Instructions for use

ESBL ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS201ING

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CE IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
03	Extended Use of the product in association with ELITe BeGenius®.and rectal swabs and blood colture matrices.	27/05/25
02	Formal corrections in “Samples and Controls” section	30/07/18
01	Extended Use of the product with Blood Culture matrix, in association with ELITe InGenius® instrument.	05/02/18
00	new product development	30/01/17

NOTE

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

TABLE OF CONTENT

1 INTENDED USE

The product **ESBL ELITe MGB® Kit** product is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of the DNA of Extended Spectrum Beta-Lactamase **CTX-M-1**, **CTX-M-9**, **CTX-M-14** and **CTX-M-15*** genes of *Enterobacteriaceae* in DNA samples extracted from rectal swabs and blood colture.

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 rectal swabs and blood culture.

The product is intended for use in the diagnosis and screening of infections of Enterobacteria positive for Extended Spectrum Beta-Lactamase genes, together with the patient's clinical data and other laboratory test results.

The product is also compatible for the characterization of Enterobacteriaceae positive for Extended Spectrum Beta-Lactamase genes in DNA samples extracted from cultural isolates.

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

*For the complete list of gene variants detected by this product, please, refer to the "Performance Characteristics" chapter.

2 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting the DNA of Extended Spectrum Beta-Lactamase **CTX-M-1**, **CTX-M-9**, **CTX-M-14** and **CTX-M-15** of *Enterobacteriaceae* isolated from specimens and amplified using the assay reagent ESBL PCR Mix, that contains primers and probes with ELITe MGB Kit technology.

The ELITe MGB Kit probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycles (Ct).

In the ELITe MGB Kit 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.

3 PRODUCT DESCRIPTION

The **ESBL ELITe MGB Kit** provides the assay reagent **ESBL PCR Mix**, an optimized and stabilized PCR Mixture that contains the specific primers and probes for:

- **CTX-M-1** and **CTX-M-15** genes family, detected in Channel **CTX-M1-9-14-15;** ; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 593 (AP593) dye,

-**CTX-M-9** and **CTX-M-14** genes family detected in Channel **CTX-M1-9-14-15**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 593 (AP593) dye,

- Internal Control (**IC)**, specific for artificial sequence IC2**,** detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 525 (AP525) dye.

The **ESBL PCR Mix** also contains buffer, magnesium chloride, the nucleotide triphosphates, the stabilizers and the enzyme Taq DNA polymerase with thermic activation (hot start).

NOTE
The four genes of Extended Spectrum Beta-Lactamase (ESBL) are detected by two different probes (one for CTX-M-1 and CTX-M-15 genes family and the other one for CTX-M-9 and CTX-M-14 genes family) labeled with the same fluorescent dye thus they cannot be differentiated.

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

The **ESBL 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
ESBL PCR Mix ref. RTS201ING	Mixture of reagents for Real-Time PCR tube with NATURAL 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 (volume range: 0.5-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.
- Trypticase Soy Broth

6 OTHER PRODUCTS REQUIRED

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

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

Table 2

Instruments and Software	Products and Reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030) **ELITe InGenius Software** version 1.3.0.19 (or later) **ESBL ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **ESBL ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **ESBL ELITe_RcS_200_100**, Assay Protocol with parameters for rectal swabs specimen analysis **ESBL ELITe_BC_200_100**, Assay Protocol with parameters for blood culture 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) **ESBL — ELITe Positive Control** (EG SpA, ref. CTR201ING) **eNAT™ kit** (Copan, ref. 606CS01R), **FecalSwab™** (Copan, ref. 470CE)
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.2.1. (or later) **ESBL ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **ESBL ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **ESBL ELITe_Be_RcS_200_100**, Assay Protocol with parameters for rectal swabs specimen analysis **ESBL ELITe_Be_BC_200_100**, Assay Protocol with parameters for blood culture 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)
ESBL PCR Mix	-20°C or below (protected from light)	one month	up to seven	up to seven separated* sessions of three hours each

*with intermediate freezing

8 SPECIMENS AND CONTROLS

8.1 Specimens and Assay Protocols

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**
blood culture	-	≤ 24 hours	-	-	-
rectal swab	collected in **eNAT™ kit**	-	≤ 1 month	≤ 6 months	> 6 months
rectal swab	collected in **FecalSwab™ kit***	-	≤ 3 days	-	-

Before the analysis dilute the blood culture sample 1:1000 in ultrapure water (at least 10 µL of samples into 10 mL of ultrapure water), mix by vortexing and transfer 0.2 mL of the diluted samples into an Extraction tube (for ELITe InGenius instrument) or into a 2 mL Sarstedt tube (for ELITe BeGenius instrument).

Before the analysis of rectal swab, 0.5 mL of sample in FecalSwab™ medium has to be transferred in a fresh eNAT™ tube with 2.0 mL of medium, mixed by vortexing. After addition of 0.5 mL of sample in FecalSwab™ medium, the eNAT™ tube can be directly loaded in the system as a primary tube. The samples diluted in eNAT™ medium can be stored under the same conditions reported in the table above.

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.

This product is compatible for use with Cultural isolates and ELITe InGenius: before the analysis with this product dilute the sample in a fresh eNAT™ tube with 2.0 mL of medium, taking with a loop an isolated colony aliquot, vortex and transfer 0.2 mL of diluted sample into Extraction tube. When nucleic acid extraction from Cultural isolates is carried out with the ELITe InGenius use the extraction protocol **ESBL ELITe_BC_200_100**.

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 Kit and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrices.

Table 5 Assay Protocols for ESBL ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
rectal swab	**ELITe InGenius**	**ESBL ELITe_RcS_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**ESBL ELITe_Be_RcS_200_100**	Positive / Negative	
blood culture	**ELITe InGenius**	**ESBL ELITe_BC_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**ESBL ELITe_Be_BC_200_100**	Positive / Negative	

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 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, 200µL of sample must be transferred into an Extraction tube (for ELITe InGenius) or into a 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 “7 WARNINGS AND PRECAUTIONS page 6” 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 17 section to check data concerning interfering substances.

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

8.2 PCR controls

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

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

NOTE

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

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

8.3 Quality controls

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

9 ELITe InGenius PROCEDURE

The procedure to use the **ESBL 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 **ESBL 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.

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
8	Select the sample loading position as “Extraction Tube” or “Primary 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.
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 separate 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.

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 **ESBL 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.

9.4 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.

9.5 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the target (channel **CTX-M1-9-14-15**) and the Internal Control (channel **IC**) with the **ESBL ELITe_RcS_200_100** and **ESBL ELITe_BC_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
ESBL Positive Control	APPROVED
2) Negative Control	**Status**
ESBL - 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
CTX-M1-9-14-15:DNA detected.	**CTX-M-1, CTX-M-15, CTX-M-9 or CTX-M-14 gene DNA was detected** in the sample.
CTX-M1-9-14-15:DNA Not detected or below LoD.	**CTX-M-1, CTX-M-15, CTX-M-9 and CTX-M-14 gene DNA was not detected** in the sample. The sample is negative for these genes or their concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Not valid assay result** due to Internal Control failure (Incorrect extraction or inhibitor 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, 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 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 26”).

Samples suitable for analysis but in which it was not possible to detect Extended Spectrum Beta-Lactamase CTX-M-1, CTX-M-15, CTX-M-9 and CTX-M-14 genes DNA are reported as: "CTX-M-1-9-14-15:DNA Not detected or below LoD". In this case it cannot be excluded that the Extended Spectrum Beta-Lactamase CTX-M-1, CTX-M-15, CTX-M-9 and CTX-M-14 genes 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”.

9.6 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 **ESBL 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 **ESBL 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 separate 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.

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 **ESBL ELITe MGB Kit** through the following procedure:

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

NOTE

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

11 PERFORMANCE CHARACTERISTICS

11.1 Analytical sensitivity: Limit of Detection

The analytical sensitivity, as Limit of Detection (LoD) of the assay, was determined on **ELITe InGenius** by testing a panel of 4 ESBL strains, one of each of the following gene types: CTX-M-1, CTX-M-9, CTX-M-14 e CTX-M-15 in association to rectal swabs samples. The LoD for each of the ESBL strains was estimated 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 (CFU / mL) for rectal swab samples and ELITe InGenius

Gene	Bacterial Isolate	LoD (CFU / mL)	95% confidence interval (CFU / mL)	
			Lower bound	Upper bound
CTX-M-1	E. coli, DICON-091	55	43	79
CTX-M-9	*E. coli*, DICON-055	29	21	46

Table 12 Limit of Detection (CFU / mL) for rectal swab samples and ELITe InGenius (continued)

Gene	Bacterial Isolate	LoD (CFU / mL)	95% confidence interval (CFU / mL)	
			Lower bound	Upper bound
CTX-M-14	*E. coli*, DICON-045	273	220	384
CTX-M-15	*E. coli*, NCTC13400	36	28	55

The calculated LoD was verified on ELITe InGenius and ELITe BeGenius instruments, by testing rectal swab and blood culture samples spiked with reference material of each target at the claimed concentration.

The results obtained confirmed the claimed concentration for all the targets of ESBL ELITe MGB Kit with rectal swab and blood culture samples on both ELITe BeGenius and ELITe InGenius.

11.2 Efficiency of detection (inclusivity)

The efficiency of detection on different variants of Extended Spectrum Beta-Lactamase genes (inclusivity) was evaluated by comparison of sequences with nucleotide database.

The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probes in the alignment of the sequences available in the database for the Extended Spectrum Beta-Lactamase genes showed their conservation and absence of significant mutations for the variants reported in the following table.

Table 13

Gene	Variants detected by the product ESBL ELITe MGB Kit
CTX-M	CTX-M-1, CTX-M-3, CTX-M-9, CTX-M-10, CTX-M-12, CTX-M-13, CTX-M-14, CTX-M-15, CTX-M-16, CTX-M-17, CTX-M-19, CTX-M-21, CTX-M-22, CTX-M-23, CTX-M-24, CTX-M-27, CTX-M-28, CTX-M-29, CTX-M-30, CTX-M-32, CTX-M-33, CTX-M-36, CTX-M-38, CTX-M-46, CTX-M-47, CTX-M-48, CTX-M-49, CTX-M-50, CTX-M-51, CTX-M-55, CTX-M-61, CTX-M-64, CTX-M-65, CTX-M-66, CTX-M-67, CTX-M-69, CTX-M-71, CTX-M-73, CTX-M-80, CTX-M-81, CTX-M-82, CTX-M-83, CTX-M-84, CTX-M-85, CTX-M-86, CTX-M-87, CTX-M-90, CTX-M-93, CTX-M-96, CTX-M-98, CTX-M-99, CTX-M-101, CTX-M-102, CTX-M-104, CTX-M-105, CTX-M-106, CTX-M-110, CTX-M-111, CTX-M-112, CTX-M-113, CTX-M-114, CTX-M-116, CTX-M-121, CTX-M-122, CTX-M-123, CTX-M-125, CTX-M-126, CTX-M-129, CTX-M-130, CTX-M-132, CTX-M-134, CTX-M-137, CTX-M-143, CTX-M-148, CTX-M-159, CTX-M-161, CTX-M-164, CTX-M-166, CTX-M-168, CTX-M-170, CTX-M-173, CTX-M-174, CTX-M-175, CTX-M-176, CTX-M-177, CTX-M-179, CTX-M-180, CTX-M-181, CTX-M-182, CTX-M-183, CTX-M-184, CTX-M-186, CTX-M-188, CTX-M-189, CTX-M-190, CTX-M-191

The efficiency of detection on different variants of Extended Spectrum Beta-Lactamase genes was also verified for a set of 14 well characterized ESBL isolates. The samples were prepared by spiking the test isolates into negative rectal matrix at concentrations close to the LoD. Three to five isolates of each CTX-M-1, CTX-M-9, CTX-M-14, CTX-M-15 gene types were tested.

The final results are reported in the following table.

Table 14 Efficiency of detection (inclusivity) of the product ESBL ELITe MGB Kit

Organism	Isolate	Gene	Concentration(CFU/mL)	Result
E. coli	DICON-091	CTX-M-1	165	Inclusive
E. coli	DICON-211	CTX-M-1	165	Inclusive
K. pneumoniae	DICON-126	CTX-M-1	165	Inclusive
K. pneumoniae	DICON-001	CTX-M-1	165	Inclusive
E. coli	DICON-003	CTX-M-1	165	Inclusive
E. coli	DICON-055	CTX-M-9	87	Inclusive

Table 14 Efficiency of detection (inclusivity) of the product ESBL ELITe MGB Kit (continued)

Organism	Isolate	Gene	Concentration(CFU/mL)	Result
E. coli	DICON-098	CTX-M-9	87	Inclusive
E. coli	DICON-085	CTX-M-9	87	Inclusive
E. coli	DICON-045	CTX-M-14	819	Inclusive
K. pneumoniae	DICON-060	CTX-M-14	819	Inclusive
E. coli	DICON-054	CTX-M-14	819	Inclusive
E. coli	NCTC13400	CTX-M-15	108	Inclusive
E. coli	NCTC13451	CTX-M-15	108	Inclusive
E. coli	NCTC13450	CTX-M-15	108	Inclusive

All tested ESBL isolates were detected and found to be inclusive by the ESBL ELITe MGB® Kit at concentrations of about 87 - 819 CFU / mL.

The efficiency of detection on different variants of Extended Spectrum Beta-Lactamase genes was also verified for a set of 24 characterized ESBL cultural isolates. Each sample was diluted in eNAT™ kit and then tested with ESBL ELITe MGB® Kit and ELITe InGenius system in Extraction + PCR mode. The cultural isolates were representative of the different genera of *Enterobacteriaceae* (e.g. *K. pneumoniae, E. coli, E. cloacae*).

The results are summarized in the following table.

Table 15

Samples	N	positive	negative	invalid
CTX-M-1 positive cultural isolates	1	1	0	0
CTX-M-3 positive cultural isolates	2	2	0	0
CTX-M-14 positive cultural isolates	3	3	0	0
CTX-M-15 positive cultural isolates	18	18	0	0

All tested ESBL isolates were detected and found to be inclusive by the ESBL ELITe MGB Kit.

11.3 Potential interfering markers

Potential cross-reactivity of the assay with other unintended targets was first evaluated by *in silico* analysis of the sequences available in the NCBI nucleotide database.

An alignment of the primer and probe sequences with the sequences available in the database including the organisms that might reasonably be expected to be present in clinical samples, such as common flora of rectal opportunistic organisms, viruses, cells, intestinal parasites, and closely related, beta-lactamase-producing organisms, showed absence of significant homologies and indicated no potential interference.

The absence of cross-reactivity with other organisms potentially found in rectal swabs was also verified by testing samples of the isolates indicated in table below at the concentration of 10^6 CFU / mL in triplicates.

Table 16 Potential interfering markers of the product ESBL ELITe MGB Kit

Organism	Isolate	Concentration (CFU/mL)	Result
K. pneumoniae	ATCC 700603	10^6	No cross-reactivity
E. coli	ATCC BAA-201	10^6	No cross-reactivity

Table 16 Potential interfering markers of the product ESBL ELITe MGB Kit (continued)

Organism	Isolate	Concentration (CFU/mL)	Result
S. marcescens	UCLA 14-13-A11	10^6	No cross-reactivity
A. baumannii	NCTC 13301	10^6	No cross-reactivity
A. lwoffii	ATCC 15309	10^6	No cross-reactivity
B. adolescentis	ATCC 15703	10^6	No cross-reactivity
B. longum	ATCC 15707	10^6	No cross-reactivity
C. jejuni	ATCC 33292	10^6	No cross-reactivity
C. albicans	Zeptometrix Z006	10^6	No cross-reactivity
C. freundii	ATCC 8090	10^6	No cross-reactivity
C. difficile	ATCC 43593	10^6	No cross-reactivity
C. perfringens	ATCC 13124	10^6	No cross-reactivity
P. mirabilis	ATCC 12453	10^6	No cross-reactivity
P. aeruginosa	ATCC 27853	10^6	No cross-reactivity
S. enterica	ATCC 700720	10^6	No cross-reactivity

All isolates were found to be negative in 3 out of 3 replicates when tested with the ESBL ELITe MGB Kit.

11.4 Interfering substances

Potentially interfering substances at their highest clinically relevant concentrations were individually spiked into negative rectal matrix containing ESBL isolates at concentration level of about 3x LoD. The substances tested were: enemas (vaseline oil), spermicidal lubricant (Nonoxynol-9), anti-diarrheal medication (Loperamide Hydrochloride, Bismuth Subsalicylate), laxatives (Sennosides), antibiotics (Vancomycin), antiacids (alginic acid / aluminum hydroxide / magnesium trisilicate, Calcium Carbonate), fecal components (Palmitic acid, Stearic acid, Mucin, Human Whole Blood). One isolate of each CTX-M-1, CTX-M-9 and CTX-M-15, gene types was tested in triplicate with the ESBL ELITe MGB Kit and ELITe InGenius.

None of the tested substances at their highest clinically relevant concentrations were found to interfere with the ESBL ELITe MGB Kit.

The possible interference during amplification reaction of 2-propanol, used in extraction process, was evaluated by testing DNA extracted from negative rectal matrix containing the ESBL isolates at concentration level of about 3x LoD. One isolate of each CTX-M-1, CTX-M-9, CTX-M-14 and CTX-M-15 gene type were tested in triplicate with the ESBL ELITe MGB Kit and ELITe InGenius.

The test showed that until 10% 2-propanol concentration the ESBL ELITe MGB Kit does not call any false negative result.

11.5 Absence of cross-contamination

The absence of cross-contamination from positive to negative samples or carry-over from one run into another was verified by performing 3 integrated runs (DNA extraction from primary tube followed by PCR) with 6 high CTX-M-1-positive samples at 10^6 CFU / mL in negative rectal matrix in eNAT medium alternated with 6 samples of negative rectal matrix in eNAT medium.

All of the tested negative rectal matrix samples resulted negative with the ESBL ELITe MGB Kit.

11.6 Whole system failure

The whole system failure rate leading to false negative results was verified analysing 50 CTX-M-9 spiked samples prepared from isolates in negative rectal matrix and resulted equal to 0%.

The 50 samples of negative rectal matrix were spiked with one CTX-M-9 isolate at a final concentration of about 3x LoD (87 CFU / mL). Each sample of the panel was tested carrying out the whole analysis procedure starting form primary tube with the ELITe InGenius system.

All of the tested samples resulted positive with the ESBL ELITe MGB Kit.

11.7 Repeatability

The Repeatability of the assay was tested on ELITe InGenius by analysis of a panel of rectal swab samples, including one negative sample and three positive samples spiked with ESBL isolates (CTX-M-1, CTX-M-9 and CTX-M-15 ESBL strains) at 3x LoD.

A summary of Repeatability results on ELITe InGenius is shown below.

Table 17 Intra - Session Repeatability on ELITe InGenius

Target	Sessions	Ct Mean	SD	% CV
CTX-M-1	Day 1	33.99	0.60	1.77
	Day 2	34.40	0.56	1.62
CTX-M-9	Day 1	34.95	0.85	2.43
	Day 2	35.34	0.43	1.21
CTX-M-15	Day 1	32.46	0.46	1.43
	Day 2	32.58	0.18	0.56
IC	Day 1	27.29	0.43	1.56
	Day 2	27.22	0.22	0.80

Table 18 Inter - Session Repeatability on ELITe InGenius

Target	Sessions	Ct Mean	SD	% CV
CTX-M-1	Days 1 + 2	34.19	0.60	1.76
CTX-M-9	Days 1 + 2	35.14	0.68	1.94
CTX-M-15	Days 1 + 2	32.52	0.35	1.07
IC	Days 1 + 2	27.25	0.33	1.21

The Repeatability of the assay was verified on ELITe BeGenius instruments by analysis of a panel of rectal swab samples, including one negative sample and four positive samples spiked with reference materials of each strain at 3xLoD.

An example of Repeatability results on ELITe BeGenius is shown in the table below.

Table 19 Intra - Session Repeatability on ELITe BeGenius

Sample	N	CTX-M1-9-14-15			% Agreement
		Ct Mean	SD	% CV	
3x LoD CTX-M-1	6	33.72	0.47	1.39	100%
3x LoD CTX-M-9	6	32.38	0.31	0.97	100%
3x LoD CTX-M-14	6	34.43	0.34	0.98	100%
3x LoD CTX-M-15	6	33.16	0.31	0.94	100%
Negative	6	-	-	-	100%

Table 20 Inter - Session Repeatability on ELITe BeGenius

Sample	N	CTX-M1-9-14-15			% Agreement
		Ct Mean	SD	% CV	
3x LoD CTX-M-1	12	33.43	0.49	1.46	100%
3x LoD CTX-M-9	12	32.20	0.44	1.36	100%
3x LoD CTX-M-14	12	34.45	0.36	1.04	100%
3x LoD CTX-M-15	12	33.20	0.26	0.79	100%
Negative	12	-	-	-	100%

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

11.8 Reproducibility

The Reproducibility of the assay was tested on **ELITe InGenius** by analysis of a panel of rectal swab samples, including one negative sample and three positive samples spiked with ESBL isolates (CTX-M-1, CTX-M-9 and CTX-M-15 ESBL strains) at 3x LoD.

A summary of Reproducibility results on ELITe InGenius is shown below

Table 21 Inter-Batch Reproducibility on ELITe InGenius

Target	Ct Mean	SD	% CV
CTX-M-1	33.99	0.58	1.70
CTX-M-9	35.30	0.60	1.70
CTX-M-15	33.30	0.33	1.00
IC	27.58	0.78	2.82

Table 22 Inter-Instrument Reproducibility on ELITe InGenius

Target	Ct Mean	SD	% CV
CTX-M-1	33.86	0.49	1.46
CTX-M-9	35.26	0.69	1.96
CTX-M-15	33.32	0.46	1.37
IC	27.97	0.75	2.67

The Reproducibility of the assay was verified on **ELITe BeGenius** instruments by analysis of a panel of rectal swab samples, including one negative sample and four positive samples spiked with ESBL reference material of each strain at 3xLoD.

An example of Reproducibility on ELITe BeGenius is shown in the table below.

Table 23 Inter-Batch Reproducibility on ELITe BeGenius

Sample	N	CTX-M1-9-14-15			% Agreement
		Mean Ct	SD	% CV	
3x LoD CTX-M-1	12	33.15	0.32	0.97	100%
3x LoD CTX-M-9	12	32.09	0.37	1.14	100%
3x LoD CTX-M-14	12	34.61	0.41	1.19	100%
3x LoD CTX-M-15	12	32.86	0.44	1.35	100%
Negative	12	-	-	-	100%

Table 24 Inter-Instrument Reproducibility on ELITe BeGenius

Sample	N	CTX-M1-9-14-15			% Agreement
		Mean Ct	SD	% CV	
3x LoD CTX-M-1	12	33.44	0.42	1.26	100%
3x LoD CTX-M-9	12	32.18	0.39	1.23	100%
3x LoD CTX-M-14	12	34.59	0.50	1.46	100%
3x LoD CTX-M-15	12	32.70	0.45	1.39	100%
Negative	12	-	-	-	100%

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

11.9 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 analyzing rectal swabs and blood culture samples negative for ESBL.

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 summarized in the following table.

Table 25

Samples	N	positive	negative	invalid	% Diagnostic Specificity
ESBL negative Rectal Swab	54	0	54	0	100%
ESBL negative Blood Culture	37	2	35	0	95%

The IC Ct cut-off value is set at 31 for rectal swabs and blood culture samples when tested with ELITe InGenius and ELITe BeGenius.

11.10 Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with **ELITe InGenius** by analyzing rectal swab samples, positive for ESBL or spiked with ESBL isolates, and blood culture samples positive for ESBL.

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 summarized in the following table.

Table 26

Samples	N	positive	negative	invalid	% Diagnostic Sensitivity
CTX-M-1 or CTX-M-15 positive Rectal Swab	38	38	0	0	99.3%
CTX-M-9 or CTX-M-14 positive Rectal Swab	9	9	0	0	
CTX-M-1 or M-15 and CTX-M-9 or M-14 positive Rectal Swab	4	3	1	0	
CTX-M-1-spiked Rectal Swab (isolate DICON-091)	24	24	0	0	
CTX-M-9-spiked Rectal Swab (isolate DICON-055)	24	24	0	0	
CTX-M-14-spiked Rectal Swab (isolate DICON-045)	24	24	0	0	
CTX-M-15-spiked Rectal Swab (isolate NCTC13400)	24	23	0	1	
Positive blood culture	51	51	0	0	100%

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 "ESBL ELITe MGB Kit", FTP RTS201ING

12 REFERENCES

Cantón R. et al., Front Microbiol. 2012 Apr 2;3:110

13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: rectal swabs and emocultures.

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

Do not use samples with too much fecal matrix with this product: samples with high turbidity inhibit the nucleic acid amplification reaction and can cause invalid results.

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

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

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

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

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

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

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 means that the target DNA is not detected in the DNA extracted from the sample, but it cannot be excluded that the target DNA has a lower titre than the product detection limit (see Performance Characteristics, page 15). In this case the result could be a false negative.

A negative result following a previously positive result may or may not indicate eradication success.

Results obtained with this product may sometimes be “Invalid” due to failed internal control and require retesting that can lead to a delay in obtaining final results.

Though rare, polymorphisms within the region of the bacterial genome covered by the product primers and probes may impair detection.

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 27

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 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 28

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 29

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 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 29 (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 30

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 31

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, 7723038, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 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 ESBL 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 **ESBL ELITe MGB® Kit** product is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of the DNA of Extended Spectrum Beta-Lactamase **CTX-M-1**, **CTX-M-9**, **CTX-M-14** and **CTX-M-15*** genes of *Enterobacteriaceae* in DNA samples extracted from rectal swabs and blood colture.

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 rectal swabs and blood culture.

The product is intended for use in the diagnosis and screening of infections of Enterobacteria positive for Extended Spectrum Beta-Lactamase genes, together with the patient's clinical data and other laboratory test results.

The product is also compatible for the characterization of Enterobacteriaceae positive for Extended Spectrum Beta-Lactamase genes in DNA samples extracted from cultural isolates.

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

*For the complete list of gene variants detected by this product, please, refer to the "Performance Characteristics" chapter.

Amplified sequence

Sequence	Gene	Fluorophore	Channel
CTX-M-1 and CTX-M-15	Extended Spectrum Beta-Lactamase CTX-M-1 and CTX-M-15	AP593	CTX-M1-9-14-15
CTX-M-9 and CTX-M-14	Extended Spectrum Beta-Lactamase CTX-M-9 and CTX-M-14	AP593	CTX-M1-9-14-15
Internal Control	artificial sequence IC2	AP525	IC

Validated matrices

› Rectal swab collected in eNAT™ kit
› Rectal swab collected in FecalSwab™ kit
› Blood Culture

Kit content and related products

ESBL ELITe MGB Kit (RTS201ING)		ESBL- ELITe Positive Control (CTR201ING)	
 X 8		 X 3	
ESBL PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube		ESBL Positive Control 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles	
Maximum shelf-life:	**24 months**	Maximum shelf-life	**24 months**
Storage temperature	**≤ -20°C**	Storage temperature	**≤ -20°C**

Other products required not provided in the kit

› ELITe InGenius instrument: INT030. › ELITe BeGenius instrument: INT040. › ELITe InGenius SP 200: INT032SP200. › ELITe InGenius SP200 Consumable Set: INT032CS. › ELITe InGenius PCR Cassette: INT035PCR. › ELITe InGenius Waste Box: F2102-000. › 300 µL Filter Tips Axigen: TF-350-L-R-S. › 1000 µL Filter Tips Tecan: 30180118.	› CPE - Internal Control: CTRCPE › eNAT™ kit (Copan, ref.606CS01R), › FecalSwab™ (COPAN,, ref. 470CE)

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	Sensitivity	Specificity
Rectal swab	CTX-M-1	55	99.3%	100%
	CTX-M-9	29		
	CTX-M-14	273		
	CTX-M-15	36		
Blood Culture	CTX-M-1	55	100%	95%
	CTX-M-9	29		
	CTX-M-14	273		
	CTX-M-15	36		

Sample preparation

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

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room tempera-ture)	**+2 / +8 °C**	**- 20 ± 10 °C**	**- 70 ± 15 °C**
blood culture	-	≤ 24 hours	-	-	-
rectal swab	collected in **eNAT™ kit**		≤ 1 month	≤ 6 months	>6 months
rectal swab	collected in **FecalSwab™ kit***		≤ 3 days	-	-

Before the analysis dilute the blood culture sample 1:1000 in ultrapure water (at least 10 µL of samples into 10 mL of ultrapure water), mix by vortexing and transfer 0.2 mL of the diluted samples into an Extraction tube (for ELITe InGenius instrument) or into a 2 mL Sarstedt tube (for ELITe BeGenius instrument).

Before the analysis of rectal swab 0.5 mL of sample in FecalSwab™ medium has to be transferred in a fresh eNAT™ tube with 2.0 mL of medium, mixed by vortexing. The samples diluted in eNAT™ medium can be stored at as reported in table above. After addition of 0.5 mL of sample in FecalSwab™ medium, the eNAT™ tube can be directly loaded in the system as a primary tube.

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** 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: ESBL ELITe_RcS_200_100 or ESBL ELITe_BC_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 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: ESBL ELITe_RcS_200_100 or ESBL ELITe_BC_200_100 or ESBL ELITe_PC or ESBL 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, 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** 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: ESBL ELITe_Be_RcS_200_100 or ESBL ELITe_Be_BC_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 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: ESBL ELITe_Be_RcS_200_100 or ESBL ELITe_Be_BC_200_100 or ESBL ELITe_Be_PC or ESBL 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	

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