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EMPOWERING IVD

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GI Parasitic PLUS ELITe MGB® Kit

reagents for DNA Real-Time PCR

REF RTS503ING

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INTENDED USE

The product **GI Parasitic PLUS ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the genomic DNA of *Giardia lamblia* (**Gla**) (alias *Giardia intestinalis*), *Cryptosporidium* spp. (**Cry**), *Entamoeba histolytica* (**Ehis**), *Encephalitozoon* spp (**Enc**), *Enterocytozoon bieneusi* (**Ent**), extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human stool specimens.

The product is intended for use as an aid in the diagnosis of gastrointestinal parasitic infections in patients suspected of having *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Enterocytozoon bieneusi*, *Encephalitozoon* spp. infection.

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

ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Enterocytozoon bieneusi*, *Encephalitozoon* spp DNAs isolated from specimens and amplified using the assay reagent **GI-P PCR Mix** 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).

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.

PRODUCT DESCRIPTION

The **GI Parasitic PLUS ELITe MGB Kit** provides the assay reagent **GI-P PCR Mix,** an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- *Giardia lamblia* **16s-like** rRNA gene, detected in Channel **Gla**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye,
- *Cryptosporidium* spp**. 18s** rRNA gene, detected in Channel **Cry;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor® 593 (AP593) dye,
- *Entamoeba histolytica* **18s** rRNA gene, detected in Channel **Ehis***;* the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 639 (AP639) dye,
- *Encephalitozoon* spp**. 16s** rRNA gene, detected in Channel **Enc***;* the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 690 (AP690) dye,
- *Enterocytozoon bieneusi* **ITS** region, detected in Channel **Ent***;* the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher and labelled by AquaPhluor 559 (AP559) dye,
- Internal Control (**IC)**, specific for artificial sequence **IC2,** 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-P PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

The **GI Parasitic PLUS ELITe MGB Kit** contains sufficient reagents for **96 tests** on the **ELITe InGenius** and **ELITe BeGenius (12 tests each tube)**, with 20 µL used per reaction.

The **GI Parasitic PLUS ELITe MGB Kit** can be also used in association with equivalent instruments.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
GI-P PCR Mix ref. RTS503ING	Mixture of reagents for Real-Time PCR tube with **WHITE cap**	**8 x 280 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~3,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Thermomixer.
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA, 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, Real-Time PCR and result interpretation of samples, the following products are required:

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA, ref. INT030). **ELITe InGenius Software** version 1.3.0.17 (or later). **GI Parasitic PLUS ELITe_PC**, Assay Protocol with parameters for Positive Control analysis. **GI Parasitic PLUS ELITe_NC**, Assay Protocol with parameters for Negative Control analysis. **GI Parasitic PLUS ELITe_ST_200_100**, Assay Protocol with parameters for Stool specimen analysis.	**ELITe InGenius SP200** (EG SpA, ref. INT032SP200). **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS). **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR). **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000). **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only. **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only. **CPE - Internal Control** (EG SpA, ref. CTRCPE). **GI Parasitic PLUS - ELITe Positive Control** (EG SpA, ref. CTR503ING). **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 with Cary Blair medium.
ELITe BeGenius (EG SpA, ref. INT040). **ELITe BeGenius Software** version 2.1.0 (or later). **GI Parasitic PLUS ELITe_Be_PC**, Assay protocol with parameters for Positive Control analysis. **GI Parasitic PLUS ELITe_ Be_NC**, Assay Protocol with parameters for Negative Control analysis. **GI Parasitic PLUS ELITe_Be_ST_200_100**, Assay Protocol with parameters for Stool specimen analysis.	

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WARNINGS AND PRECAUTIONS

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

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.

Warnings and precautions for molecular biology

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

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

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

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

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

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

Warnings and precautions specific for the components

Component	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
GI-P PCR Mix	-20°C or below (protected from light)	one month	up to seven	up to seven separate* sessions of three hours each or up to 7 consecutive hours (2 sessions of 3 hours each and the time needed to start a third session)

* with intermediate freezing

SPECIMENS AND CONTROLS

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:

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

- transfer 1 mL of InhibitEX Buffer in a 2 mL Sarstedt tube,
- 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,
- insert the swab into the 2 mL Sarstedt tube containing the InhibitEX Buffer and rotate it at least 10 times, leaning against the wall,
- discard the swab and close the tube cap,
- mix by vortexing for ~60 sec,
- incubate in a thermomixer at ~+80 °C and ~800 RPM for 10 min,
- spin at 10,000x RCF for 15 sec,
- 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:

- transfer 500 µL of InhibitEX Buffer in a 2 mL Sarstedt tube,
- transfer 500 µL of sample suspension from the FecalSwab into the 2 mL Sarstedt tube containing the InhibitEX buffer,
- cap the tube securely and mix by vortexing for ~60 sec,
- incubate in a thermomixer at ~+80 °C and ~800 RPM for 10 min,
- spin at 10,000x RCF for 15 sec,
- 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.

Assay Protocols for GI Parasitic PLUS ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
Native Stool or Stool collected in FecalSwab	**ELITe InGenius**	**GI Parasitic 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: 20 µL
	ELITe BeGenius	**GI Parasitic PLUS ELITe_Be_ST_200_100**		

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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.

PCR controls

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

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

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.

ELITe InGenius PROCEDURE

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

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	A) Validation of Positive Control and Negative Control results
		B) Validation of sample results
		C) Sample result reporting

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 (**GI-P Positive Control**, **GI-P Negative Control**) are approved and valid (Status) for the **GI-P PCR Mix** lot to be used. If no valid PCR Controls are available for the **GI-P 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.

STEP 2 - Session Setup

The **GI Parasitic 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:

Thaw the needed **GI-P PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **12 tests** in optimized conditions (2 or more tests per session). Mix gently 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.

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

	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 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.	n.a.	**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.	n.a.
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 PCR Mix** 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 PCR Mix** 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 PCR Mix** 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, 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.
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: At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

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 **GI-P Positive Control** can be used for 4 separate sessions of 3 hours each.

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

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 Parasitic 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.

A. 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 reactions with the **GI Parasitic PLUS ELITe_PC** and **GI Parasitic PLUS 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.

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B. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (channels **Gla**, **Cry**, **Ehis**, **Enc** and **Ent**) and the Internal Control (channel **IC**) with the **GI Parasitic 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.

1) Positive Control	**Status**
GI-P Positive Control	APPROVED
2) Negative Control	**Status**
GI-P 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 DNAs are either detected or not detected.

Result of sample run	Interpretation
Gla:DNA Detected	***Giardia lamblia*** **DNA was detected** in the sample.
Cry:DNA Detected	***Cryptosporidium*** **spp. DNA was detected** in the sample.
Ehis:DNA Detected	***Entamoeba histolytica*** **DNA was detected** in the sample.
Enc:DNA Detected	***Encephalitozoon*** **spp. DNA was detected** in the sample.
Ent:DNA Detected	***Enterocytozoon bieneusi*** **DNA was detected** in the sample.
Gla:DNA Not Detected or below the LoD	***Giardia lamblia*** **DNA was not detected** in the sample. The sample is negative for *Giardia lamblia* DNA, or its concentration is below the assay Limit of Detection.
Cry:DNA Not Detected or below the LoD	***Cryptosporidium*** **spp. DNA was not detected** in the sample. The sample is negative for *Cryptosporidium spp.* DNA, or its concentration is below the assay Limit of Detection.
Ehis:DNA Not Detected or below the LoD	***Entamoeba histolytica*** **DNA was not detected** in the sample. The sample is negative for *Entamoeba histolytica* DNA, or its concentration is below the assay Limit of Detection.
Enc:DNA Not Detected or below the LoD	***Encephalitozoon*** **spp. DNA was not detected** in the sample. The sample is negative for *Encephalitozoon* spp. DNA, or its concentration is below the assay Limit of Detection.
Ent:DNA Not Detected or below the LoD	***Enterocytozoon bieneusi*** **DNA was not detected** in the sample. The sample is negative for *Enterocytozoon bieneusi* DNA, 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 DNA was not efficiently detected, which could be due to problems in sample collection, pretreatment, extraction or PCR steps (e.g., incorrect sampling, degradation or loss of DNA 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:DNA Not Detected or below the LoD" are suitable for analysis but the DNA of the targets was not detected. In this case, the sample may be either negative for the DNA of the targets or the DNA of the targets is present at a concentration below the Limit of Detection of the assay (see “Performance characteristics”).

Note: The results obtained with this assay must be interpreted 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”.

C. 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.

ELITe BeGenius PROCEDURE

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

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	A) Validation of Positive Control and Negative Control results
		B) Validation of sample results
		C) Sample result reporting

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 (**GI-P Positive Control**, **GI-P Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **GI-P 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.

STEP 2 - Session Setup

The **GI Parasitic 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.

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Before to setup a run:

Thaw the needed **GI-P PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **12 tests** in optimized conditions (2 or more tests per session). Mix gently 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.

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

	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.	n.a.	**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.
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). If needed, 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). If needed, 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). If needed, for each “Position” enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
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.		-

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	n.a.	n.a.
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).	n.a.	n.a.
14	Click "Next" to continue.	n.a.	n.a.
15	**Load CPE and PCR Mix** into the "Reagent/Elution Rack".	**Load the PCR Mix** into "Reagent/Elution Rack".	**Load the PCR Mix** into "Reagent/Elution Rack".
16	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, 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). If needed, 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). If needed, 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 Basket**" with "PCR Cassette" in the Inventory Area.	Load the "**PCR Basket**" with "PCR Cassette" in the Inventory Area.	Load the **"PCR Basket**" with "PCR Cassette" in the Inventory Area.
21	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
22	**Load** the "Extraction Basket" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables.	n.a.	n.a.
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: At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

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 **GI-P Positive Control** can be used for 4 separate sessions of 3 hours each.

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

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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 Parasitic 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.

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

PERFORMANCE CHARACTERISTICS

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 *Giardia lamblia, Cryptosporidium* spp., *Entamoeba histolytica, Encephalitozoon* spp. and *Enterocytozoon bieneusi* (Microbix, ZeptoMetrix and EG SpA plasmid DNAs).

Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

Note: The LoD for the targets E. intestinalis and E. bieneusi has been calculated in copies / mL due to the use of plasmid DNAs as reference materials.

The results are reported in the following table.

Pathogen	LoD	95% confidence interval limits	
		Lower limit	Upper limit
Giardia lamblia	35 org / mL	22 org / mL	93 org / mL
Cryptosporidium parvum	176 org / mL	135 org / mL	280 org / mL
Entamoeba histolytica	390 org / mL	291 org / mL	619 org / mL
Encephalitozoon intestinalis	231 copies / mL	153 copies / mL	459 copies / mL
Enterocytozoon bieneusi	101 copies / mL	77 copies / mL	175 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 *Giardia lamblia, Cryptosporidium* spp., *Entamoeba histolytica, Encephalitozoon* spp. and *Enterocytozoon bieneusi* reference material at the claimed concentration.

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

Inclusivity: Efficiency of detection on different strain or isolates

The Inclusivity of the assay, as efficiency of detection for different strain or isolates of *Giardia lamblia, Cryptosporidium* spp., *Entamoeba histolytica, Encephalitozoon* spp. and *Enterocytozoon bieneusi*, was evaluated by *in silico* analysis. The analysis showed sequence conservation and absence of significant mutations with only exception of *E. bieneusi* genotype WR5-like. So, an efficient detection for the most of strains or isolates is expected.

The Inclusivity was also verified through the analysis of 15 reference materials of parasitic cultures from different providers (ZeptoMetrix, Vircell, ATCC and plasmid DNAs).

The results are reported in the following table.

Sample	strain	Outcome
G. lamblia	Portland-1	Gla DNA detected
	WB clone C6	Gla DNA detected
	strain H3	Gla DNA detected
C. parvum	Tyzzer	Cry DNA detected
	Bovine isolate	Cry DNA detected
C. hominis	Morgan-Ryan	Cry DNA detected
E. histolytica	HM-1:IMSS	Ehis DNA detected
	DS4-868	Ehis DNA detected
E. intestinalis	CDC:V297	Enc DNA detected
E. hellem	Didier	Enc DNA detected
E. cuniculi	Stewart	Enc DNA detected
E. bieneusi	genotype B	Ent:DNA detected
E. bieneusi	genotype S9	Ent DNA detected
E. bieneusi	genotype C	Ent DNA detected
E. bieneusi	genotype WR5	Ent:DNA not detected

All the replicates of the tested strains, genotypes or species of the five targets of interest were correctly detected except the *E. bieneusi* genotype WR5-like. However, *E. bieneusi* genotype WR5-like is mainly detected in wild animals.

The product GI Parasitic PLUS ELITe MGB Kit allows to discriminate Encephalitozoon intestinalis from the other two species (E. cuniculi and E. hellem) through melting temperature analysis. The Tm ranges are shown in the following table:

Sample	Tm interval
E. intestinalis	≥ 64.5
E. hellem	< 64.5
E. cuniculi	

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

Interference among targets

The potential interference among targets of the assay was evaluated by a test of co-amplification of *Giardia lamblia, Cryptosporidium* spp., *Entamoeba histolytica, Encephalitozoon* spp. and *Enterocytozoon bieneusi* (EG SpA plasmid DNAs).

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

Target in test	Interfering target at 2,500,000 copies / mL				
	Gla	Cry	Ehis	Ent	Enc
Gla	-	12,500 copies / mL	12,500 copies. / mL	2,500 copies / mL	2,500 copies / mL
Cry	2,500 copies / mL	-	12,500 copies / mL	2,500 copies / mL	2,500 copies / mL
Ehis	2,500 copies / mL	2,500 copies / mL	-	2,500 copies / mL	2,500 copies / mL
Ent	2,500 copies / mL	5,000 copies / mL	5,000 copies / mL	-	2,500 copies / mL
Enc	2,500 copies / mL	2,500 copies / mL	5,000 copies / mL	2,500 copies / mL	-

The GI Parasitic PLUS ELITE MGB Kit shows a minimal interference among targets. All the targets can be detected even when they are about 1000 times less than the other pathogens of interest.

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). Therefore, no cross-reactivity is expected.

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

The results are reported in the following table.

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Organism	Positive / Replicates						Outcome
	Gla	IC	Ent	Cry	Ehis	Enc	
Aeromonas hydrophila	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bacteroides fragilis	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Helicobacter pylori	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
S. cerevisiae	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Enterovirus	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Plesiomonas shigelloides	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Klebsiella pneumoniae	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Escherichia coli	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Serratia marcescens	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Acinetobacter baumannii	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bifidobacterium spp	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Candida albicans	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Citrobacter freundii	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Proteus mirabilis	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Plesiomonas aeruginosa	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Enterobacter cloacae	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Clostridium difficile	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Campylobacter spp.	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Salmonella spp.	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Shigella spp.	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Yersinia enterocolitica	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Adenovirus	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Astrovirus I	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Norovirus I	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Rotavirus	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Sapovirus	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Clostridium nexile	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity

All potentially interfering organisms tested showed no cross-reactivity for the targets using the GI Parasitic PLUS ELITe MGB Kit.

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 and DSMZ) spiked with *Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Encephalitozoon intestinalis and Enterocytozoon bieneusi* (ATCC and EG SpA).

The results are reported in the following table.

Organism	Positive / Replicates						Outcome
	Gla	IC	Ent	Cry	Ehis	Enc	
Aeromonas hydrophila	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
Enterovirus	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 spp	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Candida albicans	5 / 5	5 / 5	5 / 5	0 / 5	0 / 5	5 / 5	Interference
Citrobacter freundii	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
Pseudomonasaeruginosa	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Enterobacter cloacae	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
Campylobacter spp.	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Salmonella spp.	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Shigella spp.	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference

Organism	Positive / Replicates						Outcome
	Gla	IC	Ent	Cry	Ehis	Enc	
Yersinia enterocolitica	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Adenovirus	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Astrovirus I	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Norovirus GI	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Rotavirus	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Sapovirus	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Clostridium nexile	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference

Most of the potentially interfering organisms tested showed no inhibition for the target amplification using the GI Parasitic PLUS ELITe MGB Kit, except *Candida albicans*. The amplification of 30 genomes of the Cry and Ehis targets was impaired by the presence of high quantity of *C. albicans* genomes. In this case, the Cry and Ehis targets gave positive results when 200 genomes were amplified.

To investigate the interference of *C. albicans* on detection of Cry and Ehis targets, further tests were performed on clinical stool specimens positive for *C. albicans*. The results showed that 5x LoD of *C. parvum* (880 organisms / mL) and 3x LoD of *E. histolytica* (1170 organisms / mL) are the lowest concentrations that can be detected by the GI Parasitic PLUS ELITe MGB Kit in stool samples positive for *C. albicans*.

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.

Substance	Positive / Replicates						Outcome
	Gla	IC	Ent	Cry	Ehis	Enc	
Vaselin oil	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Nonoxynol-9	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bismuth subsalicylate	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Loperamide hydrochloride	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Bisacodyl	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Azithromycin	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Vancomycin	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Metronidazole	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Ampicillin	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Cefpodoxime	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Ciprofloxacin	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Hydrocortisone	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Calcium carbonate	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Alginic acid	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Aluminium hydroxide	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Magnesium trisilicate	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Whole blood	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Mucin	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Palmitic acid	0 / 5	5 / 5	0 / 5	0 / 5	0 / 5	0 / 5	No cross-reactivity
Stearic acid	0 / 5	5 / 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 Parasitic PLUS ELITe MGB Kit.

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 *Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Encephalitozoon intestinalis and Enterocytozoon bieneusi* (Microbix, ZeptoMetrix and EG SpA).

The results are reported in the following table.

Substance	Positive / Replicates						Outcome
	Cdif	IC	Yen	Shi	Cam	Sal	
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

Substance	Positive / Replicates						Outcome
	Cdif	IC	Yen	Shi	Cam	Sal	
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 Parasitic PLUS ELITe MGB Kit.

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 *Cryptosporidium parvum* (EG SpA) at a concentration of 1,000,000 copies / mL in 5 sessions.

The results are reported in the following table.

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

In this test with the GI Parasitic PLUS ELITe MGB Kit the cross-contamination was neither detected within sessions nor between sessions.

Whole system failure

The Whole system failure rate for the assay was evaluated by analysing 50 different negative native stool specimens and 30 stool specimens collected in FecalSwab spiked with *Giardia lamblia* (Microbix) at concentration of 3x LoD (105 organisms / mL).

The results are reported in the following table.

Samples	N	Positive	Negative	Whole system failure rate
Native Stool spiked at 3x LoD	50	50	0	0%
Stool in FecalSwab spiked at 3x LoD	30	30	0	0%

In this test with the GI Parasitic 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.

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 *Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Encephalitozoon intestinalis and Enterocytozoon bieneusi* (Microbix, ZeptoMetrix and EG SpA).

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

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	34.37	0.21	0.60	100%
3xLoD Cry+Ent		6	-	-	-	100%
3xLoD Ehis		6	-	-	-	100%
Neg	Cry (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	-	-	-	100%
3xLoD Cry+Ent		6	35.42	0.75	2.11	100%
3xLoD Ehis		6	-	-	-	100%
Neg	Ehis (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	-	-	-	100%
3xLoD Cry+Ent		6	-	-	-	100%
3xLoD Ehis		6	29.43	0.25	0.84	100%
Neg	Ent (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	-	-	-	100%
3xLoD Cry+Ent		6	34.90	1.20	3.45	100%
3xLoD Ehis		6	-	-	-	100%
Neg	Enc (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	33.52	0.26	0.78	100%
3xLoD Cry+Ent		6	-	-	-	100%
3xLoD Ehis		6	-	-	-	100%

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

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	33.88	0.29	0.87	100%
3xLoD Cry+Ent		6	-	-	-	100%
3xLoD Ehis		6	-	-	-	100%
Neg	Cry (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	-	-	-	100%
3xLoD Cry+Ent		6	34.56	0.27	0.79	100%
3xLoD Ehis		6	-	-	-	100%
Neg	Ehis (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	-	-	-	100%
3xLoD Cry+Ent		6	-	-	-	100%
3xLoD Ehis		6	29.34	0.08	0.28	100%
Neg	Ent (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	-	-	-	100%
3xLoD Cry+Ent		6	35.92	1.21	3.36	100%
3xLoD Ehis		6	-	-	-	100%
Neg	Enc (Ct)	6	-	-	-	100%
3xLoD Gla+Enc		6	33.59	0.17	0.52	100%
3xLoD Cry+Ent		6	-	-	-	100%
3xLoD Ehis		6	-	-	-	100%

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

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	33.90	0.22	0.66	100%
3xLoD Cry+Ent		12	-	-	-	100%
3xLoD Ehis		12	-	-	-	100%
Neg	Cry (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	-	-	-	100%
3xLoD Cry+Ent		12	35.15	0.57	1.64	100%
3xLoD Ehis		12	-	-	-	100%
Neg	Ehis (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	-	-	-	100%
3xLoD Cry+Ent		12	-	-	-	100%
3xLoD Ehis		12	29.76	0.23	0.79	100%
Neg	Ent (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	-	-	-	100%
3xLoD Cry+Ent		12	34.91	0.59	1.69	100%
3xLoD Ehis		12	-	-	-	100%
Neg	Enc (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	33.51	0.30	0.89	100%
3xLoD Cry+Ent		12	-	-	-	100%
3xLoD Ehis		12	-	-	-	100%

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

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	33.68	0.33	0.98	100%
3xLoD Cry+Ent		12	-	-	-	100%
3xLoD Ehis		12	-	-	-	100%
Neg	Cry (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	-	-	-	100%
3xLoD Cry+Ent		12	36.02	0.55	1.52	100%
3xLoD Ehis		12	-	-	-	100%
Neg	Ehis (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	-	-	-	100%
3xLoD Cry+Ent		12	-	-	-	100%
3xLoD Ehis		12	29.30	0.17	0.58	100%
Neg	Ent (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	-	-	-	100%
3xLoD Cry+Ent		12	34.96	0.51	1.45	100%
3xLoD Ehis		12	-	-	-	100%
Neg	Enc (Ct)	12	-	-	-	100%
3xLoD Gla+Enc		12	33.94	0.29	0.86	100%
3xLoD Cry+Ent		12	-	-	-	100%
3xLoD Ehis		12	-	-	-	100%

In the Repeatability test, the GI Parasitic PLUS ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 3.36%.

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 *Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Encephalitozoon intestinalis and Enterocytozoon bieneusi* (Microbix, ZeptoMetrix and EG SpA).

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

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	34.33	0.41	1.21	100%
3xLoD Cry+Ent		36	41.23	-	-	97%
3xLoD Ehis		36	-	-	-	100%
Neg	Cry (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	35.39	0.62	1.74	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Ehis (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	29.74	0.26	0.86	100%
Neg	Ent (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	35.25	0.76	2.15	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Enc (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	33.65	0.39	1.16	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	-	-	-	100%

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

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	33.69	0.31	0.93	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Cry (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	35.97	0.77	2.14	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Ehis (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	29.35	0.21	0.70	100%
Neg	Ent (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	34.61	0.71	2.04	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Enc (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	33.76	0.34	1.00	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	-	-	-	100%

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

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	34.42	0.50	1.45	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Cry (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	35.75	0.58	1.61	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Ehis (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	29.99	0.32	1.05	100%
Neg	Ent (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	35.29	1.08	3.07	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Enc (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	33.66	0.52	1.55	100%
3xLoD Ehis		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.

Sample	Target	N	Mean	SD	%CV	%Agreement
Neg	Gla(Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	33.73	0.30	0.90	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Cry (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	35.93	0.65	1.80	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Ehis (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	29.58	0.18	0.59	100%
Neg	Ent (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	-	-	-	100%
3xLoD Cry+Ent		36	34.27	0.58	1.69	100%
3xLoD Ehis		36	-	-	-	100%
Neg	Enc (Ct)	36	-	-	-	100%
3xLoD Gla+Enc		36	33.93	0.38	1.11	100%
3xLoD Cry+Ent		36	-	-	-	100%
3xLoD Ehis		36	-	-	-	100%

In the Reproducibility test, the GI Parasitic PLUS ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 3.07%.

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 or in modified Cary Blair medium, 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:

Negative stool tested for the target	N	Positive	Negative	% Diagnostic Specificity
Giardia lamblia	196	1	195	99.5%
Cryptosporidium spp.	196	0	196	100%
Entamoeba histolytica	100	0	100	100%
Encephalitozoon spp.	100	0	100	100%
Enterocytozoon bieneusi	100	0	100	100%

All stool samples were valid for analysis. The positive sample has a low titer, close to the LoD value of the system, and for it the retesting was not possible.

The Diagnostic Specificity of the GI Parasitic PLUS ELITe MGB Kit in association to stool, in this test, was equal to 99.5% for Gla, 100% for Cry, 100% for Ehis, 100% for Enc and 100% for Ent.

The IC Ct cut-off value is set at 30.

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 or in Cary-Blair medium, certified positive for each target or spiked with reference materials.

As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Positive/spiked stool	N	Positive	Negative	% Diagnostic Sensitivity
Positive for *Giardia lamblia*	77	76	1	98.7%
Positive for *Cryptosporidium* spp.	86	83	3	96.5%
Positive for *Entamoeba histolytica*	3	3	0	100%
Spiked for *Entamoeba histolytica*	50	50	0	
Positive for *Encephalitozoon spp.*	12	12	0	100%
Spiked for *Encephalitozoon spp.*	45	45	0	
Positive for *Enterocytozoon bieneusi*	46	43	3	95.6%
Spiked for *Enterocytozoon bieneusi*	22	22	0	

The Diagnostic Sensitivity of the GI Parasitic PLUS ELITe MGB Kit in association to stool, in this test, was equal to 98.7% for Gla, 96.5% for Cry, 100% for Ehis, 100% for Enc and 95.6% for Ent.

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 Parasitic PLUS ELITe MGB Kit”, FTP 503ING.

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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, PCR and detection of nucleic acids.

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 is not detected in the DNA extracted from the sample; however, it cannot be excluded that the target DNA 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 targeted by the product primers and probes may impair detection of target DNA.

The detection of *Cryptosporidium* spp. and *Entamoeba histolytica* could be impaired by *Candida albicans* if present in the clinical stool specimen.

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.

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TROUBLESHOOTING

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Positive Control. Check the volumes of PCR Mix and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit) Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
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.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix 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 PCR Mix.	Use a new aliquot of PCR Mix.
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.

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control, and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Prepare a new aliquot of PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the pre-treated sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the 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.

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

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination 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. Use a new tube of PCR Mix and / or CPE.

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SYMBOLS

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

NOTICE TO THE USERS

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

NOTICE TO PURCHASER: LIMITED LICENSE

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

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

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GI Parasitic PLUS ELITe MGB® Kit
used in association with Genius series® platforms
Ref: RTS503ING

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Intended use

The product **GI Parasitic PLUS ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the genomic DNA of *Giardia lamblia* (Gla) (alias Giardia intestinalis), *Cryptosporidium* spp. (Cry), *Entamoeba histolytica* (Ehis), *Encephalitozoon* spp (Enc), *Enterocytozoon bieneusi* (Ent), extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human stool specimens.

The product is intended for use as an aid in the diagnosis of gastrointestinal parasitic infections in patients suspected of having *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Enterocytozoon bieneusi*, *Encephalitozoon* spp. infection.

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

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	16s like	FAM	Gla
Target 2	18s	AP593	Cry
Target 3	18s	AP639	Ehis
Target 4	16s	AP690	Enc
Target 5	ITS	AP559	Ent
Internal Control	IC2	AP525	IC

Validated matrix

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

Kit content and related products

GI Parasitic PLUS ELITe MGB Kit (RTS503ING)	GI Parasitic PLUS - ELITe Positive Control (CTR503ING)
 X 8	 X 3
GI-P PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube	**GI-P Positive Control** 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles
Maximum shelf-life: **24 months**	Maximum shelf-life **24 months**
Storage temperature **≤ -20°C**	Storage temperature **≤ -20°C**

Other products required not provided in the kit

- ELITe InGenius instrument: INT030.
- ELITe BeGenius instrument: INT040.
- ELITe InGenius SP 200: INT032SP200.
- ELITe InGenius SP200 Consumable Set: INT032CS.
- ELITe InGenius PCR Cassette: INT035PCR.
- ELITe InGenius Waste Box: F2102-000.
- 300 µL Filter Tips Axigen: TF-350-L-R-S.
- 1000 µL Filter Tips Tecan: 30180118.
- 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	20 µL
CPE volume	10 µL	GI-P 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	Gla	**35 org. / mL**	**98.7%** (76/77)	**99.5%** (195/196)
	Cry	**176 org. / mL**	**96.5%** (83/86)	**100%** (196/196)
	Ehis	**390 org. / mL**	**100%** (53/53)	**100%** (100/100)
	Enc	**231 copies / mL**	**100%** (57/57)	**100%** (100/100)
	Ent	**101 copies / mL**	**95.6%** (65/68)	**100%** (100/100)

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.

Sample type	Transport/Storage conditions			
	+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ±10 °C**	**-70 ±15 °C**
Native stool collected without preservatives	≤ 24 hours	≤ 48 hours	≤ 1 month	≤ 2 months
Stool collected in FecalSwab (Modified Cary Blair medium)	≤ 48 hours	≤ 5 days	≤ 1 month	≤ 2 months

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, 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: **GI-P Positive Control** and **GI-P Negative Control** in the "Controls" menu. *Note:* Both must have been run, approved and not expired.	**3.** Thaw the **GI-P PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

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 Parasitic PLUS ELITe_ST_200_100	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix 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 Parasitic PLUS ELITe_ST_200_100 or GI Parasitic PLUS ELITe_PC or GI Parasitic PLUS ELITe_NC	**5.** Select the method "PCR Only" and the sample position "Elution Tube"	**6.** Load the PCR Mix 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, 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: **GI-P Positive Control** and **GI-P Negative Control** in the "Controls" menu. *Note:* Both must have been run, approved and not expired.	**3.** Thaw the **GI-P PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

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 Parasitic 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 PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load "PCR Basket" with "PCR Cassette" and the "Extraction Basket" 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.** Verify the extraction volumes: Input: "200 µL", Eluate: "100 µL"
4. Select the "Assay Protocol" of interest: GI Parasitic PLUS ELITe_Be_ST_200_100 or GI Parasitic PLUS ELITe_Be_PC or GI Parasitic PLUS ELITe_Be_NC	**5.** Load the PCR-Mix 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	