Instructions for use

C. difficile ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF M800358

UDI 08033891486563

CE IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
07	Expansion of use with ELITe BeGenius New graphics and content setting of the IFU.	22/11/24
06	ELITe InGenius - PERFORMANCE CHARACTERISTICS: Update of Potentially interfering markers to include Clostridium Nexile.	09/09/24
05	Addition of analytical data (LoD Verification, Matrix pre-treatment equivalence, Potentially interfering markers and substances) and alternative method of sample pre-treatment.	15/03/24
00–04	new product development and succeeding changes	-

TABLE OF CONTENT

1 INTENDED USE

The product ***C. difficile* ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids Real-Time PCR assay for the **detection of toxin A and toxin B genes of toxigenic *Clostridium difficile*** (***C. difficile***), including the hypervirulent epidemic NAP1/BI/027 strain, in DNA samples extracted from unformed or liquid stool 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 specimens of stool.

The assay is also validated in association with the **7500 Real-Time PCR System**, using human specimens of stool.

The product is intended for use as an aid in the diagnosis of toxigenic ***C. difficile*** in healthcare settings in conjunction with other laboratory test results and clinical data.

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

2 ASSAY EXPLANATION

Toxigenic *C. difficile* infection commonly manifests as mild-to-moderate diarrhea, occasionally with abdominal cramping. In rare cases, patients with toxigenic *C. difficile* infection can present with an acute abdomen and fulminant life-threatening colitis. Approximately 20% of individuals who are hospitalized acquire toxigenic *C. difficile* during hospitalization, and more than 30% of these patients develop diarrhea. Thus, toxigenic ***C. difficile*** colitis is currently one of the most common nosocomial infections. The diagnosis of toxigenic *C. difficile* colitis should be suspected in any patient with diarrhea who has received antibiotics within the previous 2 months and/or when diarrhea occurs 72 hours or more after hospitalization. Colonization occurs by the fecal-oral route. Pathogenic strains of toxigenic *C. difficile* produce 2 distinct toxins. Toxin A is an enterotoxin, and toxin B is a cytotoxin. Both toxin A and toxin B appear to play a role in the pathogenesis of toxigenic *C. difficile* colitis in humans.

The ***C. difficile* ELITe MGB Kit** is a triplex real-time amplification-based assay that targets the toxin A and toxin B genes of toxigenic *C. difficile*, and an internal control. Real-time amplification-based assays significantly reduce laboratory time compared with standard culture tests, improving the efficiency of the procedure.

The ***C. difficile* ELITe MGB Kit** targets both toxin A gene (tcdA) and toxin B gene (tcdB), therefore detecting all possible toxigenic genotypes (A+/B+; A+/B-; A-/B+).

3 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting **toxin A and toxin B genes of toxigenic Clostridium difficile** (*C. difficile*), including the hypervirulent epidemic NAP1/BI/027 strain, isolated from specimens and amplified using the assay reagent ***C. difficile* 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) 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.

4 PRODUCT DESCRIPTION

The ***C. difficile* ELITe MGB Kit** provides the assay reagent ***C. difficile* PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- the ***C. difficile*** **toxin A-specific gene, detected in Channel Toxin A**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor® 525 (AP525) dye.
- the ***C. difficile*** **toxin B-specific gene, detected in Channel Toxin B**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by FAM 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 642 (AP642) dye.

The reaction mixture **C. difficile PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

The product ***C. difficile*** **ELITe MGB Kit** contains sufficient reagents for **96 tests** on the **ELITe InGenius** and **ELITe BeGenius**, with 20 µL used per reaction.

The product contains sufficient reagents for **100 tests on other systems**, with 20 µL used per reaction.

5 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
C. difficile **PCR Mix ref. M800358**	Mixture of reagents for Real-Time PCR tube with **YELLOW cap**	**4 × 540 µL**	-

6 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 (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.

7 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.

Table 2

Instruments and Softwares	Products and Reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA, ref. INT030). **ELITe InGenius Software** version 1.3.0.17 (or later). **Cdiff ELITe_PC**, Assay Protocol with parameters for Positive Control analysis. **Cdiff ELITe_NC**, Assay Protocol with parameters for Negative Control analysis. **Cdiff 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). ***C. difficile* – ELITe Positive Control** (EG SpA, ref. M800373) **InhibitEX Buffer** (QIAGEN GmBH, Germany, ref. 19593), or **S.T. A.R. buffer** (Roche Diagnostics GmbH, ref. 3 335 208), or an equivalent device. **Minitip Flocked Swab**® (COPAN Italia S.p.A., Italy, ref. 518CS01) or an equivalent device.
ELITe BeGenius (EG SpA, ref. INT040). **ELITe BeGenius Software** version 2.1.0 (or later). **Cdiff ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis. **Cdiff ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis. **Cdiff ELITe_Be_ST_200_100**, Assay Protocol with parameters for stool specimen analysis.	
7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific, ref. 4406985) **NucliSENS® easyMAG®** (bioMérieux SA, ref. 200111) **ELITe STAR (**EG SpA, ref. INT010) **ELITe GALAXY** (EG SpA, ref. INT020)	**MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL** (Life Technologies, ref. 4346906) **NucliSENS® easyMAG® Reagents** (bioMérieux SA, Ref. 280130, 280131, 280132, 280133, 280134, 280135), **InviMag Universal Kit / IG** (INVITEK, ref. 2450120100). **ELITe GALAXY 300 Extraction Kit** (EG SpA, ref. INT021EX), **CPE – Internal Control** (EG SpA., ref. CTRCPE) ***C. difficile* – ELITe Positive Control** (EG SpA, ref. M800373) **S.T.A.R. buffer** (Roche Diagnostics GmbH, ref. 3 335 208).

8 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.

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

When amplification session is manually setup, it is necessary to have available lab coats, gloves and tools which are exclusively used for the extraction / preparation of the amplification reactions and for the amplification / detection of amplification products.

Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

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

Table 3

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
***Cdiff* PCR Mix**	-20 °C or below (protected from light)	one month	up to five	up to five separate* sessions of three hours each

* with intermediate freezing

9 SPECIMENS AND CONTROLS for ELITe InGenius and ELITe BeGenius

9.1 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
Native stool	collected without preservatives	≤ 24 hours	≤ 48 hours	≤ 1 month	> 1 month

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 samples pre-treatment with InhibitEX Buffer (QIAGEN, Germany. ref. 19593):

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.

Follow the instructions described below for samples pre-treatment with S.T.A.R. buffer (Roche Diagnostics GmbH, ref. 3 335 208):

Pre-treatment procedure starting from native stool collected without preservatives

- prepare a labeled 1.5 mL tube for each raw stool and dispense 0.8 mL of S.T.A.R. buffer into one tube.
- vortex the raw stool, and then use a pipettor with an aerosol resistant tip to transfer approximately 200 µL (use a wide bore tip or plastic spatula as necessary for thick stool samples) of the raw stool into the 1.5 mL tube containing the S.T.A.R. buffer.
- cap the tube securely, and then vortex the tube to homogenize mixture (20-30 sec).
- centrifuge the homogenized solution at 13.000×g (RCF) for 1 minute to clarify the sample.
- 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).
- store the clarified stool in at +2 / +8 °C for up to 7 days before proceeding with the extraction.

NOTE

The S.T.A.R. Buffer must be stored at room temperature. White precipitates may form when the buffer is stored below room temperature. Before beginning the extraction procedure check if any precipitates have formed, and if so warm the solution to 30-40 °C in a water bath or incubator until the precipitates have dissolved.

Store purified nucleic acids at +2 / +8 ° C if they will be used on the same day they were extracted or at temperature lower than -20 °C for long term storage.

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 *C. difficile* ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
Native stool collected without preservatives	**ELITe InGenius**	**Cdiff ELITe_ST_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume: 10 µL Melting analysis: optional
	ELITe BeGenius	**Cdiff ELITe_Be_ST_200_100**		

For all protocols, 200 µL of pre-treated 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 section 8 WARNINGS AND PRECAUTIONS page 6

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 section 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 18to check data concerning interfering substances.

9.2 PCR controls

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

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

9.3 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.

External controls for *Clostridium difficile* assays are provided by several manufacturers (e.g. Qnostics Ltd, UK, and ZeptoMetrix Corp., US).

10 ELITe InGenius PROCEDURE

The procedure to use the ***C. difficile*** **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

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.

STEP 2 - Session Setup

The ***C. difficile*** **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 **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (at least 5 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

Table 7

	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 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 and Elution tubes 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 7 (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

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 up to 5 work sessions of 3 hours each; 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 **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.

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 ***C. difficile*** **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.

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.

Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the target (channels **Toxin A** and **Toxin B**) and the Internal Control (channel **IC**) with the **Cdiff 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 8

1) Positive Control	**Status**
C. difficile Positive Control	APPROVED
2) Negative Control	**Status**
C. difficile 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.

Table 9

Result of Sample run	**Interpretation**
Cdiff: DNA Detected	**Toxin A gene or toxin B gene DNA of *C. difficile* was detected** in the sample. The sample is positive for *C. difficile* and it could be **toxigenic**.

Table 9 (continued)

Cdiff: DNA Not Detected or below LoD	**Toxin A gene and toxin B gene DNA of *C. difficile* was not detected** in the sample. The sample is valid negative for toxigenic *C. difficile* or its concentration is below the assay Limit of Detection.
Invalid - Retest Sample	**Invalid assay result** caused by Internal Control failure (incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as “Invalid - Retest Sample” by the **ELITe InGenius software** are not suitable for result interpretation. In this case, the Internal Control DNA was not efficiently detected due to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitor carry-over in the eluate), which may cause incorrect results.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode. (see 18 TROUBLESHOOTING page 49

Samples reported as “Cdiff DNA Not Detected or below LoD" are suitable for analysis but it was not possible to detect *C. difficile* DNA for toxin A and toxin B genes. In this case it cannot be excluded that the *C. difficile* DNA for toxin A and toxin B genes is present at a concentration below the limit of detection of the assay (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 18). Alternatively, a *C. difficile* not toxigenic could be present in the sample.

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”.

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.

11 ELITe BeGenius PROCEDURE

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

Table 10

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

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.

STEP 2 - Session Setup

The ***C. difficile*** **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:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (at least 5 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:

Table 11

	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 pre-treated 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.
3	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen	Select "**Perform Run**" from the "Home" screen.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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.		-
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 PCR Mix** into the "Reagent/Elution Rack".	**Load the PCR Mix** into "Reagent/ Elution Rack".	**Load the PCR Mix** into "Reagent/ Elution Rack".

Table 11 (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). 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 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

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 5 work sessions of 3 hours each; 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 **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.

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 ***C. difficile* 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.

12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius

12.1 Limit of Detection

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

The LoD of this assay was tested using plasmid DNA containing the amplification products whose initial concentration was measured by spectrophotometer. The plasmid DNA was diluted to a titre of about 10 copies / 10 µL in presence of 20,000 copies of Internal Control (IC) / 10 µL. This sample was tested in 18 replicates carrying out the amplification by ELITechGroup S.p.A. products in association with two different **ELITe InGenius** instruments.

The results are reported in the following table.

Table 12

Samples	N	positive	negative	Toxin A Ct mean	Toxin B Ct mean
10 copies plasmid DNA + 20,000 copies of IC	18	18	0	35.80	36.62

The LoD was verified by testing on ELITe InGenius negative clinical stool specimens spiked with reference materials (ZeptoMetrix) at 500 CFU/mL. The test was performed using both the pre-treatment procedures **InhibitEX Buffer** (QIAGEN) and **S.T.A.R. buffer** (Roche Diagnostics GmbH).

The results are reported in the following table.

Table 13

Stool Sample	Titer (C-F-U/m-L)	N	Toxin B		Toxin A		% rate of positivity
			Positive	Negative	Positive	Negative	
Pre treatment with S.T.A.R Buffer	50-0	30	30	0	29	1	100 %
Pre treatment with Inhibitex Buffer	50-0	30	30	0	30	0	100 %

The LoD value of 500 CFU/mL was verified by testing on ELITe BeGenius native stool samples spiked with reference material and the results obtained confirmed the claimed concentration.

12.2 Matrix pre-treatment equivalence

The Matrix pre-treatment equivalence between **InhibitEX Buffer** (QIAGEN) and **S. T. A. R. buffer** (Roche Diagnostics GmbH) was verified using presumably negative native stool samples collected from different subjects and the same samples spiked with reference materials (ZeptoMetrix) at 3xLoD

The results of negative percent agreement are reported in the following table.

Table 14

Negative Stool Sample	N	Toxin B		Toxin A		% Negative agreement
		Positive	Negative	Positive	Negative	
Pre-treatment with STAR Buffer	26	1	25	0	26	96.2%
Pre-treatment with Inibitex Buffer	26	1	25	0	26	96.2%

The results of positive percent agreement are reported in the following table.

Table 15

Spiked Stool Sample	N	Toxin B		Toxin A		% Positive agreement
		Positive	Negative	Positive	Negative	
Pre-treatment with STAR Buffer	25	25	0	25	0	100%
Pre-treatment with Inibitex Buffer	25	25	0	25	0	100%

12.3 Test with certified reference material

The performances of the product were evaluated using the calibrated reference material «Clostridium difficile 017 Evaluation Panel» (Qnostics, Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation by ELITechGroup S. p. A. products in association with **ELITe InGenius** instrument.

The results are reported in the following table.

Table 16 Tests with «Clostridium difficile 017 Evaluation Panel»

Sample	Sample Content	Nominal titre (CFU/mL)	Positive / Replicates
CD1421749	*C. difficile* A-B+	$8.0x10^4$	2/2
CD1421759	*C. difficile* A-B+	$8.0x10^5$	2/2
CD1421769	*C. difficile* A-B+	$8.0x10^6$	2/2
CD142CS53	*C. sordellii* A-B-	$2.1x10^5$	0/2

All samples were correctly detected.

A further test was carried out using the calibrated reference material «Clostridium difficile 027 Evaluation Panel» (Qnostics, Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation by ELITechGroup S. p. A. products in association with **ELITe InGenius** instrument. The results are reported in the following table.

Table 17 Tests with «Clostridium difficile 027 Evaluation Panel»

Sample	Sample Content	Nominal titre (CFU/mL)	Positive / Replicates
CD1422737	C. difficile A+B+	$5.0x10^3$	2/2
CD1422747	*C. difficile* A+B+	$5.0x10^4$	2/2
CD1422757	C. difficile A+B+	$5.0x10^5$	2/2
CD1422767	*C. difficile* A+B+	$5.0x10^6$	2/2

All samples were correctly detected.

The performances of the product were also evaluated using the reference material «QCMD 2013 Clostridium difficile EQA Panel» (Qnostics Ltd, UK), a panel of *C. difficile* dilutions within the concentration limit. Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, by ELITechGroup S. p. A. products in association with **ELITe InGenius** instrument.

The results are reported in the following table.

Table 18 Tests with «QCMD 2013 Clostridium difficile EQA Panel»

Sample	Sample Content	Nominal titre (CFU/mL)	Sample Status	Pos. / Rep.
CD13-01	C. difficile 027	$4.6x10^5$	Frequently detected	2/2
CD13-02	*C. difficile* 017	$8.0x10^6$	Frequently detected	2/2
CD13-03	Cd Negative	-	Negative	0/2
CD13-04	C. difficile 027	$4.6x10^6$	Detected	2/2
CD13-05	*C. difficile* 027	$4.6x10^6$	Frequently detected	2/2
CD13-06	C. difficile 017	$8.0x10^5$	Frequently detected	2/2
CD13-07	*C. difficile* 027	$4.6x10^3$	Detected	2/2
CD13-08	*C. sordellii*	$2.1x10^5$	Negative	0/2
CD13-09	*C. difficile* 027	$4.6x10^5$	Frequently detected	2/2
CD13-10	*C. difficile* 017	$8.0x10^4$	Detected	2/2

All samples were correctly detected.

12.4 Reproducibility with reference material

The Reproducibility of Ct values obtained by *C. difficile* ELITe MGB Kit in association with the ELITe InGenius was tested by analysing the reference material «NATtrol™ C. difficile Verification Panel» (ZeptoMetrix, US) containing ribotypes 002, 017, 078 and 027.

The Reproducibility was obtained through the analysis of panel samples in duplicates, in two runs per day. Three different lots of product were tested in three different days, on three different instruments by three different operators.

The Ct values of toxin A and toxin B genes were used to calculate the percentage Coefficient of Variability (%CV) in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Table 19

Sample	Toxin A gene				Toxin B gene			
	Pos. / rep.	Mean Ct	SD	%CV	Pos. / rep.	Mean Ct	SD	%CV
C. difficile 002	12/12	20.70	0.31	1.50	12/12	21.78	0.23	1.05
C. difficile 017	12/12	24.39	0.20	0.84	12/12	25.48	0.11	0.43
C. difficile 078	12/12	24.32	0.24	0.98	12/12	25.30	0.09	0.34
C. difficile 027-1	12/12	22.42	0.25	1.12	12/12	23.13	0.16	0.71
C. difficile 027-2	12/12	24.10	0.32	1.32	12/12	24.82	0.25	1.01
C. sordelli	0/12	-	-	-	0/12	-	-	-

The Reproducibility of Ct values for the two targets showed a low %CV that did not exceed 2 %.

Furthermore, the Reproducibility of melting temperature (Tm) values obtained by the ELITechGroup S. p. A. products in association with the ELITe InGenius instrument was tested by analysing the reference material «NATtrol™ C. difficile Verification Panel» (ZeptoMetrix, US).

The Reproducibility was obtained through the analysis of panel samples in duplicates, in two runs per day. Three different lots of product were tested in three different days, on three different instruments by three different operators.

The Tm values of toxin A and toxin B genes were used to calculate the % CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Table 20

Sample	Toxin A gene				Toxin B gene			
	N	Mean Tm	SD	%CV	N	Mean Tm	SD	%CV
C. difficile 002	12	64.78	0.16	0.25	12	60.73	0.22	0.37
C. difficile 017	12	64.59	0.10	0.15	12	60.58	0.29	0.48
C. difficile 078	12	64.55	0.09	0.14	12	60.57	0.28	0.47
C. difficile 027-1	12	64.73	0.17	0.26	12	62.98	0.22	0.35
C. difficile 027-2	12	64.68	0.12	0.19	12	63.12	0.23	0.37

The Reproducibility of Tm values for the two targets showed a low %CV that did not exceed 0.5 %.

Interestingly, while the Tm for the toxin A gene resulted very similar for all the *C. difficile* Ribotype analyzed, the Tm for the toxin B gene resulted clustered in two groups: one includes the *C. difficile* Ribotypes 002, 017 and 078, the other the hypervirulent *C. difficile* Ribotype 027.

The clustered analysis is reported in the table below.

Table 21

Sample	Toxin A gene				Toxin B gene			
	N	Mean Tm	SD	%CV	N	Mean Tm	SD	%CV
C. difficile 002, 017, 078	60	64.67	0.15	0.24	36	60.63	0.27	0.45
Hypervirulent *C. difficile* 027					24	63.05	0.23	0.37

This clustering is due to two nucleotide polymorphisms in the probe hybridization region of toxin B gene shared by the hypervirulent C. difficile Ribotype 027 (e. g. strain R 12087, SEQID HM062510; strain UK1, SEQID KC292158; strain CD196, SEQID FN538970) and other Clade 2 Binary Toxin positive C. difficile strains (e. g. strain R9385, Ribotype 122, SEQID HM062502; strain R10870, Ribotype 111, SEQID HM062497; strain CH6230, Ribotype 251, SEQID HM062509; strain J9965, SEQID HM062500).

On the basis of the results a Tm cut-off of 62.0 °C can be defined to identify the presumptive presence of the hypervirulent C. difficile ribotype 027 in the sample as shown in the table below.

Table 22

Toxin B gene Tm values				
Other *C. difficile*		Tm cut-off	Presumptive Hypervirulent *C. difficile* 027	
Mean Tm	Mean Tm + 4SD		Mean Tm – 4 SD	Mean Tm
60.63	61.71	62.00	62.12	63.05

12.5 Potentially interfering markers: Interference

The inhibition by potentially interfering markers was evaluated through the analysis of a panel of purified nucleic acids at high concentration (at least 10^5 copies / reaction) including the following organisms: *Aeromonas hydrophilia* (AH), *Bacteroides fragilis* (BF), *Vibrio cholera* (VC), *Helicobacter pylori* (HP), *Saccharomyces cerevisae* (SC), *Plesiomonas shigelloides* (PS), *Klebsiella pneumoniae* (KC), *Escherichia coli* (Ecoli), *Serratia marcescens* (SM), Acinetobacter baumannii (AB), *Bifidobacterium spp* (Bifido), *Candida albicans* (CA), *Citrobacter freundii* (CF), *Clostridium nexile* (Cnex), *Proteus mirabilis* (PM), *Pseudomonas aeruginosa* (PA), *Enterobacter cloacae* (EC), *Giardia lamblia* (GL), *Cryptosporidium spp* (CP), *Entamoeba histolytica* (EH), *Enterovirus*, *Adenovirus*, *Astrovirus*, *Norovirus*, *Rotavirus*, *Sapovirus*.

The genomic DNAs of each organism at high concentration (at least 10^5 copies / reaction) were spiked with genomic DNA of *Clostridioides difficile* (Vircell Microbiologists, Spain, code MBC043), as reference material at a concentration of about 3x LoD (1500 CFU / mL) and added with 20,000 copies / reaction of Internal Control template (CPE - Internal Control) to mimic the extracted clinical samples.

Reference sample with the targets of interest but without the Potential Interfering Organisms were also analyzed.

Each organism was tested in 6 replicates in randomized positions on ELITe InGenius in "*PCR Only*" mode.

A summary of results is shown in the tables below.

Table 23

Sample	N		Mean Ct			% Pos. Agreement	Outcome
	Toxin B	Toxin A	Toxin B	Toxin A	IC		
Reference (Cdif)	6/6	6/6	37.29	37.08	24.41	100%	No inhibition
Cdif + AH-BF-VC	6/6	6/6	36.97	36.57	24.27	100%	No inhibition
Cdif + HP-SC-PS	6/6	6/6	37.33	37.42	23.92	100%	No inhibition
Cdif + KP-Ecoli-SM	6/6	6/6	37.56	37.64	23.67	100%	No inhibition
Cdif + AB-Bifido-CA	6/6	6/6	37.93	38.37	24.26	100%	No inhibition
Cdif + CF-Cnex-PM	6/6	6/6	38.55	38.00	24.26	100%	No inhibition
Cdif + PA-EC-GL	6/6	6/6	38.66	38.11	24.16	100%	No inhibition
Cdiff+ EH	6/6	6/6	38.26	37.90	23.94	100%	No inhibition
Cdiff+ CP	6/6	6/6	38.03	38.29	23.97	100%	No inhibition
Cdiff-Adeno	6/6	6/6	37.81	37.49	23.64	100%	No inhibition
Cdiff-Entero	6/6	6/6	37.62	37.86	23.03	100%	No inhibition
Cdiff-Astro	6/6	6/6	37.58	37.34	24.11	100%	No inhibition
Cdiff-Noro	6/6	6/6	37.75	37.90	24.22	100%	No inhibition
Cdiff-Sapo	6/6	6/6	37.21	36.92	23.92	100%	No inhibition
Cdiff-Rota	6/6	6/6	37.74	37.45	22.90	100%	No inhibition
Cdiff-Cnex	6/6	6/6	37.59	36.77	24.03	100%	No inhibition

All potentially interfering organisms tested showed no inhibition of the *C. difficile* target amplification using the *C. difficile* ELITe MGB Kit.

12.6 Potentially interfering markers: cross-reactivity

The absence of cross-reactivity with potential interfering organisms was also verified through the analysis of a panel of purified nucleic acids from different organisms as reported in the Potentially interfering markers: Interference test above.

The genomic DNAs of each organism at high concentration (at least 10^5 copies / reaction) were added with 20,000 copies / reaction of Internal Control template (CPE - Internal Control) to mimic the extracted clinical samples.

Reference sample with the targets of interest but without the Potential Interfering Organisms were also analyzed.

Each organism was tested in 6 replicates in randomized positions on ELITe InGenius in “PCR Only” mode.

A summary of results is shown in the tables below.

Table 24

Sample	Positive / Replicates		IC mean Ct	% Negative Agreement	Outcome
	Toxin A	Toxin B			
Reference	0 / 6	0 / 6	24.55	100%	No cross-reactivity
AH-BF-VC	0 / 6	0 / 6	23.77	100%	No cross-reactivity
HP-SC-PS	0 / 6	0 / 6	23.72	100%	No cross-reactivity

Table 24 (continued)

Sample	Positive / Replicates		IC mean Ct	% Negative Agreement	Outcome
	Toxin A	Toxin B			
KP-Ecoli-SM	0 / 6	0 / 6	23.85	100%	No cross-reactivity
AB-Bifido-CA	0 / 6	0 / 6	25.58	100%	No cross-reactivity
CF-Cnex-PM	0 / 6	0 / 6	25.50	100%	No cross-reactivity
PA-EC-GL	0 / 6	0 / 6	23.90	100%	No cross-reactivity
CP-EH-Entero	0 / 6	0 / 6	23.77	100%	No cross-reactivity
Adeno-Astro-Noro	0 / 6	0 / 6	23.87	100%	No cross-reactivity
Rota-Sapo	0 / 6	0 / 6	23.84	100%	No cross-reactivity
C. nexile	0/6	6/6	23.74	0%	Cross-reactivity

All potentially interfering markers tested showed no cross-reactivity for the *C. difficile* target using the *C. difficile* ELITe MGB Kit, with the exception of *Clostridium Nexile* VPI C48-37 strain, that cross-reacted with the amplification reaction for Toxin B gene.

12.7 Potentially interfering substances

The effect of Potentially interfering substances was evaluated by analyzing endogenous or exogenous substances that may be found in native stool samples (most difficult matrix), within clinically relevant limits. In particular, the following exogenous and endogenous substances were tested in the table below as indicate in CLSI EP37 Ed1E:

Table 25

Interfering Substance	Active principle / Product	ID	Concentration
Enemas	Vaselin oil	VAS	20 mg/mL
Spermicidal lubricant	Nonoxynol-9	NON	1.2% v/v
Anti-diarrheal medications	Bismuth subsalicylate	BISM	0.87 mg/mL
	Loperamide hydrochloride	LOP	0.005 mg/mL
Laxatives	Bisacodyl	BISA	0.25 mg /mL
Antibiotics	Azithromycin	AZI	10 µg /mL
	Vancomycin	VAN	0.12 mg/mL
	Metronidazole	MET	0.12 mg/mL
	Ampicillin	AMP	0.08 mg/mL
	Cefpodoxime	CEF	4.5 µg/mL
	Ciprofloxacin	CIP	5 µg/mL
Anti-inflammatories	Hydrocortisone	HYD	3 mg/mL

Table 25 (continued)

Interfering Substance	Active principle / Product	ID	Concentration
Antiacids	Calcium carbonate	CAL	0.5 mg/mL
	Alginic acid	ALG	0.01 mg/mL
	Aluminium hydroxide	ALU	0.03 mg/mL
	Magnesium trisilicate	MAG	0.01 mg/mL
Whole Blood	Haemoglobin, immunoglobulins, nucleic acids, etc	WB	5% v/v
Fecal components	Mucin	MUC	3 mg/mL
	Palmitic acid	PAL	0.85 mg/mL
	Stearic acid	STE	0.85 mg/mL

The Potentially Interfering Substances were tested in 6 groups of 3 substances. Mucin, Cefpodoxime, Ciprofloxacin, Hydrocortisone and Whole blood have been tested alone.

Reference material Clostridioides difficile NAP1 (ZeptoMetrix). was spiked in native stool samples at a concentration of about 3xLoD.

Reference samples with the targets of interest but without the Potential Interfering Substances were also analyzed.

Each organism was tested in 6 replicates in randomized positions on ELITe InGenius in “Extract + PCR” mode”.

A summary of results is shown in the tables below.

Table 26

Sample	Positive / Replicates		Mean Ct			% Positive Agreement		Outcome
	Toxin B	Toxin A	Toxin B	Toxin A	IC	Toxin B	Toxin A	
Reference (Cdiff)	6/6	6/6	34.68	34.53	25.35	100%	100%	No inhibition
Cdiff+ VAN+MET+AMP	6/6	6/6	36.43	36.53	25.54	100%	100%	No inhibition
Cdiff+LOP+BISA+AZI	6/6	6/6	36.31	36.27	25.46	100%	100%	No inhibition
Cdiff+ ALG+ALU+MAG	6/6	6/6	36.41	36.48	25.72	100%	100%	No inhibition
Cdiff+ WB	6/6	6/6	35.64	35.41	25.72	100%	100%	No inhibition
Cdiff+VAS+NON+BISM	6/6	6/6	34.75	34.31	25.36	100%	100%	No inhibition
C.dif+CAL+PAL+STE	6/6	6/6	35.95	36.20	25.70	100%	100%	No inhibition
C.dif+MUC	6/6	6/6	36.96	37.24	25.70	100%	100%	No inhibition
Cdiff+ CEF	6/6	6/6	35.14	34.93	25.69	100%	100%	No inhibition
Cdiff+ CIP	6/6	6/6	34.96	34.54	25.79	100%	100%	No inhibition
Cdiff+ HYD	6/6	6/6	34.85	34.26	25.86	100%	100%	No inhibition

All potentially interfering substances tested showed no inhibition of the C. *difficile* target amplification using the *C. difficile* ELITe MGB Kit.

12.8 Potentially interfering substances: cross-reactivity

The absence of cross-reactivity with potential interfering substances was also verified through the analysis of endogenous or exogenous substances that may be found in native stool samples (most difficult matrix), within clinically relevant limits as reported in the Potentially interfering substances test above.

The Potentially Interfering Substances were tested in 6 groups of 3 substances. Mucin and whole blood have been tested alone.

In each sample was added with 20,000 copies / reaction of Internal Control template (CPE - Internal Control) to mimic the extracted clinical samples.

Reference sample without the potential interfering substances was also analyzed.

Each sample was extracted and amplified in 6 replicates in randomized positions in “Extract + PCR” mode on ELITe InGenius system.

Each organism was tested in 6 replicates in randomized positions on ELITe InGenius in “Extract + PCR” mode.

A summary of results is shown in the tables below.

Table 27

Sample	Positive / Replicates		IC mean Ct	% Negative Agreement	Outcome
	Toxin A	Toxin B			
Reference	0 / 6	0 / 6	25.91	100%	No cross-reactivity
LOB+BISA+AZI	0 / 6	0 / 6	25.69	100%	No cross-reactivity
VAN+MET+AMP	0 / 6	0 / 6	25.58	100%	No cross-reactivity
ALG+ALU+MAG	0 / 6	0 / 6	25.79	100%	No cross-reactivity
CEF+CIP+HYD	0 / 6	0 / 6	25.77	100%	No cross-reactivity
WB	0 / 6	0 / 6	25.50	100%	No cross-reactivity
VAS+NON+BISM	0 / 6	0 / 6	25.88	100%	No cross-reactivity
CAL+PAL+STE	0 / 6	0 / 6	25.64	100%	No cross-reactivity
MUC	0 / 6	0 / 6	25.63	100%	No cross-reactivity

All potentially interfering substances tested showed no cross-reactivity for the *C. difficile* target using the *C. difficile* ELITe MGB Kit.

12.9 Repeatability

The Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of native stool specimens negative or spiked with reference material of *C. difficile* (Zeptometrix).

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

Table 28 ELITe InGenius Intra-Session Repeatability (Day 1)

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	8	-	-	-	-	-	-	100%
3x LoD	8	36.14	0.35	0.96	35.74	0.57	1.59	100%
10x LoD	8	33.68	0.33	0.99	33.05	0.39	1.19	100%

Table 29 ELITe BeGenius Intra-Session Repeatability (Day 1)

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	8	-	-	-	-	-	-	100%
3x LoD	8	36.84	0.37	1.01	36.31	0.51	1.41	100%
10x LoD	8	34.12	0.44	1.29	33.17	0.45	1.35	100%

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

Table 30 ELITe InGenius Inter-Session Repeatability

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	16	-	-	-	-	-	-	100%
3x LoD	16	36.11	0.35	0.96	35.75	0.44	1.22	100%
10x LoD	16	33.51	0.31	0.93	32.88	0.41	1.24	100%

Table 31 ELITe BeGenius Inter-Session Repeatability

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	16	-	-	-	-	-	-	100%
3x LoD	16	36.73	0.39	1.06	36.03	0.61	1.70	100%
10x LoD	16	33.97	0.39	1.16	33.02	0.47	1.44	100%

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

12.10 Reproducibility

The Reproducibility of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of native stool specimens negative or spiked with reference material of *C. difficile* (Zeptometrix).

An example of Inter-Batch Reproducibility (on two lots) results is shown in the tables below.

Table 32 ELITe InGenius Inter-Batch Reproducibility

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	8	-	-	-	-	-	-	100%
3x LoD	8	36.22	0.67	1.85	35.24	0.28	0.78	100%
10x LoD	8	33.86	0.27	0.81	32.72	0.34	1.05	100%

Table 33 ELITe BeGenius Inter-Batch Reproducibility

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	8	-	-	-	-	-	-	100%
3x LoD	8	36.47	0.61	1.69	34.63	0.44	1.28	100%
10x LoD	8	33.92	0.24	0.72	32.20	0.23	0.72	100%

An example of Inter-Instruments Reproducibility (on two instruments) is shown in the tables below.

Table 34 ELITe InGenius Inter-Instrument Reproducibility

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	16	-	-	-	-	-	-	100%
3x LoD	16	35.74	0.27	0.75	34.92	0.34	0.96	100%
10x LoD	16	33.41	0.69	2.05	32.47	0.59	1.82	100%

Table 35 ELITe BeGenius Inter-Instrument Reproducibility

Sample	N	Toxin B			Toxin A			% Agreement
		Mean Ct	SD	%CV	Mean Ct	SD	%CV	
Neg	16	-	-	-	-	-	-	100%
3x LoD	16	36.26	0.46	1.27	34.74	0.63	1.81	100%
10x LoD	16	33.55	0.35	1.03	32.04	0.36	1.13	100%

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

12.11 Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative samples, was evaluated in association with ELITe InGenius, by analysing clinical samples of stool certified negative for *C. difficile* DNA.

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 36

Negative stool	N	Positive	Negative	%Diagnostic Specificity
C. difficile	45	1	44	97.8%

The IC cut-off value is set at 32 for both ELITe InGenius and ELITe BeGenius.

12.12 Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with ELITe InGenius by analysing clinical samples of stool certified positive for C. difficile DNA.

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 37

Positive stool	N	Positive	Negative	%Diagnostic Sensitivity
C. difficile	30	30	0	100%

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File for the "*C. difficile* ELITe MGB Kit", FTP M800358.

13 SPECIMENS AND CONTROLS FOR OTHER SYSTEMS

13.1 Specimens

This product must be used with DNA extracted from the following clinical samples: liquid or unformed stool.

The stool samples, intended for DNA extraction, should be collected following standard stool collection and handling procedures and identified according to laboratory guidelines. Raw stool should be adequately sealed in a sterile screw-cap container that can prevent accidental discharge of the contents and must be transported following all applicable regulations for the transport of etiologic agents. Store samples refrigerated (+2 / +8 °C) for up to 48 hours before processing. If stool clarification cannot be performed within 48 hours of collection, store samples at ≤-70 °C.

Prepare a labeled 1.5 mL tube for each raw stool and dispense 0.8 mL of S.T.A.R. buffer into the tube. Vortex the raw stool, and then use a pipettor with an aerosol resistant tip to transfer approximately 200 µL (use a wide bore tip or plastic spatula as necessary for thick stool samples) of the raw stool into the

1.5 mL tube containing the S. T. A. R. buffer. Cap the tube securely, and then vortex the tube to homogenize mixture (20-30 sec). Centrifuge the homogenized solution at 13.000×g (RCF) for 1 minute to clarify the sample. Carefully transfer 400-650 µL of the clarified stool supernatant into a labeled secondary tube, being careful not to disturb the pelleted fecal material. Please refer to the extraction kit instruction for use to identify the volumes and the secondary tubes compatible with the extraction systems. Store the clarified stool in at +2 / +8 °C for up to 7 days before proceeding with the extraction.

NOTE

The S.T.A.R. Buffer must be stored at room temperature. White precipitates may form when the buffer is stored below room temperature. Before beginning the extraction procedure check if any precipitates have formed, and if so warm the solution to 30-40 °C in a water bath or incubator until the precipitates have dissolved.

NOTE

To process clarified stool samples with **ELITe STAR System** and with **software version 3.4.13** (or later equivalent versions) use the extraction protocol **UUNI_E100_S200_ELI**, that uses 200 µL of sample and elutes the extract in 100 µL (the elution takes actually place in 115 µL of which 100 µL are recovered).After transfer in the secondary tube, load the clarified samples on **ELITe STAR**. A minimum volume of 400-600 µL is always required for each sample. Add **50 µL** of **CPE - Internal Control** and **750 µL of molecular biology grade** water into **Proteinase-Carrier tube** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Store purified nucleic acids at +2 / +8 °C if they will be used on the same day they were extracted or at temperature lower than -20 °C for long term storage.

NOTE

To process clarified stool samples with the **ELITe GALAXY System** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses 300 µL of sample and elutes the extract in 100 µL (the elution takes actually place in 110 µL of which 100 µL are recovered).After transfer in the secondary tube, load the clarified samples on **ELITe GALAXY**. A minimum volume 400-650 µL, dependent on the tube class used, is always required for each sample. Add **2,5 µL / sample** of **CPE - Internal Control** and **7,5 µL / sample** of **molecular biology grade water** into **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Store purified nucleic acids at +2 / +8 °C if they will be used on the same day they were extracted or at temperature lower than -20 °C for long term storage.

NOTE

To process clarified stool samples with **NucliSENS® easyMAG®**, transfer 20 µL of each clarified stool into the **NucliSENS easyMAG** disposable sample vessel (8 samples per vessel, and up to three vessels per run) as established in the instrument work list.

Set up an extraction request on the **«NucliSENS easyMAG» system** as follows:

- Matrix = Stool;
- Protocol = Generic 2.0.1;
- Volume (mL) = 0.02 mL;
- Eluate (µL) = 110 µL;
- Type = Primary.

Create a new extraction run that has a univocal and recognisable file name (e.g. "year-month-day-Extract01"), and then carefully add the extraction requests to the run in the order they were placed into the disposable, and then begin the 10 minute lysis process.

During the 10 minute lysis process, prepare the magnetic silica suspension for 8 samples by mixing **550 µL** of **NucliSENS® easyMAG® Magnetic Silica**, **545 µL** of **molecular biology grade water** and **5 µL** of ***C. difficile* Internal Control**. For each sample, use the BioHit pipettor or a manual pipettor to dispense 125 µL of the magnetic silica suspension into the NucliSENS easyMAG Strip for Premix. Use the BioHit pipettor to transfer 100 µL of the magnetic silica suspension into each sample in the 8-well disposable sample vessel, mix well by pipetting up and down three times, and then start the automated extraction procedure. After the process is completed, remove the isolated nucleic acids from the disposable within 30 minutes of the extraction completion to prevent contamination of the samples with the magnetic silica.

13.2 Interfering substances

Presence of human blood, mucin, fecal fats, over the counter and prescription medicines in samples has been shown not to interfere with the detection of toxigenic **C. difficile** by the **C. difficile ELITe MGB® Kit** used in association with **NucliSENS® easyMAG®**.

Table 38

Potentially Interfering Substance	Active Ingredient	Interferes?
Anti-Fungal /Anti-Itch Vaginal	Nystatin	No
Creams / Ointments / Suppositories	Hydrocortisone, 1%	No
Anti-Hemorrhoid Creams / Ointments	Phenylephrine, 0.25%	No
Antacids	Calcium Carbonate	No
Enema	Mineral oil	No

Table 38 (continued)

Potentially Interfering Substance	Active Ingredient	Interferes?
Spermicidal lubricant	Nonoxynol 9	No
Anti-Diarrheal Medication	Loperamide Hydrochloride	No
Anti-Diarrheal Medication	Bismuth Subsalicylate	No
Laxatives	Sennosides	No
Antibiotics (Oral or Injectable)	Metronidazole (Oral)	No
	Ciprofloxacin (Oral)	No
	Azithromycin (Oral)	No
	Vancomycin (Oral)	No
	Zosyn (Injectable)	No
Non-Steroidal Anti-Inflammatory	Naproxen Sodium	No
Steroidal Anti-Inflammatory	Prednisone	No
Moist Towelette	Benzalkonium Chloride 0.40%	No
Moist Towelette	Isopropyl Alcohol, 70%	No
Fecal Fat (Palmitic acid)	Palmitic acid	No
Fecal Fat (Stearic acid)	Stearic acid	No
Blood	Glucose, Hormones, Enzymes, Ions, Iron, etc	No
Mucus	Immunoglobulins, Lysozyme, Polymers, etc	No

No data concerning inhibition caused by other antiviral, antibiotic and chemotherapeutic or immunosuppressant drugs are available.

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

13.3 Amplification controls

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

For the negative control, use molecular biology grade water (not provided).

For the positive control, use **«*C. difficile* - ELITe Positive Control»** ref M800373 (not provided).

13.4 Quality controls

It is recommended to validate the entire analysis procedure of each extraction and amplification session by processing a negative tested sample and a positive tested sample or a calibrated reference material.

14 OTHER SYSTEMS PROCEDURE

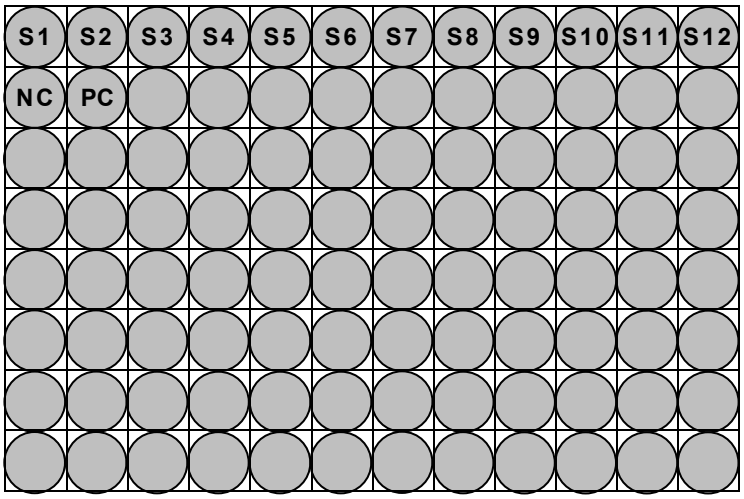
Setting of the real-time amplification session

(To perform in the amplification / detection of amplification products area)

Before starting the session, follow the manufacturer recommendations provided in the instrument documentation and:

- switch on the computer, switch on the real-time thermal cycler, run the dedicated software, open an "absolute quantification" session;
- when the Applied Biosystem® **7500 Fast Dx Real-Time PCR Instrument** or Applied Biosystem® **7500 Fast Real-Time PCR System** is used, choose “Run mode: Fast 7500”;
- when the Applied Biosystem® **7500 Real-Time PCR System** is used “Run mode: Standard 7500” is automatically selected;
- create a new “detector” or set the appropriate "detector" in the Tool menu by selecting the Detector Manager:
  - set the "detector" for the toxin A gene probe with the "reporter" = "VIC" (AP525 is similar to VIC), the "quencher" = "none" (non-fluorescent) and call it “Toxin A”;
  - Set the "detector" for the toxin B gene probe with the "reporter" = "FAM", the "quencher" = "none" (non-fluorescent) and call it “Toxin B”;
  - Set the "detector" for the Internal Control probe with the "reporter" = "CY5" (AP642 is similar to Cy5), the "quencher" = "none" (non-fluorescent) and call it “IC”;
- go to “View” menu, select the “Well Inspector” and, for each well in use in the microplate, set the "detector" (type of fluorescence that is to be measured), the "passive reference" = "ROX" (AP593 is similar to ROX) for normalisation of the measured fluorescence and the type of reaction (sample, amplification negative control, amplification positive control). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

See below an example of how a qualitative analysis of 12 samples can be organised.



Legend: S1 - **S12**: Samples to be analysed; **NC**: amplification Negative Control; **PC**: amplification Positive Control.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol >Thermal Profile) the parameters of the **thermal cycle**:

- add to the amplification stage (Add Step) an **extension step at 72 °C**,

NOTE

The fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the hybridisation step at 56°C.

- modify timing as indicated in the table **"Thermal cycle"** below,
- set the number of cycles to **45**,
- set the reaction volume to **30 µL**,
- optional: add dissociation stage (Add Dissociation Stage) and set temperature from 40 °C to 80 °C.

Table 39

Thermal Cycling Parameters		
Stage	**Temperatures**	**Timing**
Decontamination	50 °C	2 min.
Initial Denaturation	93 °C	2 min.
Amplification and Detection (45 cycles)	93 °C	10 sec.
	56 °C (data collection)	30 sec.
	72 °C	15 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	1 min.
	80 °C	15 sec.
	60 °C	15 sec.

Amplification set-up

(To be performed in the extraction / preparation of the amplification reaction area)

Before starting the session, it is necessary to:

- thaw the tubes containing the processed samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice,
- thaw the ***C. difficile* PCR Mix** tubes required for the session, remembering that each tube is sufficient to prepare **25 reactions**. Mix gently, spin down the content for 5 seconds and keep them on ice for a maximum of four hours,
- thaw a ***C. difficile* Positive Control** tube (not provided).Mix gently, spin down the content for 5 seconds and keep on ice for a maximum of four hours,
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powderless gloves and not to damage the wells.

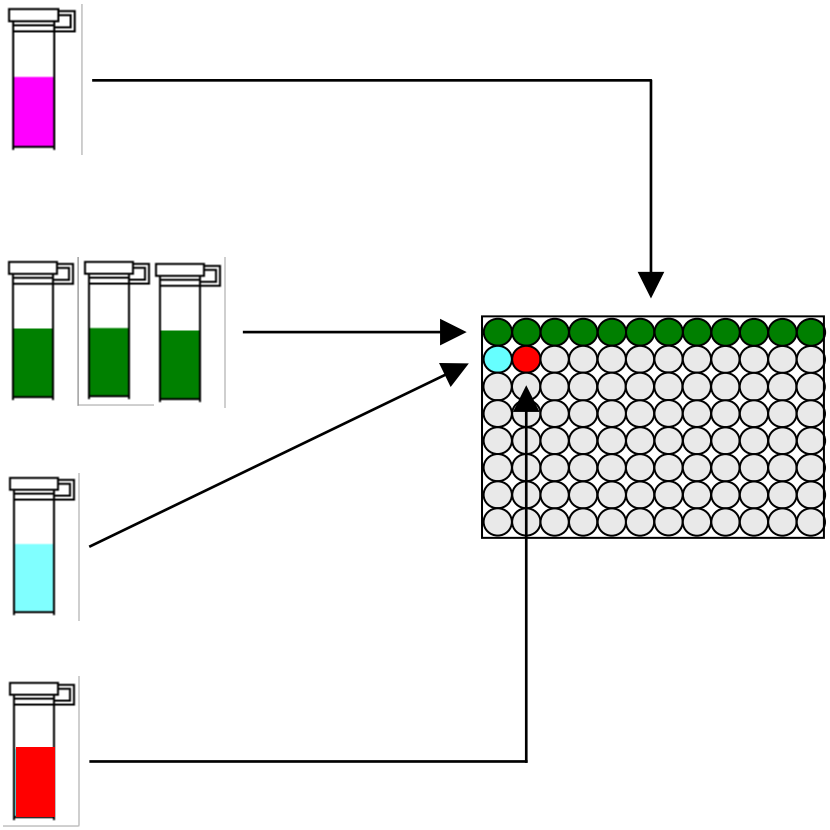
1. Accurately dispense **20 µL** of the ***C. difficile* PCR Mix** into the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

NOTE

If not all the reaction mixture is used, store the remaining volume in the dark at -20 °C for no longer than one month. Freeze and thaw the reaction mixture up to **five times**.

2. Add to the reaction mixture **10 µL** of the first processed sample in the designated well, as previously established in the **Work Sheet**. Proceed in the same way with the other extracted samples.
3. Add to the reaction mixture **10 µL** of **molecular biology grade water** (not provided) in the negative control well, as previously established in the **Work Sheet**.
4. Add to the reaction mixture **10 µL** of **C. difficile Positive Control** in the designated well, as previously established in the **Work Sheet**.
5. Accurately seal the **Amplification microplate** with the **Amplification Adhesive Sheet**.
6. If a centrifuge with a plate adaptor is available spin down the **Amplification microplate** to collect the reaction mixture at the bottom of the wells.
7. Transfer the **Amplification microplate** into the real-time thermal cycler in the amplification / detection of amplification products area and start the thermal cycle for the amplification. Save the session setting with an univocal and recognisable file name (e.g. "year-month-day-C. difficile-EGSpA").

The following figure illustrates the set up of the amplification reaction.



1. **Add 20 µL of PCR Mix to each well**
2. **Add 10 µL of extracted DNAs**
3. **Add 10 µL of Negative Control**
4. **Add 10 µL of the Positive Control**

NOTE

if the preparation of the amplification reaction is performed with the **ELITe GALAXY** instrument, load the elution microplate, the Q-PCR Mix and the amplification microplate as indicated in the instrument user manual and following the steps required by the GUI.

NOTE

At the end of the thermal cycle the **Amplification microplate** with the reaction products must be removed from the instrument and discarded without producing environmental contamination. In order to avoid spilling the reaction products, the **Sealing Optical Adhesive Sheet must not be removed from the Amplification microplate**.

Qualitative analysis of the results

The recorded values of the fluorescence emitted during the amplification reactions by the toxin A gene probe (VIC detector “Toxin A”), by the toxin B gene probe (FAM detector "Toxin B") and by the Internal Control probe (CY5 detector "IC") must be analysed by the instrument software.

Before starting the analysis, follow the manufacturer recommendations provided in the instrument documentation and:

- set (Results > Amplification plot > delta Rn vs. Cycle) the **Analysis Settings** for all detectors to **Auto Baseline** and **Manual Ct**, with the **Threshold** set to **0.05**. Push the **Analyze** button and **save** the results.

The values of fluorescence emitted by the specific probes during the amplification reaction and the **Threshold** value of fluorescence allow determining the **Threshold cycle (Ct)**. The Ct is the cycle when the fluorescence reached the **Threshold** value and it is proportional to the initial target quantity.

In the **Positive Control** amplification reaction, the **Ct** values of Toxin A and Toxin B detectors (Results Report) are used to validate the amplification and detection, as described in the following table:

Table 40

Positive Control Reaction Detector VIC "Toxin A"	Assay Result	Amplification / Detection
Ct < 35	POSITIVE	CORRECT

Table 41

Positive Control Reaction Detector FAM "Toxin B"	Assay Result	Amplification / Detection
Ct < 35	POSITIVE	CORRECT

If the result of the Positive Control amplification is Ct ≥ 35 or Ct Undetermined for Toxin A or for Toxin B detectors, then the target DNA has not been detected. It means that problems occurred during the amplification or the detection step (incorrect dispensing of the reaction mix or the positive controls, degradation of the reaction mix or the positive controls, incorrect setting of the positive control position, incorrect setting of the thermal cycle), which may lead to incorrect results. The entire run is invalid and has to be repeated starting from the amplification step.

In the **Negative Control** amplification reaction, the Ct values of Toxin A, Toxin B and IC detectors (Results Report) are used to validate the amplification and the detection as described in the following table:

Table 42

Negative Control Reaction Detector VIC "Toxin A"	Assay Result	Amplification / Detection
Ct Undetermined or Ct ≥ 40	NEGATIVE	CORRECT

Table 43

Negative Control Reaction Detector FAM "Toxin B"	Assay Result	Amplification / Detection
Ct Undetermined or Ct ≥ 40	NEGATIVE	CORRECT

Table 44

Negative Control Reaction Detector Cy5 "IC"	Assay Result	Amplification / Detection
Ct Undetermined or Ct ≥ 34	NEGATIVE	CORRECT

If the result of the **Negative Control** amplification is **Ct < 40**, for Toxin A or Toxin B detectors, or **Ct < 34**, for IC detector, then the target DNA has been incorrectly detected. It means that problems have occurred during the amplification step (contamination), which may lead to false positive results. The session is not valid and needs to be repeated starting from the amplification step.

In each **sample** amplification reaction, the **Ct** values of Toxin A or Toxin B detectors are used to detect the target DNA while the Internal Control **Ct** value is used to validate extraction, amplification and detection.

NOTE

Verify on the instrument software (Results à Amplification plot à delta Rn vs. Cycle) that the **Ct** was determined by a prompt and regular increase of the fluorescence and not by peaks or an increase of the background (irregular or high background).

The amplification reactions **Ct** values of each **sample** (Results à Report) are used as described in the following table:

Table 45

Sample Reaction			Assay Result
Detector VIC "Toxin A"	**Detector FAM "Toxin B"**	**Detector Cy5 "IC"**	
Undetermined or Ct ≥ 40	Undetermined or Ct ≥ 40	Ct < 34	Negative
		Undetermined or Ct ≥ 34	Invalid
Determined, Ct < 40	Undetermined or Ct ≥ 40	Not Applicable	Positive
Undetermined or Ct ≥ 40	Determined, Ct < 40	Not Applicable	Positive
Determined, Ct < 40	Determined, Ct < 40	Not Applicable	Positive

Table 46

Assay Result	Result Interpretation
Negative	**Toxin A gene or of toxin B gene DNA of *C. difficile* was not detected** in the sample. The sample is negative for toxigenic *C. difficile* or its concentration is below the Limit of Detection of the assay.
Invalid	**Invalid assay result.** Repeat run from extraction of the same sample or perform a new extraction from a new sample.
Positive	**Toxin A gene or of toxin B gene DNA of *C. difficile* was detected** in the sample. The sample is positive for *C. difficile* and it could be **toxigenic**.

The presence of either (toxin A gene or toxin B gene) or both markers is indicative of toxigenic C. difficile.

If the result of the sample amplification reaction is **Ct Undetermined** or **Ct ≥ 40** for Toxin A and Toxin B detectors and **Ct Undetermined** or **Ct ≥ 34** for the IC detector, it means that it was impossible to detect efficiently the targets and the Internal Control DNA. In this case problems have occurred during the amplification step (inefficient or no amplification) or during the extraction step (degradation of DNA, loss of DNA during extraction or carry-over of inhibitors in the extracted DNA), which may lead to incorrect results and false negatives. In this case the sample is not suitable, the run is invalid and it needs to be repeated starting from the extraction of the sample or of a new sample from the same patient.

If the result of the sample amplification is **Ct Undetermined** or **Ct ≥ 40** for Toxin A or Toxin B detectors and **Ct < 34** for the IC detector, it means that the toxigenic *C. difficile* DNA is not detected in the processed sample. The sample is presumed to be negative. However, the number of organisms in the sample may be below the detection limit of the product (see Performance Characteristics). In this case the result could be a false negative.

NOTE

When Toxin A and/or Toxin B DNA is detected in a sample, the IC detector may be **Ct Undetermined** or **Ct ≥ 34**. In fact, the high efficiency of the DNA amplification of toxin A and toxin B gene may compete with the low efficiency of the Internal Control amplification. In this case the sample is suitable and the positive result of the assay is valid.

15 PERFORMANCE CHARACTERISTICS WITH OTHER SYSTEMS

15.1 Clinical Performances

Performance characteristics of the ***C. difficile* ELITe MGB Kit** used in association to stools samples and **ELITe STAR** system were determined by analysis of a panel of clinical samples previously tested by *C. difficile* cytotoxicity culture test as a method of reference. A total of 82 fecal specimens, 30 positive and 52 negative, were tested. Each sample was used to carry out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

Table 47

	Reference method		
***C. difficile* ELITe MGB Kit**	**Tx *C. difficile* Positive**	**Tx *C. difficile* Negative**	**Total**
Toxigenic *C. difficile* Positive	29	4	33
Toxigenic *C. difficile* Negative	1	48	49
Specimen Total valid for analysis	30	52	82
Diagnostic Sensitivity (Positive Percent Agreement)	97%	-	
Diagnostic Specificity (Negative Percent Agreement)	-	92%	

Compared to the reference method ***C. difficile* ELITe MGB Kit** identified 97% of the specimens positive for toxigenic *C. difficile* and 92% of the negative specimens. The assay did not confirm positive sample that was positive in the culture test. The assay identifies 4 positive samples that were negative in the culture test. Melting curve analysis confirmed 4 samples to be true positives for toxigenic *C. difficile*.

Discrepant result resolution was carried out by an independent molecular test (Xpert® C. difficile system, Cepheid).The final result is reported in the table below.

Table 48

	Reference methods		
***C. difficile* ELITe MGB Kit**	**Tx *C. difficile* Positive**	**Tx *C. difficile* Negative**	**Total**
Toxigenic *C. difficile* Positive	33	0	33
Toxigenic *C. difficile* Negative	0	49	49
Specimen Total valid for analysis	33	49	82
Diagnostic Sensitivity (Positive Percent Agreement)	100%	-	
Diagnostic Specificity (Negative Percent Agreement)	-	100%	

Performance characteristics of the ***C. difficile* ELITe MGB Kit** used in association to stools samples and **ELITe GALAXY** system were determined by analysis of a panel of clinical samples previously tested with cytotoxicity culture test as a method of reference. A total of 82 *C. difficile* fecal specimens, 30 positive and 52 negative, were tested. Each sample was used to carry out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

Table 49

	Reference method		
***C. difficile* ELITe MGB Kit**	**Tx *C. difficile* Positive**	**Tx *C. difficile* Negative**	**Total**
Toxigenic *C. difficile* Positive	28	4	32
Toxigenic *C. difficile* Negative	2	48	50
Specimen Total valid for analysis	30	52	82
Diagnostic Sensitivity (Positive Percent Agreement)	93%	-	
Diagnostic Specificity (Negative Percent Agreement)	-	92%	

Compared to the reference method ***C. difficile* ELITe MGB Kit** identified 93% of the specimens positive for toxigenic *C. difficile* and 92% of the negative specimens. The assay did not confirmed positive 2 samples that was positive in the culture test. The assay identifies 4 positive samples that were negative in the culture test. Melting curve analysis confirmed 4 samples to be true positives for toxigenic *C. difficile*.

Discrepant result resolution was carried out by an independent molecular test (Xpert® C. difficile system, Cepheid). The final result are reported in the table below.

Table 50

	Reference methods		
***C. difficile* ELITe MGB Kit**	**Tx *C. difficile* Positive**	**Tx *C. difficile* Negative**	**Total**
Toxigenic *C. difficile* Positive	32	0	32
Toxigenic *C. difficile* Negative	1	49	50
Specimen Total valid for analysis	33	49	82
Diagnostic Sensitivity (Positive Percent Agreement)	100%	-	
Diagnostic Specificity (Negative Percent Agreement)	-	100%	

Performance characteristics of the ***C. difficile* ELITe MGB Kit** were determined by a prospective investigational study comparing the results obtained with ***C. difficile* ELITe MGB Kit**used in association with NucliSENS® easyMAG® (BioMeriéux) and 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems) with a cytotoxicity culture test as a method of reference.

A total of 100 *C. difficile*-positive and negative fecal specimens, that were evaluated by culture test, were then tested with the ***C. difficile* ELITe MGB Kit**. Compared to the method of reference, ***C. difficile* ELITe MGB Kit** identified 100% of the specimens positive for toxigenic *C. difficile* and 86% of the negative specimens.

Table 51

	Reference method		
***C. difficile* ELITe MGB Kit**	**Tx *C. difficile* Positive**	**Tx *C. difficile* Negative**	**Total**
Toxigenic *C. difficile* Positive	50	7	57
Toxigenic *C. difficile* Negative	0	43	43
Specimen Total valid for analysis	50	50	100
Diagnostic Sensitivity (Positive Percent Agreement)	100%	-	
Diagnostic Specificity (Negative Percent Agreement)	-	86%	

Compared to the reference method ***C. difficile* ELITe MGB Kit** identified 100% of the specimens positive for toxigenic *C. difficile* and 86% of the negative specimens. The assay identifies 7 positive samples that were negative in the culture test.

Discrepant analysis testing was conducted by PCR using primers specific for toxin B gene but unrelated to the ones of ***C. difficile* ELITe MGB Kit**. The final result are reported in the table below.

Table 52

	Reference methods		
***C. difficile* ELITe MGB Kit**	**Tx *C. difficile* Positive**	**Tx *C. difficile* Negative**	**Total**
Toxigenic *C. difficile* Positive	50	5	55
Toxigenic *C. difficile* Negative	0	45	45
Specimen Total valid for analysis	50	50	100
Diagnostic Sensitivity (Positive Percent Agreement)	100%	-	
Diagnostic Specificity (Negative Percent Agreement)	-	90%	

15.2 Non-Clinical Performances

15.2.1 Limit of Detection

The LoD of ***C. difficile* ELITe MGB Kit** was calculated by Probit regression statistical analysis. LoD for each strain was calculated as the concentrations at which the probability of a positive result is the 95%.

The LoD of ***C. difficile* ELITe MGB Kit** used in association to clarified stools samples and **ELITe STAR** system was determined using two strains of toxigenic *C. difficile*.

Reference materials containing *C. difficile* 027 or *C. difficile* 017 (Qnostics Ltd, UK) were diluted into negative clarified stool. Multiple dilutions in a range from 1000 CFU / mL to 1 CFU / mL were tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The LoD values as CFU/mL are reported below.

Table 53 Limit of Detection for clarified stool samples and ELITe STAR system

Strain	CFU / mL
C. difficile 027, toxin A+B+, toxinotype III	187
C. difficile 017, toxin A-B+, toxinotype VIII	278

The LoD of ***C. difficile* ELITe MGB Kit** used in association to clarified stools samples and **ELITe GALAXY** system was determined using two strains of toxigenic *C. difficile*.

Reference materials containing C. difficile 027 or C. difficile 017 (Qnostics Ltd, UK) were diluted into negative clarified stool. Multiple dilutions in a range from 178 CFU / mL to 1.78 CFU / mL were tested carrying out the whole analysis procedure, extraction, PCR setup and amplification, by ELITechGroup S.p.A. products.

The Lod as values CFU/mL are reported below.

Table 54 Limit of Detection for clarified stool samples and ELITe GALAXY system

Strain	CFU / mL
C. difficile 027, toxin A+B+, toxinotype III	88
C. difficile 017, toxin A-B+, toxinotype VIII	84

The LoD of ***C. difficile* ELITe MGB Kit** used in association to clarified stools samples and **NucliSENS® easyMAG®** system was determined using two strains of toxigenic *C. difficile.*

Fresh cultures of *C. difficile* strain VPI 10463 and strain 4118 were quantified and diluted in negative clarified stool. Multiple dilutions were tested carrying out the whole analysis procedure, extraction and amplification, by BioMeriéux and ELITechGroup S.p.A. products.

After determination, LoD for each strain was then verified by testing 20 replicates at the claimed concentration. The Lod as values CFU/mL are reported below.

Table 55 Limit of Detection for clarified stool samples and NucliSENS® easyMAG® system

Strain	CFU / mL
ATCC 43255, Strain VPI 10463, Toxinotype 0	1.121
ATCC BAA-1870, Strain 4118, Toxinotype III	3.750

15.2.2 Analytical sensitivity: reproducibility with calibrated reference material

The reproducibility of the assay results compared with results obtained using other assays in different laboratories was checked by testing a proficiency panel.

A test was carried out using as reference material the QCMD 2012 *C. difficile* DNA EQA Panel (Qnostics Ltd, UK). Each sample was tested carrying out the extraction by ELITe STAR system and amplification using ELITechGroup S.p.A. products.

The results are reported in the following table.

Table 56 Tests with proficiency panel and ELITe STAR system

Sample	type	CFU / mL	Result
CD12-01	*C. difficile* 027	4.6 x 10^4	POS
CD12-02	*C. sordellii*	-	NEG
CD12-03	*C. difficile* 027	4.6 x 10^3	POS
CD12-04	*C. difficile* 027	4.6 x 10^5	POS
CD12-05	*C. difficile* 027	4.6 x 10^5	POS
CD12-06	Negative	-	NEG
CD12-07	*C. difficile* 027	4.6 x 10^6	POS
CD12-08	*C. difficile* 017	8 x 10^6	POS
CD12-09	*C. difficile* 017	8 x 10^4	POS
CD12-10	*C. difficile* 017	8 x 10^5	POS

All samples were correctly detected

Further test was carried out using as reference material the QCMD 2012 *C. difficile* DNA EQA Panel (Qnostics Ltd, UK). Each sample was tested carrying out the extraction and PCR setup by **ELITe GALAXY system** and amplification using ELITechGroup S.p.A. products.

The results are reported in the following table.

Table 57 Tests with proficiency panel and ELITe GALAXY system

Sample	type	CFU / mL	Result
CD12-01	*C. difficile* 027	4.6 x 10^4	POS
CD12-02	*C. sordellii*	-	NEG
CD12-03	*C. difficile* 027	4.6 x 10^3	POS
CD12-04	*C. difficile* 027	4.6 x 10^5	POS

Table 57 Tests with proficiency panel and ELITe GALAXY system (continued)

Sample	type	CFU / mL	Result
CD12-05	*C. difficile* 027	4.6 x 10^5	POS
CD12-06	Negative	-	NEG
CD12-07	*C. difficile* 027	4.6 x 10^6	POS
CD12-08	*C. difficile* 017	8 x 10^6	POS
CD12-09	*C. difficile* 017	8 x 10^4	POS
CD12-10	*C. difficile* 017	8 x 10^5	POS

All samples were correctly detected.

The last test was carried out using as reference material the QCMD 2013 *C. difficile* DNA EQA Panel (Qnostics Ltd, UK). Each sample was tested carrying out the extraction by NucliSENS® easyMAG® and amplification using ELITechGroup S.p.A. products.

The results are reported in the following table.

Table 58 Tests with calibrated reference materials and NucliSENS® easyMAG®

Sample	type	CFU/mL	Pos./ Rep.	Result
CD13-01	*C. difficile* 027	4.6 x 10^5	2/2	POS
CD13-02	*C. difficile* 017	8 x 10^6	2/2	POS
CD13-03	Negative	-	0/2	NEG
CD13-04	*C. difficile* 027	4.6 x 10^4	2/2	POS
CD13-05	*C. difficile* 027	4.6 x 10^6	2/2	POS
CD13-06	*C. difficile* 017	8 x 10^5	2/2	POS
CD13-07	*C. difficile* 027	4.6 x 10^3	2/2	POS
CD13-08	*C. sordellii*	2.1 x 10^5	0/2	NEG
CD13-09	*C. difficile* 027	4.6 x 10^5	2/2	POS
CD13-10	*C. difficile* 017	8 x 10^4	2/2	POS

All samples were correctly detected.

15.2.3 Genotype detection efficiency (inclusivity)

Performances of the ***C. difficile*** **ELITe MGB Kit** correctly identified 30 toxigenic *C. difficile* isolates representative of the global genetic diversity, including seven different toxinotypes (0, III, V, VIII, X, XII and XXII), and thirteen different PCR ribotypes (001, 002, 010, 012, 014, 017, 020, 027, 036, 056, 087, 110 and 154). All of the strains were obtained from and quantitated by ATCC, US, with the exception of *C. difficile* strain CCUG 8864 (toxinotype X, A-/B+, kindly donated by Thermo-Fisher Diagnostics). All strains tested were added to the negative clarified stool at near the detection limit and tested with ***C. difficile*** **ELITe MGB Kit**in association with NucliSENS® easyMAG® system. All strains tested positive.

Table 59

ATCC	Strain	Isolated	Toxin A / B	Toxino type	PCR Ribotype
9689	90556-M6S	(type strain)	A+B+	0	001
17857	870, N4	Unknown	A+B+	0	001
43255	VPI 10463	Abdominal wound	A+B+	0	087
43594	W1194	Human Feces, Belgium	A+B+	0	005
43596	545	Human Feces, Belgium	A+B+	0	012
43597	Unknown	Human Feces, Belgium	A+B+	0	014
43598	1470 (F)	Human Feces, Belgium	A–B+	VIII	017
43599	2022	Human Feces, Belgium	A+B+	0	001
43600	2149	Human Feces, Belgium	A+B+	0	014
51695	BDMS 18 AN	Unknown	A+B+	0	001
700792	14797-2	Michigan, Female Feces, 1977	A+B+	0	005
BAA-1382	630	Clinical Isolate, Switzerland, 1982	A+B+	0	012
BAA-1803	Unknown	Quest Clinical isolate	A+B+	III**c**	027
BAA-1804	Unknown	Quest Clinical isolate	A+B+	0	053
BAA-1805	Unknown	Quest Clinical isolate	A+B+	III**b**	027
BAA-1806	Unknown	Quest Clinical isolate	A+B+	0	220
BAA-1808	Unknown	Quest Clinical isolate	A+B+	0	020
BAA-1811	Unknown	Quest Clinical isolate	A+B+	0	057
BAA-1812	Unknown	Quest Clinical isolate	A+B+	XII	024
BAA-1813	Unknown	Quest Clinical isolate	A+B+	0	002
BAA-1814	Unknown	Quest Clinical isolate	A+B+	XXII	251
BAA-1870	4118	Human, Maine, 2004 (hypervirulent strain NAP1/BI/027)	A+B+	III**b**	027
BAA-1871	4111	Human New Jersey	A+B+	0	001
BAA-1872	4206	Human, Maine	A+B+	0	207
BAA-1873	5283	Human, New York	A+B+	0	053
BAA-1874	4205	Human, Oregon	A+B+	0	002
BAA-1875	5325	Human, Georgia	A+B+	V	078
BAA-2155	LBM 0801058	Human Feces, Albuquerque, NM, USA	A+B+	XXII	251
BAA-2156	LBM 0801040	Human Feces, Cambridge, UK 2007	A+B+	0	118
-	CCUG 8864	Prof. M. Delmee, C. U. Louvain, Brussels	A–B+	X	036

15.2.4 Cross-reactivity and interference

The analytical specificity of the ***C. difficile* ELITe MGB Kit** was evaluated for cross-reactivity by testing non-toxigenic *C. difficile*, species phylogenetically related to *C. difficile*, and other pathogenic microorganisms commonly present in normal gastrointestinal microflora. Tested species consisted of bacterial and fungal microorganisms (tested at concentrations of 1×10^6 CFU when possible), viral and intracellular parasites (tested at concentrations of 1×10^5 PFU or IFU). Also human genomic DNA was tested (at 1×10^6 cells). All organisms (130) tested, using the ***C. difficile* ELITe MGB Kit** in association with NucliSENS® easyMAG® system, resulted in no cross-reactivity, with the exception of *Clostridium nexile*, that cross-reacted with the amplification reaction for toxin B gene.

C. nexile was observed in approximately 3% of the clinical samples that were tested. *C. nexile* does not express Toxin B, but there is enough sequence homology to produce a signal in the assay. Overall analytical specificity was 99% (129/130).

The analytical specificity of the ***C. difficile* ELITe MGB Kit** was also evaluated for microbial interference by testing the same species used for cross-reactivity. The microorganisms were tested as described above in cross-reactivity testing, but also spiked individually with each of two different toxigenic *C. difficile* strains (ATCC 43255, strain VPI 10463, Toxinotype 0 or ATCC BAA-1870, strain 4118, Toxinotype III) at near the detection limit. All organisms tested using the ***C. difficile* ELITe MGB Kit** in association with NucliSENS® easyMAG® system resulted in no microbial interference.

Table 60

Genera and Species	Ref.	Provider	Amount	Interferes?
Abiotrophia defectiva	49176	ATCC	1×10^6	No
Acinetobacter baumannii	19606	ATCC	1×10^6	No
Acinetobacter lwoffii	15309	ATCC	1×10^6	No
Aeromonas hydrophila	7966	ATCC	1×10^6	No
Alcaligenes faecalis subsp. faecalis	15554	ATCC	1×10^6	No
Anaerococcus tetradius	35098	ATCC	1×10^6	No
Bacillus cereus	13472	ATCC	1×10^6	No
Bacteroides caccae	43185	ATCC	1×10^6	No
Bacteroides merdae	43184	ATCC	1×10^6	No
Bacteroides stercoris	43183	ATCC	1×10^6	No
Bifidobacterium adolescentis	15703	ATCC	1×10^6	No
Bifidobacterium longum	15707	ATCC	1×10^6	No
Campylobacter coli	BAA-370	ATCC	1×10^6	No
Campylobacter jejuni subsp. jejuni	33292	ATCC	1×10^6	No
Candida albicans	10231	ATCC	1×10^6	No
Cedecea davisae	33431	ATCC	1×10^6	No
Chlamydia trachomatis	8017775	Zeptometrix	1×10^5	No
Citrobacter amalonaticus	25405	ATCC	1×10^6	No
Citrobacter freundii	8090	ATCC	1×10^6	No
Citrobacter koseri	27028	ATCC	1×10^6	No
Citrobacter sedlakii	51115	ATCC	1×10^6	No

Table 60 (continued)

Genera and Species	Ref.	Provider	Amount	Interferes?
Clostridium beijerinckii	8260	ATCC	1×10^6	No
Clostridium bifermentans	638	ATCC	1×10^6	No
Clostridium bolteae	BAA-613	ATCC	1×10^6	No
Clostridium butyricum	19398	ATCC	1.5×10^5	No
*Clostridium chauvoe*i	11957	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	43593	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	43601	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	43602	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	43603	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	700057	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	BAA-1801	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	BAA-1807	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	BAA-1809	ATCC	1×10^6	No
Clostridium difficile (non toxigenic)	BAA-1810	ATCC	1×10^6	No
Clostridium difficile toxinotype XIa, A-/B-	R11402	Thermo-Fisher Diagnostics	1×10^6	No
Clostridium difficile toxinotype XIb, A-/B-	IS58	Thermo-Fisher Diagnostics	1×10^6	No
Clostridium fallax	19400	ATCC	1×10^6	No
Clostridium haemolyticum	9650	ATCC	1×10^6	No
Clostridium histolyticum	19401	ATCC	1×10^6	No
Clostridium innocuum	14501	ATCC	1×10^6	No
Clostridium methylpentosum	43829	ATCC	1×10^6	No
Clostridium nexile	27757	ATCC	1×10**6**	Yes
Clostridium novyi	19402	ATCC	1×10^6	No
Clostridium paraputrificum	25780	ATCC	1×10^6	No
Clostridium perfringens	13124	ATCC	1×10^6	No
Clostridium ramosurn	25582	ATCC	1×10^6	No
Clostridium scindens	35704	ATCC	1×10^6	No
Clostridium septicum	12464	ATCC	1×10^6	No
Clostridium sordellii	9714	ATCC	1×10^6	No
Clostridium sphenoides	19403	ATCC	1×10^6	No
Clostridium spiroforme	29899	ATCC	1×10^6	No

Table 60 (continued)

Genera and Species	Ref.	Provider	Amount	Interferes?
Clostridium sporogenes	15579	ATCC	1×10^6	No
Clostridium symbiosum	14940	ATCC	1×10^6	No
Clostridium tertium	14573	ATCC	1×10^6	No
Clostridium tetani	19406	ATCC	1×10^6	No
Collinsella aerofaciens	25986	ATCC	1×10^6	No
Corynebacterium genitalium	33798	ATCC	1×10^6	No
Desulfovibrio piger	29098	ATCC	1×10^6	No
Edwardsiella tarda	15947	ATCC	1×10^6	No
Eggerthella lenta	25559	ATCC	1×10^6	No
Enterobacter aerogenes	51697	ATCC	1×10^6	No
Enterobacter cloacae	13047	ATCC	1×10^6	No
Enterococcus casseliflavus (vanC2)	25788	ATCC	1×10^6	No
Enterococcus cecorum	43198	ATCC	1×10^6	No
Enterococcus dispar	51266	ATCC	1×10^6	No
Enterococcus faecalis (vanB)	51299	ATCC	1×10^6	No
Enterococcus faecium (vanA)	700221	ATCC	1×10^6	No
Enterococcus gallinarum (vanC)	700425	ATCC	1×10^6	No
Enterococcus hirae	10541	ATCC	1×10^6	No
Enterococcus raffinosus	49427	ATCC	1×10^6	No
Escherichia coli	35218	ATCC	1×10^6	No
Escherichia fergusonii	35469	ATCC	1×10^6	No
*Escherichia hermann*ii	33650	ATCC	1×10^6	No
Flavonifractor plautii	49531	ATCC	1×10^6	No
Fusobacterium varium	8501	ATCC	1×10^6	No
Gardnerella vaginalis	14019	ATCC	1×10^6	No
Gemella morbillorum	27824	ATCC	1×10^6	No
Hafnia alvei	13337	ATCC	1×10^6	No
Helicobacter fennelliae	35683	ATCC	1×10^6	No
Helicobacter pylori	43504	ATCC	1×10^6	No
Homo sapiens, Human Cells	-	Seattle Children Hospital Research Institute	1×10^6	No
Klebsiella oxytoca	8724	ATCC	1×10^6	No

Table 60 (continued)

Genera and Species	Ref.	Provider	Amount	Interferes?
Klebsiella pneumoniae subsp. pneumoniae	13883	ATCC	1×10^6	No
Lactobacillus acidophilus	4356	ATCC	1×10^6	No
Lactobacillus reuteri	23272	ATCC	1×10^6	No
Lactococcus lactis	11454	ATCC	1×10^6	No
Leminorela grimontii	33999	ATCC	1×10^6	No
Listeria grayi	19120	ATCC	1×10^6	No
Listeria innocua	33090	ATCC	1×10^6	No
Listeria monocytogenes	13932	ATCC	1×10^6	No
Peptostreptococcus anaerobius	27337	ATCC	1×10^6	No
Plesiomonas shigelloides	14029	ATCC	1×10^6	No
Porphyromonas asaccharolytica	25260	ATCC	1×10^6	No
Prevotella melaninogenica	25845	ATCC	1×10^6	No
Proteus mirabilis	12453	ATCC	1×10^6	No
Proteus penneri	35198	ATCC	1×10^6	No
Providencia alcalifaciens	9886	ATCC	1×10^6	No
Providencia rettgeri	9250	ATCC	1×10^6	No
Providencia stuartli	33672	ATCC	1×10^6	No
Pseudomonas aeruginosa	27853	ATCC	1×10^6	No
Ruminococcus bromii	27255	ATCC	1.75×10^5	No
Salmonella choleraesuis (typhimurium)	14028	ATCC	1×10^6	No
Salmonella enterica subsp. Arizonae	13314	ATCC	1×10^6	No
Salmonella enterica subsp. Enterica	700720	ATCC	1×10^6	No
Serratia liquefaciens	27592	ATCC	1×10^6	No
Serratia marcescens	274	ATCC	1×10^6	No
Shigella boydii	9207	ATCC	1×10^6	No
Shigella dysenteriae	11835	ATCC	1×10^6	No
Shigella sonnei	25931	ATCC	1×10^6	No
Staphylococcus aureus	BAA-1556	ATCC	1×10^6	No
Staphylococcus epidermidis	12228	ATCC	1×10^6	No
Stenotrophomonas maltophilia	13637	ATCC	1×10^6	No
Streptococcus agalactiae	12973	ATCC	1×10^6	No
Streptococcus dysgalactiae	43078	ATCC	1×10^6	No

Table 60 (continued)

Genera and Species	Ref.	Provider	Amount	Interferes?
Streptococcus intermedius	27335	ATCC	1×10^6	No
Streptococcus uberis	19436	ATCC	1×10^6	No
Trabulsiella guamensis	49490	ATCC	1×10^6	No
Veillonella parvula	10790	ATCC	1×10^6	No
Vibrio cholerae	25870	ATCC	1×10^6	No
Vibrio parahaemolyticus	17802	ATCC	1×10^6	No
Yersinia bercovieri	43970	ATCC	1×10^6	No
Yersinia rohdei	43380	ATCC	1×10^6	No
Adenovirus	VR-1516	ATCC	1×10^5	No
Coxsackievirus	VR-28	ATCC	1×10^5	No
Cytomegalovirus	VR-1590	ATCC	1×10^5	No
Echovirus	VR-41	ATCC	1×10^5	No
Enterovirus	VR-836	ATCC	1×10^5	No
Norovirus Type I	0810086CF	ZeptoMetrix	1×10^5	No
Rotavirus	VR-2018	ATCC	1×10^5	No

15.2.5 Reproducibility

A 7-member panel comprised of a negative clarified stool specimen and varying concentrations of two toxigenic *C. difficile* strains (ATCC 43255 and BAA-1870) was tested in three replicates by two operators. For each *C. difficile* strain, the panel included a specimen below the LoD (expected to yield a positivity rate between 20 to 80%), low positive (at LoD, expected to yield a 95% positivity rate) and a moderate positive (three times LoD, expected to have 100% positivity rate).Each of the two operators performed one run per day for 12 days on three reagent lots at one site (7 specimens × 3 replicates × 12 days × 2 runs).Each sample was tested carrying out the extraction by NucliSENS® easyMAG® system and amplification by ELITechGroup S.p.A. products.

The negative panel member yielded negative results 100%, the below LoD specimen positivity rate was 78%, the low positive specimen positivity rate was 98%, and the moderate positive panel member positivity rate was 100%.

Table 61 Day to Day Reproducibility

Specimen Type	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Total % Agree-ment
Negative	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	72 / 72 (100%)
Below LoD	6 / 12	12 / 12	8 / 12	10 / 12	7 / 12	7 / 12	11 / 12	10 / 12	12 / 12	9 / 12	11 / 12	10 / 12	113 / 144 (78%)
Low Positive	10 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	11 / 12	12 / 12	12 / 12	141 / 144 (98%)
Moderate Positive	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	12 / 12	144 / 144 (100%)

Table 62 Lot to Lot Reproducibility

Specimen Type	Lot 1	Lot 2	Lot 3	Total %Agreement
Negative	30 / 30	27 / 27	15 / 15	72 / 72 (100%)
Below LoD	50 / 60	38 / 54	25 / 30	113 / 144 (78%)
Low Positive	57 / 60	54 / 54	30 / 30	141 / 144 (98%)
Moderate Positive	60 / 60	54 / 54	30 / 30	144 / 144 (100%)

15.2.6 Carry-Over / Cross-Contamination

An analytical study was performed to evaluate the potential for cross-contamination between high *C. difficile* (5×10^8 CFU/mL) specimens and negative specimens throughout the ***C. difficile* ELITe MGB® Kit** used in association with NucliSENS® easyMAG® (BioMeriéux) and 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems). Two operators performed five runs of 24 sample extraction (11 high titre *C. difficile* samples, 11 negative samples, 1 Positive Control sample, and 1 Negative Control sample per run) in a checkerboard pattern (high *C. difficile* samples interrupted by completely negative samples). The processed samples were then amplified in five separate runs using two different checkerboard patterns. The cross-contamination testing resulted in no false positive result from 55 *C. difficile* negative samples.

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics are recorded in the Section 7 of the Product Technical File "*C. difficile* ELITe MGB® Kit", FTP M800358.

16 REFERENCES

Cloud J. and Kelly C. P. (2007) Cur. Opin. Gastroenterology 23: 4 - 9.

Cohen, S. H. et al. (1998) Clin. Infect. Diseases 26: 410 - 412.

Kuijper, E. J. et al. (2006) Clin. Microb. and Infection 12: 2 - 18.

Article about LoB and LoD statistics: K. Linnet and M. Kondratovich (2004) Clin. Chem. 50: 732 - 740.

CLSI EP37, Ed1E: “Supplemental Tables for interference Testing in Clinical Chemistry”

17 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: unformed or liquid human stool 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

It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results.

This product requires the use of special clothing and instruments for extraction / preparation of amplification reactions and for amplification / detection of amplification products to avoid false positive results.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A positive result obtained with this product does not indicate the presence of viable toxigenic *C. difficile* but is presumptive for the presence of toxigenic *C. difficile*. Therefore, a positive result does not necessarily indicate intervention eradication failure since non-viable DNA may persist.

A negative result obtained with this product indicates that the toxin A or toxin B gene DNA is not detected in the DNA extracted from the sample; however it cannot be excluded that the toxigenic *C. difficile* 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.

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.

18 TROUBLESHOOTING

ELITe InGenius and ELITe BeGenius

Table 63

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 5 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 5 consecutive sessions (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.

Table 63 (continued)

Invalid Positive Control reaction	
Possible Causes	**Solutions**
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 64

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.

Table 65

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 5 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 5 consecutive sessions (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.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 66

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 67

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

Table 68

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.

Open Platform:

Table 69

Target DNA not detected in the Positive Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Take care when dispensing reactions into the microplate wells and comply with the work sheet. Check the volumes of PCR Mix dispensed. Check the volumes of positive control dispensed.
PCR Mix degradation.	Use a new aliquot of PCR Mix.

Table 69 (continued)

Target DNA not detected in the Positive Control reaction	
Possible Causes	**Solutions**
Positive Control degradation.	Use a new aliquot of Positive Control.
Instrument setting error.	Check the position settings for the positive control reaction on the instrument. Check the thermal cycle settings on the instrument.

Table 70

Target DNA detected in the Negative Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, Negative Controls, positive controls into the microplate wells and comply with the work sheet.
Error while setting the instrument	Check the position settings of the samples, Negative Controls, Positive Controls or standards on the instrument
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of molecular biology grade water.	Use a new aliquot of sterile water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction / preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

Table 71

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing or inadequate mixing of sample.	Take care, by pipetting three times, when mixing samples, Negative Controls and Positive Controls into the reaction mixture. Avoid creating bubbles.
Baseline setting error.	Set the baseline calculation range within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g from cycle 6 to cycle 15. Use the automatic baseline calculation by setting the "Auto Baseline" option.

Table 72

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

19 SYMBOLS

REF Catalogue Number.


Upper limit of temperature.

LOT Batch code.


Use by (last day of month).

IVD *in vitro* diagnostic medical device.

CE Fulfilling the requirements of the European Directive 98\79\EC for in vitro diagnostic medical device.

UDI Unique Device Identification


Contains sufficient for "N" tests.


Caution, consult instructions for use.

CONT Contents.


Keep away from sunlight.


Manufacturer.

20 NOTICE TO PURCHASER: LIMITED LICENSE

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

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

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

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 *C. difficile* 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 ***C. difficile* ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids Real-Time PCR assay for the **detection of toxin A and toxin B genes of toxigenic *Clostridium difficile*** (***C. difficile***), including the hypervirulent epidemic NAP1/BI/027 strain, in DNA samples extracted from unformed or liquid stool 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 specimens of stool.

The assay is also validated in association with the **7500 Real-Time PCR System**, using human specimens of stool.

The product is intended for use as an aid in the diagnosis of toxigenic ***C. difficile*** in healthcare settings in conjunction with other laboratory test results and clinical data.

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

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	*C. difficile* toxin A-specific gene	AP525	Toxin A
Target 2	*C. difficile* toxin B-specific gene	FAM	Toxin B
Target 3	IC2	AP642	IC

Validated matrix

- Native stool collected without preservatives

Kit content and related products

***C. difficile* ELITe MGB Kit (M800358)**		***C. difficile* - ELITe Positive Control (M800373)**	
 X 4		 X 2	
***C. difficile* PCR Mix** 4 tubes of 540 µL 24 reactions per tube 96 reactions per kit 5 freeze-thaw cycles per tube		***C. difficile* Positive Control** 2 tubes of 160 µL 4 reactions per tube 8 reactions per kit 4 freeze-thaw cycles per tube	
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 S. T. A. R. buffer (Roche Diagnostics GmbH, ref. 3 335 208) or an equivalent buffer • Minitip Flocked Swab® (COPAN Italia S. p. A., Italy, ref. 518CS01) or an equivalent device.

ELITe InGenius and ELITe BeGenius Protocol

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

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Sensitivity	Specificity
Native Stool	500 CFU/mL	100% (30/30)	97.8% (44/45)

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 73

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	> 1 month

ELITe InGenius Procedures

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

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify 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.

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: C.diff 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: C. diff ELITe_ST_200_100 or C. diff ELITe_PC or C. diff 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 (GUI) of ELITe BeGenius software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR) or PCR Only.

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify 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.

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: C.diff 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 Rack” 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

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 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: C.diff ELITe_Be_ST_200_100 or C.diff ELITe_Be_PC or C.diff ELITe_Be_NC	**5.** Load the PCR-Mix in the Reagent/ Elution Rack and insert it in the Cooler Unit	**6.** Load “PCR Rack” with “PCR Cassette”
7. Close the door. Start the run	**8.** View, approve and store the results	

ELITechGroup S.p.A.
C.so Svizzera, 185, 10149 Torino ITALY
Tel. +39-011 976 191
Fax +39-011 936 76 11
E. mail: emd.support@elitechgroup.com
WEB site: www.elitechgroup.com