Not detected or below the LoD	**Norovirus 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.

Table 10 (continued)

Result of sample run	Interpretation
SV:RNA Detected	**Sapovirus RNA was detected** in the sample.
SV:RNA Not detected or below the LoD	**Sapovirus 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.
RV:RNA Detected	**Rotavirus RNA was detected** in the sample.
RV:RNA Not detected or below the LoD	**Rotavirus 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.
ADV:DNA Detected Serotype F40	**Adenovirus DNA was detected** in the sample and typed as Serotype F40.
ADV:DNA Detected Serotype F41	**Adenovirus DNA was detected** in the sample and typed as Serotype F41.
ADV:DNA Detected Typing not determined	**Adenovirus DNA was detected** in the sample, but the analysis for genogroup typing was not feasible. The test should be repeated.
ADV:DNA Not detected or below the LoD	**Adenovirus DNA was not detected** in the sample. The sample is negative for the target DNA, or its concentration is below the assay Limit of Detection.
ASV:RNA detected.	**Astrovirus RNA was detected** in the sample.
ASV:RNA Not detected or below the LoD	**Astrovirus 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, pretreatment, 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 “Troubleshooting”).

Samples reported as “XXX:RNA/DNA Not detected or below the LoD” are suitable for analysis but the DNA/RNA of the targets was not detected. In this case, the sample may be either negative for the DNA/RNA of the targets or the DNA/RNA of the targets is present at a concentration below the Limit of Detection of the assay (see “Performance characteristics”).

Samples reported as “XXX:RNA/DNA Detected Typing not determined” are not suitable for typing of Genogroup I or II of Norovirus and of Serotype F40 or F41 of Adenovirus. However, samples are positive for Norovirus RNA and/or Adenovirus DNA.

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”.

9.3.3 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 **GI Viral PLUS ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 11

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) 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 “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 **GI Viral PLUS ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

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

All the 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 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 **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session).Mix 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**. 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 **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 12

Sample 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. 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 has to be freshly prepared for each work session and **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 three types of run follow the steps below while referring to the GUI:

Table 13

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature. Pre-treat the samples according to procedure described in the “Specimens and Controls” section. For this assay, **200 µL of sample** must be transferred in a 2mL Sarstedt tube previously labelled.	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.	**Thaw Positive Control** tubes at room temperature for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.
2	**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.	Not applicable	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.

Table 13 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
3	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen	Select "**Perform Run**" from the "Home" screen.
4	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	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
5	Select the "Run mode": "**Extract + PCR".**	Select the "Run mode": "**PCR Only**".	Select the "Run mode": "**PCR Only**".
6	**Load the samples** into the "Sample Rack". When secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack".	**Load the samples** into the "Elution Rack".	**Load the Positive Control and Negative Control** tubes into the "Elution Rack".
7	**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).	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). 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).
8	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
9	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL.
10	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
11	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
	Note: When more than 12 samples are processed, repeat the procedure from point 6.		Not applicable
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	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	Not applicable
14	Click "Next" to continue.	Not applicable	Not applicable
15	**Load CPE** and the **complete reaction mixture** into the "Reagent/Elution Rack".	**Load** the **complete reaction mixture** into "Reagent/Elution Rack".	**Load** the **complete reaction mixture** into "Reagent/Elution Rack".

Table 13 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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 reagent and / or CPE enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). For each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). For each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
17	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
18	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.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
19	Click “Next” to continue.	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.	Load the **“PCR Rack**” with “PCR Cassette” in the Inventory Area.
21	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
22	Load the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables.	Not applicable.	Not applicable.
23	Close the instrument door.	Close the instrument door.	Close the instrument door.
24	Press “Start”.	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 has to be freshly prepared for each work session and **cannot** be stored for re-use.

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 **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 **GI Viral PLUS ELITe MGB Kit** through the following procedure:

1. Validation of Positive Control and Negative Control results,
2. Validation of sample results,
3. 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 for ELITe BeGenius and ELITe InGenius instruments by testing native stool samples spiked with reference material of Norovirus GI, Norovirus GII, Astrovirus, Adenovirus F40, Adenovirus F41 and Rotavirus (ZeptoMetrix and ATCC) and Sapovirus GI/II/IV and Sapovirus GV (EG SpA plasmids).

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.

Table 14

Pathogen	LoD	95% confidence interval limits	
		Lower limit	Upper limit
Norovirus GI	219 $TCID_{50}$/mL	146 $TCID_{50}$/mL	507 $TCID_{50}$/mL
Norovirus GII	151 $TCID_{50}$/mL	114 $TCID_{50}$/mL	321 $TCID_{50}$/mL
Astrovirus	690 $TCID_{50}$/mL	409 $TCID_{50}$/mL	2782 $TCID_{50}$/mL
Adenovirus F40	0.082 $TCID_{50}$/mL	0.058 $TCID_{50}$/mL	0.149 $TCID_{50}$/mL
Adenovirus F41	0.006 $TCID_{50}$/mL	0.004 $TCID_{50}$/mL	0.09 $TCID_{50}$/mL
Rotavirus	2.4 $TCID_{50}$/mL	1.9 $TCID_{50}$/mL	3.5 $TCID_{50}$/mL
Sapovirus GV	792 copies/mL	583 copies/mL	1267 copies/mL
Sapovirus GI/II/IV	1119 copies/mL	859 copies/mL	1897 copies/mL

The calculated LoD value was verified by testing on ELITe BeGenius and ELITe InGenius native stool samples and stool samples collected in FecalSwab spiked with Norovirus GI, Norovirus GII, Astrovirus, Adenovirus F40, Adenovirus F41, Rotavirus, Sapovirus GI/II/IV and Sapovirus GV reference materials at the claimed concentration.

The results obtained confirmed the claimed concentration for all the targets of GI Viral PLUS MGB Kit with the two matrices on both ELITe BeGenius and ELITe InGenius.

11.2 Inclusivity: Efficiency of detection on different strain or isolates

The Inclusivity of the assay, as efficiency of detection for different genotypes or isolates of Norovirus (GI/GII), Astrovirus, Adenovirus (F40/F41), Rotavirus and Sapovirus (GI/II/IV/GV) was evaluated by in silico analysis. The analysis showed conservation and absence of significant mutations for all the targets of interest, except for Norovirus. So, different detection efficiencies are expected for some Norovirus genotypes or isolates.

The Inclusivity was also verified through the analysis of 10 reference materials (Qnostics, Vircell, ZeptoMetrix and ATCC) and testing 14 plasmid DNAs representative of main genomic variants of Norovirus GI and Norovirus GII.

The results with reference materials are reported in the following table.

Table 15

Target	Provider	Positive / Replicates	Outcome
Norovirus GI	ZeptoMetrix	6 / 6	NV:RNA Detected Genogroup I
Norovirus GII	Vircell	6 / 6	NV:RNA Detected Genogroup II
Adenovirus F40	ZeptoMetrix	6 / 6	ADV:DNA Detected Serotype F40
Adenovirus F41	Vircell	6 / 6	ADV:DNA Detected Serotype F41
Adenovirus F41	Qnostics	6 / 6	ADV:DNA Detected Serotype F41
Rotavirus	Vircell	6 / 6	RV:RNA Detected
Rotavirus	Qnostics	6 / 6	RV:RNA Detected
Sapovirus	ATCC	6 / 6	SV:RNA Detected
Astrovirus I	ATCC	6 / 6	ASV:RNA Detected
Astrovirus V	ATCC	6 / 6	ASV:RNA Detected

All samples were correctly detected by the GI Viral PLUS ELITe MGB Kit.

The results with plasmid DNAs are reported in the following table.

Table 16

Sample	Copies / reaction	Positive / Replicates	Outcome
Plasmid Norovirus GI (SEQ ID MN938461)	750	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GI (SEQ ID KP027330)	750	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GI (SEQ ID MZ470608)	$4x10^4$	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GI (SEQ ID OK562729)	$3x10^4$	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GI (SEQ ID MN421562)	$1x10^5$	6 / 6	NV:RNA Detected Genogroup II
Plasmid Norovirus GI (SEQ ID LC378987)	$1x10^7$	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GI (SEQ ID MW647681)	750	6 / 6	NV:RNA Detected Genogroup I

Table 16 (continued)

Sample	Copies / reaction	Positive / Replicates	Outcome
Plasmid Norovirus GI (SEQ ID OK147886)	750	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GI (SEQ ID MW362461)	750	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GI (SEQ ID EU085525)	750	6 / 6	NV:RNA Detected Genogroup I
Plasmid Norovirus GII (SEQ ID MK328934)	$1x10^2$	6 / 6	NV:RNA Detected Genogroup II
Plasmid Norovirus GII (SEQ ID MG674721)	$1x10^2$	6 / 6	NV:RNA detected Genogroup I
Plasmid Norovirus GII (SEQ ID MG495078)	$1x10^2$	6 / 6	NV:RNA detected Genogroup I
Plasmid Norovirus GII (SEQ ID KC464491)	$1x10^2$	6 / 6	NV:RNA detected Genogroup II

With some Norovirus GI variants, the sensitivity of the product can change up to 10,000-fold.

With the Norovirus GI genotype 9, the product will give a wrong typing as “Norovirus GII”.

With the Norovirus GII genotypes 6 and 7, the product will give a wrong typing as “Norovirus GI”.

11.3 Interference among targets

The potential interference among targets of the assay was evaluated by a test of co-amplification of Norovirus GI, Norovirus GII, Astrovirus, Adenovirus F40, Adenovirus F41, Rotavirus, Sapovirus GI/II/IV and Sapovirus GV (EG SpA plasmid DNAs).

For each target, the lower concentration detectable in all replicates is reported in the following table.

Table 17

Target in test (low copies)	Interfering target at high concentration (50,000 copies / reaction)						
	NV1	**NV2**	**SV124**	**SV5**	**RV**	**ADV-F40**	**ASV**
NV1	-	-	50 c / rxn	50 c / rxn	50 c / rxn	50 c / rxn	50 c / rxn
NV2	-	-	100 c / rxn	100 c / rxn	100 c / rxn	100 c / rxn	100 c / rxn
SV124	100 c / rxn	100 c / rxn	-	-	100 c / rxn	100 c / rxn	100 c / rxn
SV5	50 c / rxn	50 c / rxn	-	-	50 c / rxn	50 c / rxn	50 c / rxn
RV	250 c / rxn	250 c / rxn	250 c / rxn	250 c / rxn	-	250 c / rxn	250 c / rxn
ADV-F40	50 c / rxn	50 c / rxn	50 c / rxn	50 c / rxn	50 c / rxn	-	50 c / rxn
ASV	250 c / rxn	250 c / rxn	250 c / rxn	250 c / rxn	250 c / rxn	250 c / rxn	-

The GI Viral PLUS ELITe MGB Kit shows a minimal interference among targets. All the targets can be detected even when they are about 200 times less than the other pathogens of interest.

11.4 Potentially interfering organisms: Cross-reactivity

The potential cross-reactivity of unintended organisms that may be found in clinical stool specimens was evaluated for the assay by in silico analysis. The analysis showed no significant homology with other unintended organisms (viruses, bacteria, protozoa, and fungi) and therefore, no cross-reactivity is expected, except for some Adenovirus different from F40 and F41.

The absence of cross-reactivity with potential interfering organisms was also verified through the analysis of a panel of unintended organisms (ATCC, ZeptoMetrix, DSMZ, and plasmid DNAs).

The results are reported in the following table.

Table 18

Sample	Positive / Replicates					Outcome
	NV	SV	RV	ADV	ASV	
Aeromonas hydrophilia	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bacteroides fragilis	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Helicobacter pylori	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Saccharomyces cerevisiae	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Plesiomonas shigelloides	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Klebsiella pneumoniae	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Escherichia coli	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Serratia Marcescens	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Acinetobacter baumannii	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bifidobacterium adolescentis	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Candida albicans	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Citrobacter freundii	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Clostridium difficile	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Proteus mirabilis	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Pseudomonas aeruginosa	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Enterobacter cloacae	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Giardia lamblia	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Cryptosporidium parvum	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Entamoeba histolytica	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Yersinia enterocolitica	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Salmonella enterica	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Shigella flexneri	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Vibrio cholera	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Campylobacter jejuni	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Enterovirus B E4	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Adenovirus species C (SEQ ID OR777170)	0 / 5	0 / 5	0 / 5	5 / 5	0 / 5	Cross-reactivity for ADV
Adenovirus species A (SEQ ID KX868289)	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Adenovirus species B (SEQ ID AF542110)	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity

Table 18 (continued)

Sample	Positive / Replicates					Outcome
	NV	SV	RV	ADV	ASV	
Adenovirus species C (SEQ ID MN398196)	0 / 5	0 / 5	0 / 5	5 / 5	0 / 5	Cross-reactivity for ADV
Adenovirus species D (SEQ ID JN226752)	0 / 5	0 / 5	0 / 5	5 / 5	0 / 5	Cross-reactivity for ADV
Adenovirus species E (SEQ ID KY996446)	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Adenovirus species G (SEQ ID DQ923122)	0 / 5	0 / 5	0 / 5	5 / 5	0 / 5	Cross-reactivity for ADV

All potentially interfering organisms tested showed no cross-reactivity for the target's amplification except for the Adenovirus C, D and G, using the GI Viral PLUS ELITe MGB Kit.

11.5 Potentially interfering organisms: Inhibition

The potential inhibition of unintended organisms that may be found in clinical stool specimens was evaluated for the assay through the analysis of a panel of unintended organisms (ATCC, ZeptoMetrix, DSMZ and plasmid DNAs) spiked with Norovirus (GI/GII), Astrovirus, Adenovirus (F40/F41), Rotavirus, Sapovirus (GI/II/IV/GV) (EG SpA plasmid DNAs).

The results are reported in the following table.

Table 19

Organism	Positive / Replicates						Outcome
	NV1	NV2	RV	ADV-F40	ADV-F41	ASV	
Aeromonas hydrophilia	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Bacteroides fragilis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Helicobacter pylori	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Saccharomyces cerevisiae	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Plesiomonas shigelloides	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Klebsiella pneumoniae	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Escherichia coli	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Serratia Marcescens	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Acinetobacter baumannii	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Bifidobacterium adolescentis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Candida albicans	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Citrobacter freundii	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Clostridium difficile	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Proteus mirabilis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Pseudomonas aeruginosa	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference

Table 19 (continued)

Organism	Positive / Replicates						Outcome
	NV1	NV2	RV	ADV-F40	ADV-F41	ASV	
Enterobacter cloacae	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Giardia lamblia	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Cryptosporidium parvum	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Entamoeba histolytica	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Yersinia enterocolitica	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Salmonella enterica	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Shigella flexneri	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Vibrio cholera	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Campylobacter jejuni	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Enterovirus B E4	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus species C (SEQ ID OR777170)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus species A (SEQ ID KX868289)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus species B (SEQ ID AF542110)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus species C (SEQ ID MN398196)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus species D (SEQ ID JN226752)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus species E (SEQ ID KY996446)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus species G (SEQ ID DQ923122)	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference

All potentially interfering organisms tested showed no inhibition of the target amplification using the GI Viral PLUS ELITe MGB Kit.

11.6 Potentially interfering substances: Cross-reactivity

The cross-reactivity by potentially interfering substances (endogenous and exogenous) that might be found in stool specimens was evaluated for the assay by analysis of a panel of substances at relevant concentration.

The results are reported in the following table.

Table 20

Substance	Positive / Replicates						Outcome
	NV1	NV2	RV	ADV-F40	ADV-F41	ASV	
Vaselin oil	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Nonoxynol-9	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bismuth subsalicylate	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Loperamide hydrochloride	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bisacodyl	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Azithromycin	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Vancomycin	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Metronidazole	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Ampicillin	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Cefpodoxime	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Ciprofloxacin	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Hydrocortisone	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Calcium carbonate	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Alginic acid	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Aluminium hydroxide	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Magnesium trisilicate	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Whole blood	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Mucin	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Palmitic acid	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Stearic acid	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity

The test showed that all the tested substances do not cross-react with the targets using the GI Viral PLUS ELITe MGB Kit.

11.7 Potentially interfering substances: Inhibition

The potential inhibition of interfering substances (endogenous and exogenous) that might be found in clinical stool specimens was evaluated for the assay by analysis of a panel of substances at relevant concentration in samples spiked with Norovirus GI, Norovirus GII, Astrovirus, Adenovirus F40, Adenovirus F41, Rotavirus, Sapovirus GV and Sapovirus GI/GII/GIV reference materials (ZeptoMetrix, ATCC and EG SpA plasmids).

The results are reported in the following table.

Table 21

Substance	Positive / Replicates						Outcome
	NV1	NV2	RV	ADV-F40	ADV-F41	ASV	
Vaselin oil	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Nonoxynol-9	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Bismuth subsalicylate	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Loperamide hydrochloride	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Bisacodyl	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Azithromycin	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Vancomycin	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Metronidazole	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Ampicillin	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Cefpodoxime	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Ciprofloxacin	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Hydrocortisone	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Calcium carbonate	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Alginic acid	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Aluminium hydroxide	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Magnesium trisilicate	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Whole blood	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Mucin	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Palmitic acid	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Stearic acid	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference

The test showed that the tested substances do not inhibit the targets detection using the GI Viral PLUS ELITe MGB Kit.

11.8 Cross-contamination

The possibile Cross-contamination during analysis was evaluated for the assay by testing 60 replicates of a negative stool specimen alternated to 60 replicates of the same specimen spiked with Adenovirus F40 (ADV-F40) reference material (ZeptoMetrix) at about 1 x 10^4 $TCID_{50}$ / mL.

The results are reported in the following table.

Table 22

Samples	N	Positive	Negative	%Agreement
Positive	60	60	0	100%
Negative	60	0	60	100%

None of the tested negative samples gave false positive results. In this test with the GI Viral PLUS ELITe MGB Kit the cross-contamination was neither detected within sessions nor between sessions.

11.9 Whole system failure

The Whole system failure rate for the assay was evaluated by analysing 52 different negative native stool specimens and 52 stool specimens collected in FecalSwab spiked with Norovirus GII reference material (Zeptometrix) at 3x LoD concentration.

The results are reported in the following table.

Table 23

Samples	N	Positive	Negative	Whole system failure rate
Native Stool spiked at 3x LoD	52	52	0	0%
Stool in FecalSwab spiked at 3x LoD	52	52	0	0%

In this test with the GI Viral PLUS ELITe MGB Kit, the 100% of the native stool specimens and the 100% of the stool samples collected in FecalSwab were confirmed positive. In this test the whole system failure rate was equal to 0% for native stool specimens and 0% for stool samples collected in FecalSwab.

11.10 Repeatability

The Repeatability of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of native stool specimens negative or spiked with reference materials of Norovirus GII, Adenovirus F40, Rotavirus, Astrovirus, and Sapovirus GI/II/IV (ZeptoMetrix, ATCC, and EG SpA plasmid DNA).

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

Table 24

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	31.25	0.42	1.34	100%
	NV (Tm)	6	68.4	0.17	0.25	100%
3xLoD ADV-F40+ASV	NV (Ct)	6	-	-	-	100%
3xLoD SV124		6	-	-	-	100%
Neg	SV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	-	-	-	100%
3xLoD ADV-F40+ASV		6	-	-	-	100%
3xLoD SV124		6	32.36	0.50	1.55	100%
Neg	RV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	30.18	0.29	0.95	100%
3xLoD ADV-F40+ASV		6	-	-	-	100%
3xLoD SV124		6	-	-	-	100%

Table 24 (continued)

Neg	ADV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	-	-	-	100%
3xLoD ADV-F40+ASV		6	34.17	0.81	2.37	100%
	ADV (Tm)	6	70.4	0.27	0.38	100%
3xLoD SV124	ADV (Ct)	6	-	-	-	100%
Neg	ASV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	-	-	-	100%
3xLoD ADV-F40+ASV		6	27.14	0.36	1.32	100%
3xLoD SV124		6	-	-	-	100%

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

Table 25

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	29.91	0.41	1.38	100%
	NV (Tm)	6	69.0	0.05	0.07	100%
3xLoD ADV-F40+ASV	NV (Ct	6	-	-	-	100%
3xLoD SV124		6	-	-	-	100%
Neg	SV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	-	-	-	100%
3xLoD ADV-F40+ASV		6	-	-	-	100%
3xLoD SV124		6	32.94	0.83	2.52	100%
Neg	RV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	30.01	0.23	0.76	100%
3xLoD ADV-F40+ASV		6	-	-	-	100%
3xLoD SV124		6	-	-	-	100%
Neg	ADV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	-	-	-	100%
3xLoD ADV-F40+ASV		6	34.32	0.64	1.86	100%
	ADV (Tm)	6	71.0	0.16	0.23	100%
3xLoD SV124	ADV (Ct)	6	-	-	-	100%

Table 25 (continued)

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	ASV (Ct)	6	-	-	-	100%
3xLoD NV+RV		6	-	-	-	100%
3xLoD ADV-F40+ASV		6	27.26	0.46	1.70	100%
3xLoD SV124		6	-	-	-	100%

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

Table 26

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	30.97	0.43	1.39	100%
	NV (Tm)	12	68.4	0.16	0.24	100%
3xLoD ADV-F40+ASV	NV (Ct)	12	-	-	-	100%
3xLoD SV124		12	-	-	-	100%
Neg	SV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	-	-	-	100%
3xLoD ADV-F40+ASV		12	-	-	-	100%
3xLoD SV124		12	32.37	0.45	1.39	100%
Neg	RV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	30.07	0.27	0.89	100%
3xLoD ADV-F40+ASV		12	-	-	-	100%
3xLoD SV124		12	-	-	-	100%
Neg	ADV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	-	-	-	100%
3xLoD ADV-F40+ASV		12	33.80	0.71	2.10	100%
	ADV (Tm)	12	70.3	0.24	0.34	100%
3xLoD SV124	ADV (Ct)	12	-	-	-	100%
Neg	ASV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	-	-	-	100%
3xLoD ADV-F40+ASV		12	27.02	0.33	1.22	100%
3xLoD SV124		12	-	-	-	100%

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

Table 27

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	30.08	0.46	1.53	100%
	NV (Tm)	12	68.9	0.11	0.16	100%
3xLoD ADV-F40+ASV	NV (Ct	12	-	-	-	100%
3xLoD SV124		12	-	-	-	100%
Neg	SV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	-	-	-	100%
3xLoD ADV-F40+ASV		12	-	-	-	100%
3xLoD SV124		12	32.74	0.78	2.37	100%
Neg	RV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	30.18	0.40	1.32	100%
3xLoD ADV-F40+ASV		12	-	-	-	100%
3xLoD SV124		12	-	-	-	100%
Neg	ADV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	-	-	-	100%
3xLoD ADV-F40+ASV		12	34.44	0.63	1.82	100%
	ADV (Tm)	12	71.0	0.14	0.19	100%
3xLoD SV124	ADV (Ct)	12	-	-	-	100%
Neg	ASV (Ct)	12	-	-	-	100%
3xLoD NV+RV		12	-	-	-	100%
3xLoD ADV-F40+ASV		12	27.03	0.41	1.51	100%
3xLoD SV124		12	-	-	-	100%

In the Repeatability test, the GI Viral PLUS ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

11.11 Reproducibility

The Reproducibility of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of native stool specimens negative or spiked with reference materials of Norovirus GII, Adenovirus F40, Rotavirus, Astrovirus and Sapovirus GI/II/IV (ZeptoMetrix, ATCC and EG SpA plasmid DNA).

The results of Inter-Batch Reproducibility (on six days and three lots) on ELITe BeGenius are shown in the table below.

Table 28

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	31.95	0.91	2.85	100%
	NV (Tm)	36	68.5	0.17	0.25	100%
3xLoD ADV-F40+ASV	NV (Ct	36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%
Neg	SV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	32.49	0.39	1.19	100%
Neg	RV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	30.60	0.56	1.82	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%
Neg	ADV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	33.12	0.75	2.27	100%
	ADV (Tm)	36	70.3	0.41	0.58	100%
3xLoD SV124	ADV (Ct)	36	-	-	-	100%
Neg	ASV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	28.15	0.97	3.44	100%
3xLoD SV124		36	-	-	-	100%

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

Table 29

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	30.54	0.55	1.80	100%
	NV (Tm)	36	69.1	0.21	0.30	100%
3xLoD ADV-F40+ASV	NV (Ct)	36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%

Table 29 (continued)

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	SV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	32.75	0.57	1.74	100%
Neg	RV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	30.41	0.41	1.35	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%
Neg	ADV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	33.66	0.78	2.31	100%
	ADV (Tm)	36	70.8	0.30	0.43	100%
3xLoD SV124	ADV (Ct)	36	-	-	-	100%
Neg	ASV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%

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

Table 30

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	31.73	0.93	2.93	100%
	NV (Tm)	36	68.6	0.24	0.36	100%
3xLoD ADV-F40+ASV	NV (Ct)	36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%
Neg	SV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	32.45	0.44	1.35	100%

Table 30 (continued)

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	RV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	30.80	0.53	1.73	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%
Neg	ADV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	32.91	0.68	2.07	100%
	ADV (Tm)	36	70.5	0.37	0.52	100%
3xLoD SV124	ADV (Ct)	36	-	-	-	100%
Neg	ASV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	28.26	0.95	3.35	100%
3xLoD SV124		36	-	-	-	100%

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

Table 31

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	NV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	30.74	0.62	2.02	100%
	NV (Tm)	36	69.1	0.21	0.30	100%
3xLoD ADV-F40+ASV	NV (Ct)	36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%
Neg	SV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	32.66	0.47	1.43	100%
Neg	RV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	30.28	0.42	1.38	100%
3xLoD ADV-F40+ASV		36	-	-	-	100%
3xLoD SV124		36	-	-	-	100%

Table 31 (continued)

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	ADV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	32.78	0.63	1.91	100%
	ADV (Tm)	36	70.8	0.37	0.52	100%
3xLoD SV124	ADV (Ct)	36	-	-	-	100%
Neg	ASV (Ct)	36	-	-	-	100%
3xLoD NV+RV		36	-	-	-	100%
3xLoD ADV-F40+ASV		36	25.57	0.88	3.18	100%
3xLoD SV124		36	-	-	-	100%

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

11.12 Diagnostic Specificity: Confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with ELITe InGenius by analysing clinical samples of stool collected without preservatives, certified negative for each 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 32

Negative stool tested for the target	N	Positive	Negative	% Diagnostic Specificity
Adenovirus F40/F41	101	0	101	100%
Norovirus GI/GII	101	0	101	100%
Rotavirus	101	0	101	100%
Astrovirus	100	0	100	100%
Sapovirus	101	0	101	100%

All stool samples were negative and valid for analysis.

The Diagnostic Specificity of the GI Viral PLUS ELITe MGB Kit in association to stool, in this test, was equal to 100% for all the targets.

The IC Ct cut-off value is set at 34 for ELITe InGenius and 35 for ELITe BeGenius.

11.13 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 analysing clinical samples of stool collected without preservatives, certified positive for each 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 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 33

Positive stool tested for the target	N	Positive	Negative	% Diagnostic Sensitivity
Adenovirus F40	15	15	0	100%
Adenovirus F41	35	35	0	
Norovirus GI	20	13	7	92.9%
Norovirus GII	120	117	3	
Rotavirus	50	50	0	100%
Astrovirus	50	50	0	100%
Sapovirus	55	51	4	92.7%

The Diagnostic Sensitivity of the GI Viral PLUS ELITe MGB Kit in association to stool, in this test, was equal to 100% for ADV F40/F41, 92.9% for NV GI/GII, 100% for RV, 100% for ASV and 92.7% for SV.

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 “GI Viral PLUS ELITe MGB Kit”, FTP 501ING.

12 REFERENCES

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13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: native stool or stool collected in FecalSwab.

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

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

In case of co-infections, the sensitivity for one target can be affected by the amplification of a second target (see Performance Characteristics).

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

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

In case of occurrence of Adenovirus species C or G in the sample, the product will detect it as Adenovirus target without the typing determination.

In case of occurrence of Adenovirus species D in the sample, the product will detect it and typing as Adenovirus serotype F41.

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 34

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture, and Positive Control. Check the volumes of complete reaction mixture, and Positive Control.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.

Table 34 (continued)

Degradation of complete reaction mixture or of its components.	Do not re-use the complete reaction mixture, prepare it freshly for each work session. 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.
Positive Control degradation.	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 a new aliquot of Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 35

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 36

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 re-use the complete reaction mixture, prepare it freshly for each work session. 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.

Table 36 (continued)

Invalid Sample reaction	
Possible Causes	**Solutions**
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 pre-treated sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 37

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 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 38

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 pre-treated sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 39

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 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.
	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. To inform ELITechGroup S. p. A., manufacturer of this device, please use the following mail address: egspa. vigilance@elitechgroup.com..

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, 7541454, 7671218, 7723038, 7767834, 8163910, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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

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

Appendix A GI Norovirus PLUS 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 **GI Viral PLUS ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids reverse transcription and Real-Time PCR assay for the **detection and identification** of the genomic DNA of Adenovirus (ADV), and the genomic RNA of Norovirus (NV), Rotavirus (RV), Astrovirus (ASV) and Sapovirus (SV), extracted from clinical specimens.

The assay is able to detect the DNA of Adenovirus belonging to serotypes F40 and F41 (typed by melting analysis), the RNA of Norovirus belonging to genogroups GI and GII (typed by melting analysis), Rotavirus belonging to group A, human Astrovirus and human Sapovirus.

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 stool specimens.

The product is intended for use as an aid in the diagnosis of gastrointestinal viral infections in patients suspected of having Adenovirus, Norovirus, Rotavirus, Astrovirus or Sapovirus infection.

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

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	GI and GII Polyprotein	FAM	NV
Target 2	capsid protein	AP690	ASV
Target 3	F40 and F41 Hexon protein	AP639	ADV
Target 4	group A NSP3	AP593	RV
Target 5	GI/GII/GIV and GV Polyprotein	AP559	SV
Internal Control	phage MS2	AP525	IC

Validated matrix

- Native stool collected without preservatives
- Stool collected in FecalSwab (Modified Cary Blair medium)

Kit content and related products

GI Viral PLUS ELITe MGB Kit (RTS501ING)		GI Viral PLUS - ELITe Positive Control (CTR501ING)	
 X 4	 X 2	 X 3	
GI-V PCR Mix 4 tubes of 600 µL 24 reactions per tube 96 reactions per kit 5 freeze-thaw cycles per tube	**RT EnzymeMix** 2 tubes of 20 µL 48 reactions per tube 96 reactions per kit 10 freeze-thaw cycles	**GI-V Positive Control** 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles	
Maximum shelf-life:	**18 months**	Maximum shelf-life	**24 months**
Storage temperature	**≤ -20°C**	Storage temperature	**≤ -20°C**

Other products required not provided in the kit

Table 40

- ELITe InGenius instrument: INT030.
- ELITe BeGenius instrument: INT040.
- ELITe InGenius SP 200: INT032SP200.
- **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use)
- CPE - Internal Control: CTRCPE
- InhibitEX Buffer (QIAGEN GmBH, Germany, ref. 19593) or an equivalent device.
- Minitip Flocked Swab® (COPAN Italia S.p.A., Italy, ref. 518CS01) or an equivalent device.
- FecalSwab™ (COPAN Italia S. p. A., Italy, ref. 470CE,) or an equivalent device.

ELITe InGenius and ELITe BeGenius Protocol

• Sample volume	• 200 µL	• Eluate PCR input volume	• 10 µL
• CPE volume	• 10 µL	• GI-NV PCR Mix volume	• 20 µL
• Total elution volume	• 100 µL	• Frequency of controls	• 15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Target	Limit of Detection	Sensitivity	Specificity
Native Stool / Stool collected in FecalSwab	Norovirus GI	**219 $TCID_{50}$/mL**	**92.9%** (130/140)	**100%** (101/101)
	Norovirus GII	**151 $TCID_{50}$/mL**		
	Astrovirus	**690 $TCID_{50}$/mL**	**100%** (50/50)	**100%** (100/100)
	Adenovirus F40	**0.082 $TCID_{50}$/mL**	**100%** (50/50)	**100%** (101/101)
	Adenovirus F41	**0.006 $TCID_{50}$/mL**		
	Rotavirus	**2.4 $TCID_{50}$/mL**	**100%** (50/50)	**100%** (101/101)
	Sapovirus GV	**792 copies/mL**	**92.7%** (51/55)	**100%** (101/101)
	Sapovirus GI/II/IV	**1119 copies/mL**		

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.

Table 41

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**- 20 ± 10 °C**	**70 ± 15 °C-**
Stool	collected without preservatives	≤ 24 hours	≤ 48 hours	≤ 1 month	≤ 2 months
	collected in FecalSwab	≤ 48 hours	≤ 5 days	≤ 1 month	≤ 2 months

NOTE

The specimens have to be pre-treated before use according to the procedure described in the complete IFU.

ELITe InGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe InGenius software to setup the run. All the steps: extraction, reverse transcription, 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 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.

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

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: GI Viral PLUS ELITe_ST_200_100	**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

NOTE

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

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

1. Select “Perform Run” on the touch screen	**2.** Verify the extraction volumes: Input: “200 µL”, elution: “100 µL”	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the “Assay Protocol” of interest: GI Viral PLUS ELITe_ST_200_100 or GI Viral PLUS ELITe_PC or GI Viral PLUS ELITe_NC	**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 of ELITe BeGenius software to setup the run. All the steps: extraction, reverse transcription, 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 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.

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

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: GI Viral PLUS ELITe_Be_ST_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

Procedure 2: PCR Only (e.g., eluates, 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.** For Controls: 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). For eluates: for each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).
4. Select the “Assay Protocol” of interest: GI Viral PLUS ELITe_Be_ST_200_100 or GI Viral PLUS ELITe_Be_PC or GI Viral PLUS ELITe_Be_NC	**5.** Load the Complete reaction mixture in the Reagent/Elution Rack and insert it in the Cooler Unit	**6.** Load “PCR Basket” with “PCR Cassette”
7. Close the door. Start the run	**8.** View, approve and store the results	

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