Instructions for use

Meningitis Bacterial ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS300ING

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CE IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
03	Expansion of use of the product in association with ELITe BeGenius instrument (REF INT040) New graphics and content setting of the IFU.	20/01/25
02	Update of section “Procedure Limitation Description of IC cut off value already adopted in the Assay protocol of the product	11/05/23
00 — 01	new product development and succeeding changes	-

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

TABLE OF CONTENT

1 INTENDED USE

The product **Meningitis Bacterial ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the genomic DNA of **Neisseria meningitidis**, **Streptococcus pneumoniae**, **Haemophilus influenzae** and **Haemophilus influenzae type B** in clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®**instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of Cerebrospinal fluid (CSF) and whole blood (WB) collected in EDTA.

The product is intended for use as an aid in the diagnosis of central nervous system and systemic infections of *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae and Haemophilus influenzae* type B.

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

2 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae and Haemophilus influenzae type B, isolated from specimens and amplified using the assay reagent **MB 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.

3 PRODUCT DESCRIPTION

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

- the **ctrA** gene of encapsulated N. meningitidis, detected in Channel **Nmen;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 593 (AP593) dye,
- the **lytA** gene of encapsulated S. pneumoniae, detected in Channel **Spne;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye,
- the **fucK** gene of H. influenzae, detected in Channel **Hinf;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 639 (AP639) dye,
- the **bcsB** gene of encapsulated H. influenzae type B, detected in Channel **HinfB;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 525 (AP525) dye,
- the **IC2** artificial sequence of exogenous Internal Control (IC), detected in Channel **IC;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 680 (AP680) dye.

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

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

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

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
MB PCR Mix **Ref. RTS300ING**	Mixture of reagents for Real-Time PCR in tube with **WHITE cap**	8 x 280 µL	-

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.

- Disposable nitrile powder-free gloves or similar material.

- Vortex mixer.

- Bench centrifuge (~5,000 RPM).

- Bench microcentrifuge (~13,000 RPM).

- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).

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

- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample 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 1.3.0.19 (or later). **MB ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **MB ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **MB ELITe_WB_200_100** Assay Protocol with parameters for whole blood specimen analysis **MB ELITe_CSF_200_100** Assay Protocol with parameters for cerebrospinal fluid specimen analysis	**ELITe InGenius SP200** (EG SpA, ref. INT032SP200). **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS). **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR). **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000). **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only. **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only. **CPE - Internal Control** (EG SpA, ref. CTRCPE). **Meningitis Bacterial - ELITe Positive Control** (EG SpA, ref. CTR300ING).
ELITe BeGenius (EG SpA, ref. INT040). **ELITe BeGenius Software** version 2.2.1 (or later). **MB ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis. **MB ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis. **MB ELITe_Be_WB_200_100** Assay Protocol with parameters for whole blood specimen analysis **MB ELITe_Be_CSF_200_100** Assay Protocol with parameters for cerebrospinal fluid specimen analysis	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

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

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

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

Never pipette solutions by mouth.

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

Carefully wash hands after handling samples and reagents.

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

Carefully read all the instructions provided before running the assay.

While running the assay, follow the product instructions provided.

Do not use the product after the indicated expiry date.

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

Do not use reagents from different batches.

Do not use reagents from other manufacturers.

7.2 Warnings and precautions for molecular biology

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

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

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

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

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

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

7.3 Warnings and precautions specific for the components

Table 3

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

* with intermediate freezing

8 SPECIMENS AND CONTROLS

Specimens and Assay Protocols

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens, 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
cerebrospinal fluid	Avoid contamination with patient blood	≤ 3 days	≤ 3 days	≤ 1 month	long period
whole blood	EDTA	≤ 3 days	≤ 3 days	≤ 1 month	long period

It is recommended to divide the specimens into aliquots before freezing to prevent repeated freeze / thaw cycles.

When using frozen samples, thaw the samples just before the extraction to avoid possible nucleic acid degradation.

To perform samples testing on the **ELITe InGenius** and **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 Meningitis Bacterial ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
CSF	**ELITe InGenius**	**MB ELITe_CSF_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MB ELITe_Be_CSF_200_100**		
Whole blood	**ELITe InGenius**	**MB ELITe_WB_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MB ELITe_Be_WB_200_100**		

NOTE

Verify if the primary tube and the volume of the sample are compatible with ELITe InGenius or ELITe BeGenius, following the Instruction for use of the extraction kit **ELITeInGeniusSP200** (EG SpA, ref. INT032SP200).

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

If required, 200 of sample must be transferred into an **Extraction tube** for ELITe InGenius; 200 µL of sample must be transferred into **2 mL Sarstedt Tube** for ELITe BeGenius.

NOTE

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

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

Refer to “Potentially Interfering Substances” in the 11 PERFORMANCE CHARACTERISTICS page 17section to check data concerning interfering substances.

PCR controls

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

- for the Positive Control, use the product Meningitis Bacterial – ELITe Positive Control (not provided with this kit) with the MB ELITe_PC or MB ELITe_Be_PC Assay Protocols
- for the Negative Control, use molecular biology grade water (not provided with this kit) with the MB ELITe_NC or MB ELITe_Be_NC Assay Protocols.

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the PCR control validation for each lot of PCR reagent. PCR control results expire after **15 days**, at which time it is necessary to re-run the Positive and Negative Controls. The PCR controls must be re-run if any of the following events occur:

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

Quality controls

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

9 ELITe InGenius PROCEDURE

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

Table 6

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

9.1 STEP 1 - Verification of the system readiness

Before starting the session:

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

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

9.2 STEP 2 - Session Setup

The **Meningitis Bacterial 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 **12 tests** in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE
Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

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

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. If required, transfer **200 µL of sample** 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.

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
15	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
16	Close the instrument door.	Close the instrument door.	Close the instrument door.
17	Press “Start”.	Press “Start”.	Press “Start”.

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

NOTE

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

NOTE

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

NOTE

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

NOTE

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

NOTE

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

9.3 STEP 3 - Review and approval of results

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

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

NOTE

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

The **ELITe InGenius** generates results with the **Meningitis Bacterial 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.

9.3.1 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius Software** interprets the PCR results for the targets of the Positive Control and Negative Control reaction with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct values are used to verify the system (reagents lot and instrument).

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

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

The results of the Positive Control and Negative Control amplification are used by the **ELITe InGenius software** to set up the Control Charts monitoring the amplification step performances. Refer to the instrument manual for more details.

NOTE

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

NOTE

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

9.3.2 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (channels **Nmen**, **Spne**, **Hinf** and **HinfB**) and the Internal Control (channel **IC**) with the **MB ELITe_CSF_200_100** and **MB ELITe_WB_200_100** Assay Protocols 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**
MB Positive Control	APPROVED
2) Negative Control	**Status**
MB 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**
Nmen: DNA detected.	***N. meningitidis*** **DNA was detected** in the sample.
Spne: DNA detected.	***S. pneumoniae*** **DNA was detected** in the sample.
Hinf: DNA detected.	***H. influenzae*** **DNA was detected** in the sample.

Table 9 (continued)

Result of sample run	Interpretation
HinfB: DNA detected.	***H. influenzae* type B DNA was detected** in the sample. **N.B.:** when ***H. influenzae* type B** DNA is detected, the generic ***H. influenzae*** DNA could also be detected.
Nmen: DNA not detected or below LoD.	***N. meningitidis* DNA was not detected** in the sample. The sample is negative for N. meningitidis DNA or its concentration is below the assay Limit of Detection.
Spne: DNA not detected or below LoD.	***S. pneumoniae* DNA was not detected** in the sample. The sample is negative for S. pneumoniae DNA or its concentration is below the Limit of Detection of the assay.
Hinf: DNA not detected or below LoD.	***H. influenzae* DNA was not detected** in the sample. The sample is negative for ***H. influenzae* DNA** or its concentration is below the assay Limit of Detection.
HinfB: DNA not detected or below LoD.	***H. influenzae* DNA type B was not detected** in the sample. The sample is negative for H. influenzae type B DNA or its concentration is below the assay Limit of Detection.
Invalid - Retest Sample.	**Not valid assay result** caused by Internal Control failure (due to e.g., incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, pretreatment, extraction or PCR steps (e. g., incorrect sampling, degradation or loss of DNA during the extraction or inhibitors in the eluate), which may cause incorrect results.

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see 14 TROUBLESHOOTING page 27)

When *Haemophilus influenzae* type B DNA is detected in a sample, sometimes the generic *Haemophilus influenzae* DNA could not be detected due to differences in the sensitivity of the two reactions. However, the sample is positive for *Haemophilus influenzae* type B DNA.

Samples reported as “Xxx: DNA not detected or below the LoD" are suitable for analysis but the DNA of the targets was not detected. In this case, the sample may be either negative for target DNA or the target DNA is present at a concentration below the Limit of Detection of the assay (see 11 PERFORMANCE CHARACTERISTICS page 17).

NOTE

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

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

9.3.3 Sample result reporting

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

10 ELITe BeGenius PROCEDURE

The procedure to use the **Meningitis Bacterial 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

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

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

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

10.2 STEP 2 - Session Setup

The **Meningitis Bacterial 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 12 tests in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

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

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). If required, transfer **200 µL of sample** 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. 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 the ELITe InGenius SP 200 Consumable Set.
3	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen	Select "**Perform Run**" from the "Home" screen.
4	Remove all the Racks from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
5	Select the "Run mode": "**Extract + PCR".**	Select the "Run mode": "**PCR Only**".	Select the "Run mode": "**PCR Only**".
6	**Load the samples** into the "Sample Rack". When secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack".	**Load the samples** into the "Elution Rack".	**Load the Positive Control and Negative Control** tubes into the "Elution Rack".
7	**Insert** the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert the "Sample ID" (SID) for each "Position" used (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3). If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
8	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
9	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL.
10	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
11	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
	Note: When more than 12 samples are processed, repeat the procedure from point 6.		Not applicable
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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”.
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 7 hours (2 sessions of about 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

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

NOTE

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

NOTE

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

10.3 STEP 3 - Review and approval of results

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

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

NOTE

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

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

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

NOTE

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

11 PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) of the Meningitis Bacterial ELITe MGB Kit was defined in association with CSF samples and whole blood collected in EDTA and ELITe InGenius system.

The LoD was calculated by testing a panel of CSF samples and whole blood collected in EDTA samples spiked with a reference material of *N. meningitidis, S. pneumoniae, H. influenzae* and *H. influenzae* type B at known titre (provided by an external laboratory). The LoD was obtained by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call.

The final results are reported in the following table.

Table 12 Limit of Detection (organisms / mL) for CSF samples and ELITe InGenius system

Pathogen	LoD	95% confidence interval	
		Lower bound	Upper bound
N. meningitidis	34	21	172
S. pneumoniae	34	22	134
H. influenzae	95	53	426
H. influenzae type B	66	41	197

Table 13 Limit of Detection (organisms / mL) for whole blood samples and ELITe InGenius system

Pathogen	LoD	95% confidence interval	
		Lower bound	Upper bound
N. meningitidis	56	37	130
S. pneumoniae	189	119	473
H. influenzae	172	112	400
H. influenzae type B	77	50	186

The calculated LoD value in association to WB and CSF was verified on the ELITe InGenius and ELITe BeGenius instruments, testing a pool of matrix samples spiked with reference material of each pathogen at the claimed concentration.

Efficiency of detection (inclusivity)

The efficiency of detection of the assay for ctrA gene of encapsulated *N. meningitidi*s, lytA gene of encapsulated *S. pneumoniae*, fucK gene of *H. influenzae,* bcsB gene of encapsulated *H. influenzae* type B (inclusivity) was evaluated by comparison of sequences with nucleotide database.

The analysis of the regions chosen for the hybridization of the primers and of the fluorescent probes in the alignment of the sequences available in the database for the pathogens of interest showed their conservation and absence of significant mutations.

The detection of strains of *N. meningitidis, S. pneumoniae, H. influenzae*, and *H. influenzae* type B was also verified by analysis of certified genomic DNA from clinical samples (provided by an external laboratory).

The Certified genomic DNA samples were diluted to a Ct value of about 30 and analysed in association with ELITe InGenius system.

The final results are reported in the following table.

Table 14 Inclusivity

Organisms	Sample	Positive	Negative
N. meningitidis	12 clinical isolates	12	0
S. pneumoniae	12 clinical isolates	12	0
H. influenzae	10 clinical isolates	10	0
H. influenzae type B	12 clinical isolates	12	0

Potential interfering markers

The potential cross-reactivity with other unintended organisms of the assay was evaluated by *in silico* analysis of sequences available in the EBI ENA nucleotide database.

The regions chosen for the hybridisation of the primers and the fluorescent probes were checked on the alignment of the sequences of other prokaryotic and eukaryotic organisms. The hybridisation regions showed absence of significant homologies and indicated no potential interference.

The absence of cross-reactivity with other organisms that can be found in CSF and whole blood samples was also verified by testing a panel of certified materials (ATCC, Vircell, NIBSC and clinical isolate).

Other organism genomic DNA samples were analysed in duplicate for each potentially cross-reactive organism in association with ELITe InGenius.

The final results are reported in the following table.

Table 15 Potential cross-reactivity

Organism	Strain	Outcome
E. coli	Clinical isolate	No cross-reactivity
B. burgdorferi	IRS	No cross-reactivity
L. monocytogenes	53 XXIII	No cross-reactivity
S. agalactiae	G19	No cross-reactivity
HSV1	McIntyre	No cross-reactivity
HSV2	G	No cross-reactivity
VZV	Ellen	No cross-reactivity
JCV	International Standard	No cross-reactivity
BKV	International Standard	No cross-reactivity
T. gondii	RH	No cross-reactivity
Enterovirus	Pesascek	No cross-reactivity

All the organisms were negative for the targets when tested by Meningitis Bacterial ELITe MGB Kit.

The absence of interference by other organisms that can be found in whole blood and CSF samples collected in EDTA samples was also verified by testing a panel of certified materials (ATCC, Vircell, NIBSC and clinical isolate) spiked with certified DNA of *N. meningitidis, S. pneumoniae* and *H. influenzae* type B to a final concentration of about 10 copies/reaction.

Other organism genomic DNA samples spiked with the targets were analysed in duplicate for each potentially interfering organism in association with ELITe InGenius.

The final results are reported in the following table.

Table 16 Potential interference

Organism	Strain	Outcome
E. coli	Clinical isolate	No interference
B. burgdorferi	IRS	No interference
L. monocytogenes	53 XXIII	No interference
S. agalactiae	G19	No interference

Table 16 Potential interference (continued)

Organism	Strain	Outcome
HSV1	McIntyre	No interference
HSV2	G	No interference
VZV	Ellen	No interference
JCV	International Standard	No interference
BKV	International Standard	No interference
T. gondii	RH	No interference
Enterovirus	Pesascek	No interference

All the organisms did not interfere with the amplification of the targets when tested by the Meningitis Bacterial ELITe MGB Kit.

Potential interference among targets

The potential interference among targets of the assay was evaluated by a test of co-amplificationof plasmid DNA containing the target sequences of encapsulated *N. meningitidis* (ctrA gene) of encapsulated *S. pneumoniae*, (lytA gene), of *H. influenzae* (fucK gene) and encapsulated *H. influenzae* type B (bcsB gene).

The panel included samples with plasmid DNAs for *N. meningitidis, S. pneumoniae, H. influenzae*, or *H. influenzae* type B at high concentration (10^5 copies / reaction) and the other pathogens of interest at low concentration levels (e.g. 10^3, 10^2, 10 copies / reaction).

Each condition was analysed in duplicate in association with ELITe InGenius.

For each target, the lowest concentration detectable in duplicate in co-amplification reaction (copies /reaction, c./rxn) is reported in the following table.

Table 17 Interference among targets

Target in test	Interfering target at ~10^5 copies / reaction			
	N. meningitidis	***S. pneumoniae***	***H. influenzae***	***H. influenzae* type B**
N. meningitidis detectable at	-	10 c./rxn	10 c./rxn	10^2 c./rxn
S. pneumoniae detectable at	10 c./rxn	-	10 c./rxn	10^2 c./rxn
H. influenzae detectable at	10^2 c./rxn	2 x 10^3 c./rxn	-	n.a.*
H. influenzae type B detectable at	10 c./rxn	10 c./rxn	10^2 c./rxn	-

* not applicable as the *H. influenzae* type B gives positive results for generic *H. influenzae*.

Interfering substances

A panel of potentially interfering substances at relevant concentrations was tested with the product Meningitis Bacterial ELITe MGB Kit. The substances tested were EDTA, Rifampicin and Ampicillin.

The substances were individually added to CSF (Rifampicin and Ampicillin) and to whole blood (EDTA**)** spiked with each reference materials of *N. meningitides, S. pneumoniae*, *H. influenzae* and *H. influenzae* type B (provided by an external laboratory) at concentration of 3x LoD.Samples were processed in three replicates on ELITe InGenius in “Extract + PCR” mode.

The results are reported in the following table.

Table 18 Interfering substances: %CV (reference + test)

Substance	*N. meningitides*	*S. pneumoniae*	*H. influenzae*	*H. influenzae* type B	IC
Ampicillin	0.86	1.27	1.83	2.46	0.77
Rifampicin	1.14	1.28	1.36	2.75	0.81
EDTA	0.83	0.71	2.02	1.33	1.11

All the samples resulted positive for the target of interest. The percentage Coefficient of Variability (%CV) of *Ct* values were lower than 5%. None of the tested substances at the tested concentrations were found to interfere with the target detection by Meningitis Bacterial ELITe MGB Kit

Repeatability

The Repeatability of the assay obtained was evaluated on ELITe InGenius and ELITe BeGenius by analysing a panel of whole blood collected in EDTA samples including one negative sample and positive samples spiked with the reference materials of each target at concentration of about 3-4x LoD.

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

Table 19 Intra-Session Repeatability with whole blood samples on ELITe InGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	6	34.07	0.25	0.74	100%
S. pneumoniae	6	32.08	0.14	0.45	
H. influenzae	6	31.98	0.29	0.92	
H. influenzae type B	6	30.58	0.27	0.88	
Internal Control	18	27.03	0.19	0.70	100%

Table 20 Intra-Session Repeatability with whole blood samples on ELITe BeGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	6	34.28	0.29	0.83	100%
S. pneumoniae	6	31.83	0.17	0.53	
H. influenzae	6	32.41	0.12	0.37	
H. influenzae type B	6	31.30	0.25	0.80	
Internal Control	18	28.88	0.69	2.40	100%

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

Table 21 Inter-Session Repeatability with whole blood samples on ELITe InGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	12	34.03	0.19	0.56	100%
S. pneumoniae	12	32.13	0.14	0.43	
H. influenzae	12	31.87	0.25	0.78	
H. influenzae type B	12	30.56	0.22	0.73	
Internal Control	36	27.13	0.23	0.86	100%

Table 22 Inter-Session Repeatability with whole blood samples on ELITe BeGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	12	34.07	0.45	1.32	100%
S. pneumoniae	12	31.70	0.54	1.69	
H. influenzae	12	32.51	0.27	0.82	
H. influenzae type B	12	31.33	0.29	0.94	
Internal Control	36	28.78	0.63	2.20	100%

In the Repeatability test, the Meningitis Bacterial ELITe MGB Kit showed, for each target, a maximum variability of target Ct values as %CV lower than 5%.

Reproducibility

The Inter-Batch and Inter – Instrument Reproducibility of results obtained by the product Meningitis Bacterial ELITe MGB Kit in association with the ELITe InGenius was tested by analysing a panel of CSF samples and a panel of whole blood collected in EDTA samples. Each panel was spiked with the reference materials of each target at concentration of about 3x LoD.

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

Table 23 Inter-Batch reproducibility with CSF samples on ELITe InGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	18	37.18	0.52	1.41	100%
S. pneumoniae	18	36.41	0.72	1.98	
H. influenzae	18	38.23	0.64	1.68	
H. influenzae type B	18	35.59	0.48	1.36	
Internal Control	71/72	27.93	0.95	3.40	98.6%

Table 24 Inter-Batch Reproducibility with whole blood samples on ELITe InGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	18	36.81	0.39	1.05	100%
S. pneumoniae	18	34.40	0.30	0.88	
H. influenzae	18	38.38	0.56	1.45	
H. influenzae type B	18	35.72	0.61	1.72	
Internal Control	71/72	28.96	0.55	1.91	98.6%

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

Table 25 Inter – Instrument Reproducibility with CSF samples on ELITe InGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	18	37.24	0.53	1.41	100%
S. pneumoniae	18	36.78	0.95	2.59	
H. influenzae	18	38.32	0.57	1.49	
H. influenzae type B	18	35.70	0.55	1.53	
Internal Control	72/72	28.55	1.11	3.90	100%

Table 26 Inter – Instrument Reproducibility with whole blood samples on ELITe InGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	18	36.95	0.58	1.57	100%
S. pneumoniae	18	34.44	0.45	1.29	
H. influenzae	18	38.55	0.61	1.59	
H. influenzae type B	18	35.48	0.54	1.51	
Internal Control	72/72	29.36	1.04	3.54	100%

The Inter- Batch and Inter-Instrument Reproducibility in association with ELITe BeGenius was evaluated by analysing only a panel of whole blood collected in EDTA including one negative sample and positive samples spiked with the reference materials of each target at concentration of about 3-4x LoD.

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

Table 27 Inter – Batch Reproducibility with whole blood samples on ELITe BeGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	12	34.07	0.36	1.07	100%
S. pneumoniae	12	31.75	0.30	0.94	
H. influenzae	12	32.53	0.21	0.63	
H. influenzae type B	12	31.27	0.23	0.73	
Internal Control	36	28.59	0.62	2.15	100%

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

Table 28 Inter – Instrument Reproducibility with whole blood samples on ELITe BeGenius

Target	N	Mean Ct	SD	%CV	% Agreement
N. meningitidis	12	33.81	0.25	0.75	100%
S. pneumoniae	12	31.82	0.43	1.34	
H. influenzae	12	32.49	0.27	0.82	
H. influenzae type B	12	31.37	0.28	0.89	
Internal Control	36	28.15	0.39	1.39	100%

In the Reproducibility test, the Meningitis Bacterial ELITe MGB Kit showed, for each target, a maximum variability of target Ct values as %CV lower than 5%.

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 CSF and whole blood collected in EDTA. The samples were certified positive or spiked with certified materials of each target.

As ELITe BeGenius showed equivalent analytical performances to ELITe InGenius, it can be assumed that the results of Diagnostic sensitivity obtained in association with ELITe InGenius are applicable also to ELITe BeGenius.

The results are summed up in the following tables.

Table 29 Diagnostic Sensitivity with CSF samples

Positive /spiked CSF samples	N	Positive	Negative	Invalid	% Diagnostic Sensitivity
N. meningitidis	50	50	0	0	100 %
S. pneumoniae	50	50	0	0	100 %
H. influenzae	50	50	0	0	100 %
H. influenzae type B	20	20	0	0	100 %

Table 30 Diagnostic Sensitivity with whole blood samples

Positive /spiked whole blood samples	N	Positive	Negative	Invalid	% Diagnostic Sensitivity
N. meningitidis	50	50	0	0	100 %
S. pneumoniae	57	57	0	0	100 %
H. influenzae	50	48	2	0	96.0 %
H. influenzae type B	20	20	0	0	100 %

Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with ELITe InGenius by analysing clinical samples of CSF and whole blood collected in EDTA, certified negative for each target.

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

The results are summed up in the following tables.

Table 31 Diagnostic Specificity with CSF samples

Negative CSF samples	N	Positive	Negative	Invalid	% Diagnostic Specificity
N.meningitidis	150	0	150	0	100 %
S. pneumoniae	150	1	149	0	99.3 %
H. influenzae	148	0	148	0	100 %
H. influenzae type B	150	0	150	0	100 %

Table 32 Diagnostic Specificity with whole blood samples

Negative whole blood samples	N	Positive	Negative	Invalid	% Diagnostic Specificity
N.meningitidis	153	0	153	0	100 %
S. pneumoniae	149	4	145	0	97.3 %
H. influenzae	151	3	148	0	98.0 %
H. influenzae type B	153	0	153	0	100 %

The Internal Control Ct (IC Ct) cut-off value is set at 34 for CSF and whole blood, in association to ELITe InGenius and ELITe BeGenius.

Robustness: clinical samples invalid results

The assay robustness, as evaluation of invalid results in the first sample analysis, was verified analysing the results obtained from clinical samples of different matrices.

The percentage of invalid samples was verified using the results of Diagnostic Sensitivity and Diagnostic Specificity tests. The results are reported in the following table.

Table 33

Sample	N	Invalid result	%
CSF samples	321	0	0 %
Whole Blood samples	326	11	3.4 %

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File Meningitis Bacterial ELITe MGB Kit, FTP RTS300ING.

12 REFERENCES

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13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: cerebrospinal fluid (CSF) and whole blood collected in EDTA.

Do not use this product with samples containing heparin: heparin inhibits the amplification reaction of nucleic acids and can cause invalid results.

Currently there are no data available concerning product performance with the following clinical samples: nasopharyngeal swabs, nasopharyngeal aspirate, sputum, Broncho-alveolar lavage (BAL), Broncho aspirate (BA).

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

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

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

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

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

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

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

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

In case of co-infections, the sensitivity for one target can be affected by the amplification of a second target (see 11 PERFORMANCE CHARACTERISTICS page 17).

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.

14 TROUBLESHOOTING

Table 34

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Positive Control. Check the volumes of PCR Mix and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Positive Control degradation.	Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use a new aliquot of Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 35

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 36

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

Table 36 (continued)

Invalid Sample reaction	
Possible Causes	**Solutions**
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 37

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

Table 38

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

Table 39

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

15 SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE	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.
	Consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

16 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.

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Appendix A Meningitis Bacterial 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 at www.elitechgroup.com.

INTENDED USE

The product **Meningitis Bacterial ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the genomic DNA of **Neisseria meningitidis**, **Streptococcus pneumoniae**, **Haemophilus influenzae** and **Haemophilus influenzae type B** in clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®**instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of Cerebrospinal fluid (CSF) and whole blood (WB) collected in EDTA.

The product is intended for use as an aid in the diagnosis of central nervous system and systemic infections of *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae and Haemophilus influenzae* type B.

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

Amplified sequence

Sequence	Gene	Fluorophore	Channel
N. meningitidis	ctrA	AP593	Nmen
S. pneumoniae	lytA	FAM	Spne
H. influenzae	fucK	AP639	Hinf
H. influenzae type B	bcsB	AP525	HinfB
Internal Control	IC2	AP680	IC

Validated matrix

- Whole blood collected in EDTA
- CSF

Kit content and related products

Meningitis Bacterial ELITe MGB Kit Kit (RTS300ING)	Meningitis Bacterial - ELITe Positive Control (CTR300ING)
 X 8	 X 3
MB PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube	MB Positive Control 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles

Meningitis Bacterial ELITe MGB Kit Kit (RTS300ING)		Meningitis Bacterial - ELITe Positive Control (CTR300ING)	
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.

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	20 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Target	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
CSF	N. meningitidis	34	100% 50/50*	100% 150/150*
	S. pneumoniae	34	100% 50/50*	99.3% 149/150*
	H. influenzae	95	100% 50/50*	100% 148/148*
	H. influenzae type B	66	100% 20/20*	100% 150/150*
WB	*N. meningitidis*	56	100% 50/50*	100% 153/153*
	S. pneumoniae	189	100% 57/57*	97.3% 145/149*
	H. influenzae	172	96% 48/50*	98% 148/151*
	H. influenzae type B	77	100% 20/20*	100% 153/153*

**confirmed samples/ tested samples*

Sample preparation

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

Table 40

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ±10 °C	-70 ±15 °C
cerebrospinal fluid	Avoid contamination with patient blood	≤ 3 days	≤ 3 days	≤ 1 month	long period
whole blood	EDTA	≤ 3 days	≤ 3 days	≤ 1 month	long period

ELITe InGenius Procedures

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

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** 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: MB ELITe_CSF_200_100 or MB ELITe_WB_200_100	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix in the Inventory Block
7. Load PCR Cassettes, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**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: MB ELITe_PC or MB ELITe_NC or MB ELITe_CSF_200_100 or MB ELITe_WB_200_100	**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, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	8. Close the door. Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

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

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** 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: MB ELITe_Be_CSF_200_100 or MB ELITe_Be_WB_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 in the Reagent/ Elution Rack and insert it in the Cooler Unit
7. Load "PCR Rack" with "PCR Cassette" and the "Extraction Rack" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE

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

Procedure 2: PCR Only (e.g., eluates, 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: MB ELITe_Be_PC or MB ELITe_Be_NC or MB ELITe_Be_CSF_200_100 or MB ELITe_Be_WB_200_100	**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	

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