Instructions for use

CRE ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS200ING

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CE IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
05	Extension of the use of the product in association with «ELITe BeGenius®» instrument (REF INT040). Update of the paragraph “Other product required”. Update of the paragraph “Notice to the purchaser: limited licence” Update of the paragraph “Symbols” with the symbol “Consult instructions for use” New graphics and content setting of the IFU.	24/11/25
04	Change of the probe used for OXA-48-like genes detection	14/11/18
03	Formal corrections in “Samples and Controls” section.	30/07/18
02	Extended Use of the product with Blood Culture matrix, in association with ELITe InGenius instrument.	22/12/17
00-01	New product development and succeeding changes	-

NOTE

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

TABLE OF CONTENT

1 INTENDED USE

The product **CRE ELITe MGB® Kit** 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 carbapenem-resistance genes* (KPC, NDM, VIM, IMP, OXA-48-like) of *Enterobacteriaceae.*

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 as an aid in the diagnosis and screening of infections of *Enterobacteriaceae* positive for carbapenem-resistance genes, in association with the patient's clinical data and other laboratory test results.

The product is also compatible for the characterization of Enterobacteriaceae positive for carbapenem-resistance genes in DNA samples extracted from cultural isolate.

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 11 PERFORMANCE CHARACTERISTICS page 17

2 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting the DNA of carbapenem-resistance genes **KPC, NDM, VIM, IMP, OXA-48-like** of *Enterobacteriaceae* isolated from specimens and amplified using the assay reagent CRE 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.

NOTE
The fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

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

- **KPC** gene family, detected in Channel **KPC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye,

- **NDM** gene family detected in Channel **NDM VIM IMP**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 593 (AP593) dye,

- **VIM** gene family detected in Channel **NDM VIM IMP**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 593 (AP593) dye,

- **IMP** gene family detected in Channel **NDM VIM IMP**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 593 (AP593) dye,

- **OXA-48-like** gene family detected in Channel **OXA**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 639 (AP639) 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 **CRE PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, the stabilizers and the enzyme Taq DNA polymerase with thermic activation (hot start).

NOTE
The three genes of Metal Beta-Lactamase family, NDM, VIM and IMP, are detected by different probes with the same fluorescent dye and then detected by the same NDM VIM IMP Channel and cannot be distinguished.

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

The **CRE 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
CRE PCR Mix ref. RTS200ING	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).
- 50 mL tube with screwcap (Sarstedt, Germany, ref. 62.547.254).
- 15 mL tube with screwcap (Sarstedt, Germany, ref. 62.554.502).
- 2.0 mL skirted tube with screwcap (Sarstedt, 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, 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) **CRE ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **CRE ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **CRE ELITe_RcS_200_100**, Assay Protocol with parameters for nasal swabs specimen analysis **CRE ELITe_BC_200_100**, Assay Protocol with parameters for blood culture specimen analysis	**CRE — ELITe Positive Control** (EG SpA, ref. CTR200ING) **ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instructions for use) **CPE – Internal Control** (EG SpA, ref. CTRCPE) **eNAT™ kit** (Copan, ref. 608CS01R), **FecalSwab™** (COPAN Italia S.p.A., ref. 470CE),
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.3.0. (or later) **CRE ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **CRE ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **CRE ELITe_Be_RcS_200_100**, Assay Protocol with parameters for nasal swabs specimen analysis **CRE 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 to prevent dispersion into the environment and to avoid contamination of the instrument's working area.

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

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
CRE PCR Mix	-20°C or below (protected from light)	one month	up to four	up to four separate* 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 with **eNAT™ kit**	—	≤ 1 month	≤ 6 months	Long periods
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 collected in.FecalSwab™, 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 Coltural 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 Coltural isolates is carried out with the ELITe InGenius use the extraction protocol **CRE 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 Kits and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrices.

Table 5 Assay Protocols for CRE ELITe MGB Kit

Assay Protocols for CRE ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
rectal swab	**ELITe InGenius**	**CRE 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	**CRE ELITe_Be_RcS_200_100**	Positive / Negative	
blood culture	**ELITe InGenius**	**CRE 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	**CRE ELITe_Be_BC_200_100**	Positive / Negative	

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

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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 4 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 **CRE 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 (channels **KPC, NDM, VIM, IMP, OXA**) and the Internal Control (channel **IC**) with the **CRE ELITe_RcS_200_100** and **CRE 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**
CRE Positive Control	APPROVED
2) Negative Control	**Status**
CRE- 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
KPC:DNA detected	**KPC gene DNA was detected** in the sample.
NDM VIM IMP:DNA detected	**NDM, VIM or IMP gene DNA was detected** in the sample.
OXA:DNA detected	**OXA gene DNA was detected** in the sample.
KPC:DNA Not detected or below the LoD	**KPC gene DNA was not detected** in the sample. The sample is negative valid for this gene or its concentration is below the Limit of Detection of the assay.
NDM VIM IMP:DNA Not detected or below the LoD	**NDM, VIM and IMP gene DNA were not detected** in the sample. The sample is negative valid for these genes or their concentration is below the Limit of Detection of the assay.
OXA:DNA Not detected or below the LoD	**OXA gene DNA was not detected** in the sample. The sample is negative valid for this gene or its 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 27”).

Samples reported as: "xxx:DNA Not detected or below the LoD" are suitable for analysis but it was not possible to detect the target DNA. In this case it cannot be excluded that 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”.

9.6 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 **CRE 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 **CRE 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	Not applicable	Not applicable
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 4 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 **CRE 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

As ELITe BeGenius has been demonstrated to have equivalent analytical performances to ELITe InGenius, the results reported in the following sections are also considered applicable to ELITe BeGenius.

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 6 CRE strains, one of each of the following gene types: KPC, IMP, VIM, NDM, OXA-48 and OXA-181, in association to rectal swab samples.

The LoD for each of the CRE 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

Target	Bacterial Isolate	LoD (CFU / mL)	95% confidence interval (CFU / mL)	
			Lower bound	Upper bound
KPC	*C. freundii*, UCLA 14-13-A2	99	69	217
NDM	*E. coli*, ATCC BAA-2469	144	110	228
VIM	*K. pneumoniae*, NCTC 13439	399	338	579
IMP	*E. coli*, NCTC 13476	273	234	351
OXA-48	*E. coli*, ATCC BAA-2523	300	241	456
OXA-181	*K. pneumoniae*, JMI 18	179	139	287

The calculated LoD was verified on rectal swab samples on the ELITe InGenius instrument by testing rectal swab samples spiked with reference material of each target at the claimed concentration.

The results obtained confirmed the claimed concentration for all the targets of CRE ELITe MGB Kit with rectal swab samples on ELITe InGenius.

11.2 Efficiency of detection (inclusivity)

The efficiency of detection on different variants of carbapenem-resistance genes was evaluated by *in silico* comparison of the assay probe and primer sequences with the sequences available in the NCBI nucleotide database.

The analysis showed high sequence conservation and absence of significant mutations for the variants reported in the following table.

Table 13

target	Variants expected to be detected by the product CRE ELITe MGB® Kit
KPC	KPC-01, KPC-02, KPC-03, KPC-04, KPC-05, KPC-06, KPC-07, KPC-08, KPC-09, KPC-10, KPC-11, KPC-12, KPC-13, KPC-14, KPC-15, KPC-16, KPC-17, KPC-18, KPC-19, KPC-21, KPC-22, KPC-25, KPC-33, KPC-47e, KPC-56a, KPC-63d, KPC-272, KPC-484, KPC-629, KPC-727, KPC-860.
NDM	NDM-01, NDM-02, NDM-03, NDM-04, NDM-05, NDM-06, NDM-07, NDM-08, NDM-09, NDM-10, NDM-12, NDM-13, NDM-15, NDM-16, NDM-17, NDM-32, NDM-40, NDM-221, NDM-255, NDM-264, NDM-265
VIM	VIM-01, VIM-02, VIM-03, VIM-04, VIM-05, VIM-06, VIM-07, VIM-08, VIM-09, VIM-10, VIM-11, VIM-12, VIM-13, VIM-14, VIM-15, VIM-16, VIM-17, VIM-18, VIM-19, VIM-20, VIM-23, VIM-24, VIM-25, VIM-26, VIM-27, VIM-28, VIM-31, VIM-33, VIM-34, VIM-35, VIM-36, VIM-37, VIM-38, VIM-39, VIM-40, VIM-42, VIM-43, VIM-44, VIM-45, VIM-46, VIM-47, VIM-49, VIM-50, VIM-51
IMP	IMP-01, IMP-02, IMP-03, IMP-04, IMP-05, IMP-06, IMP-07, IMP-08, IMP-09, IMP-10, IMP-11, IMP-13, IMP-14, IMP-15, IMP-16, IMP-18, IMP-19, IMP-20, IMP-21, IMP-22, IMP-24, IMP-25, IMP-26, IMP-28, IMP-29, IMP-32, IMP-33, IMP-34, IMP-37, IMP-38, IMP-40, IMP-41, IMP-42, IMP-45, IMP-48, IMP-49, IMP-51, IMP-54, IMP-56, IMP-58
OXA	OXA-48, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245, OXA-370, OXA-405, OXA-416, OXA-439, OXA-484

The efficiency of detection on different variants of carbapenem-resistance genes was also verified for a set of 18 well characterized CRE isolates. Contrived samples were prepared by spiking the test isolates into negative rectal matrix at concentrations close to LoD. Two to three isolates of each KPC, NDM, VIM, IMP, OXA-48-like gene types were tested.

The final results are reported in the following table.

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

Organism	Isolate	CRE Marker	Concentration(CFU/ mL)	Result
K. pneumoniae	CDC-ARIB-0034	IMP	798	Inclusive
P. aeruginosa	CDC-ARIB-0103	IMP-1	762	Inclusive
P. aeruginosa	CDC-ARIB-0092	IMP-14	762	Inclusive
K. pneumoniae	NCTC 13438	KPC	297	Inclusive
K. pneumoniae	BAA-1898	KPC-2	297	Inclusive
K. pneumoniae	BAA-1904	KPC-3	347	Inclusive
K. pneumoniae	BAA-2146	NDM1	294	Inclusive
E. coli	CDC-ARIB-0150	NDM-5	294	Inclusive
E. coli	CDC-ARIB-0137	NDM-6	294	Inclusive
K. pneumoniae	ST-14 (alias R20)	OXA-181	537	Inclusive
K. pneumoniae	CDC-ARIB-0140	OXA-181	537	Inclusive
K. pneumoniae	CDC-ARIB-0066	OXA-232	900	Inclusive
K. pneumoniae	CDC-ARIB-0075	OXA-232	900	Inclusive
K. pneumoniae	BAA-2524	OXA-48	900	Inclusive
K. pneumoniae	CDC-ARIB-0160	OXA-48	900	Inclusive
K. pneumoniae	NCTC 13440	VIM-1	843	Inclusive
P. aeruginosa	NCTC 13437	VIM-10	843	Inclusive
P. aeruginosa	CDC-ARIB-0054	VIM-4	843	Inclusive

All tested CRE isolates were detected and found to be inclusive by the CRE ELITe MGB Kit at concentrations of about 300 - 900 CFU / mL.

The efficiency of detection on different variants of carbapenem-resistance genes was also verified for a set of 114 characterized CRE cultural isolates. Each sample was diluted in eNAT™ kit and then tested with CRE ELITe MGB Kit and ELITe InGenius in Extraction + PCR mode. The cultural isolates were representative of the different genera of Enterobacteriaceae (e.g. *K. pneumoniae, E. coli, E. cloacae, C. koseri*).

The results are summarized in the following table.

Table 15 Efficiency of detection (inclusivity) of the product CRE ELITe MGB Kit

Samples	N	Positive	Negative	Invalid
KPC-positive cultural isolates	22	22	0	0
OXA-48 like positive cultural isolates	35	35	0	0
NDM-positive cultural isolates	23	23	0	0
IMP-positive cultural isolates	11	11	0	0

Table 15 Efficiency of detection (inclusivity) of the product CRE ELITe MGB Kit (continued)

Samples	N	Positive	Negative	Invalid
VIM- positive cultural isolates 17 17 0 0	17	17	0	0
OXA-48 and NDM positive cultural isolates	6	6	0	0

All tested CRE isolates were detected and found to be inclusive by the CRE 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 closely related organisms (related resistance) 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 CRE ELITe MGB Kit

Organism	Isolate	Antibiotic Resistance Marker	Concentration (CFU/ mL)	Result
K. pneumoniae	700603	SHV	10^6	No cross-reactivity
E. coli	BAA-202	SHV	10^6	No cross-reactivity
E. coli	BAA-201	TEM	10^6	No cross-reactivity
S. marcescens	14-13-A11	SME	10^6	No cross-reactivity
S. marcescens	14-13-A12	SME	10^6	No cross-reactivity
A. baumannii	13301	OXA-23	10^6	No cross-reactivity
A. baumannii	13302	OXA-25	10^6	No cross-reactivity
A. baumannii	13303	OXA-26	10^6	No cross-reactivity
A. baumannii	13304	OXA-27	10^6	No cross-reactivity
A. baumannii	13305	OXA-58	10^6	No cross-reactivity
A. baumannii	13420	OXA-51-like SE clone	10^6	No cross-reactivity
K. pneumoniae	DICON-185	CTX-M-1	10^6	No cross-reactivity
E. coli	DICON-003	CTX-M-1	10^6	No cross-reactivity
E. coli	DICON-178	CTX-M-9	10^6	No cross-reactivity
K. pneumoniae	DICON-005	CTX-M-9	10^6	No cross-reactivity
E. cloacae	NCTC 13464	CTX-M-9	10^6	No cross-reactivity
E. coli	13353	CTX-M-15	10^6	No cross-reactivity
K. pneumoniae	CDC-ARIB-0044	CTX-M-15	10^6	No cross-reactivity

All but *K. pneumoniae* DICON185 isolates were found to be negative in 3 out of 3 replicates when tested with the CRE ELITe MGB Kit. The *K. pneumoniae* DICON185 isolate had positive signal in one out of 3 replicates. When re-tested with 5 replicates the isolate gave 5 out of 5 negatives and it was considered not cross-reacting.

11.4 Interfering substances

Potentially interfering substances at their highest clinically relevant concentrations were individually spiked into negative rectal matrix containing CRE isolates at concentration level of about 3x LoD. The substances tested were: enemas (vaseline oil), spermicidal lubricant (Nonoxynol-9), anti-diarrheal medication (Bismuth Subsalicylate), laxatives (Sennosides), antibiotics (Vancomycin), antiacids (alginic acid / aluminum hydroxide / magnesium trisilicate, Calcium Carbonate, Cimetidine, Omeprazole), fecal components (Palmitic acid, Stearic acid, Mucin, Human Genomic DNA, Human Leukocytes, Human Whole Blood). One isolate of each KPC, NDM, VIM, IMP, OXA-48 and OXA-181 gene types was tested in triplicate with the CRE ELITe MGB Kit and ELITe InGeniussystem.

None of the tested substances were found to interfere with the CRE ELITe MGB® Kit.

The possible interference during amplification reaction of 2-propanol, used in extraction process, was evaluated testing DNA extracted from negative rectal matrix containing the CRE isolates at concentration level of about 400 CFU / mL. One isolate of each KPC, NDM, VIM, IMP, OXA-48 and OXA-181 gene type were tested in triplicate with the CRE ELITe MGB Kit and ELITe InGenius system.

The test showed that until 10% 2-propanol concentration the CRE ELITe MGB Kit product do 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 KPC-positive samples at 10^6 CFU / mL in eNAT medium alternated with 6 negative samples of eNAT medium.

No inter-track cross-contamination or inter-run carry-over was found after testing 18 positive samples and 18 negative samples in a checkerboard configuration with ELITe InGenius™ system

11.6 Whole system failure

The whole system failure rate leading to false negative results was verified analysing 50 IMP 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 IMP isolate at a final concentration of about 400 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 CRE 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 a negative sample and three positive samples (KPC + NDM + OXA, VIM and IMP CRE strains) at low and moderate concentration.

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

Table 17 Repeatability on ELITe InGenius

Target	Sample type	Ct Mean	SD	%CV
KPC	Low Positive	34.82	0.48	1.4%
	Moderate Positive	33.84	0.49	1.4%
NDM	Low Positive	36.87	0.51	1.4%
	Moderate Positive	35.21	0.24	0.7%

Table 17 Repeatability on ELITe InGenius (continued)

Target	Sample type	Ct Mean	SD	%CV
OXA	Low Positive	34.80	0.32	0.9%
	Moderate Positive	33.17	0.32	1.0%
VIM	Low Positive	35.26	0.19	0.5%
	Moderate Positive	33.47	0.20	0.6%
IMP	Low Positive	35.72	0.73	2.0%
	Moderate Positive	33.90	0.43	1.3%
IC	n.a.	29.56	0.43	1.5%
	n.a.	29.81	0.61	2.0%

The Repeatability of the assay was verified on **ELITe BeGenius** 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 low concentration.

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

Table 18 Intra-session Repeatability on ELITe BeGenius

Sample	N	NDM VIM IMP			KPC			OXA			% Agreement
		Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	
Negative	6	-	-	-	-	-	-	-	-	-	100%
NDM + OXA-48	6	33.85	0.15	0.44	-	-	-	33.33	0.45	1.35	100%
VIM + KPC	6	30.91	0.21	0.67	30.02	0.31	1.03	-	-	-	100%
IMP + OXA-181	6	32.38	0.23	0.72	-	-	-	34.45	0.24	0.70	100%

Table 19 Inter-session Repeatability on ELITe BeGenius

Sample	N	NDM VIM IMP			KPC			OXA			% Agreement
		Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	
Negative	12	-	-	-	-	-	-	-	-	-	100%
NDM + OXA-48	12	34.01	0.31	0.92	-	-	-	33.33	0.38	1.13	100%
VIM + KPC	12	31.06	0.48	1.53	30.52	0.60	1.96	-	-	-	100%
IMP + OXA-181	12	32.94	0.68	2.05	-	-	-	34.52	0.44	1.28	100%

In the Repeatability test, the CRE 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 (KPC + NDM + OXA, VIM and IMP CRE strains) at low and moderate concentration.

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

Table 20 Reproducibility on ELITe InGenius

Target	Sample type	Ct Mean	Instrument to instrument		Batch to batch	
			SD	% CV	SD	% CV
KPC	Low Positive	35.00	0.289	0.83%	0.214	0.61%
	Moderate Positive	34.53	0.428	1.24%	0.312	0.90%
NDM	Low Positive	36.44	0.223	0.61%	0.000	0.00%
	Moderate Positive	35.32	0.317	0.90%	0.000	0.00%
OXA	Low Positive	33.65	0.140	0.42%	0.089	0.26%
	Moderate Positive	33.16	0.169	0.51%	0.207	0.62%
VIM	Low Positive	36.02	0.444	1.23%	0.489	1.36%
	Moderate Positive	34.50	0.289	0.84%	0.412	1.19%
IMP	Low Positive	37.18	0.291	0.78%	0.549	1.48%
	Moderate Positive	35.21	0.575	1.63%	0.783	2.22%
IC	n.a.	29.17	0.397	1.36%	0.911	3.12%

The Reproducibility of the assay was verified on **ELITe BeGenius** 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 low concentration.

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

Table 21 Inter-batch Reproducibility on ELITe BeGenius

Sample	N	NDM VIM IMP			KPC			OXA			% Agreement
		Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	
Negative	12	-	-	-	-	-	-	-		-	**100%**
NDM + OXA-48	12	34.10	0.30	0.89	-	-	-	33.34	0.40	1.20	**100%**
VIM + KPC	12	31.14	0.31	1.00	30.10	0.24	0.80	-	-	-	**100%**
IMP + OXA-181	12	32.69	0.43	1.31	-	-	-	34.35	0.25	0.74	**100%**

Table 22 Inter-instrument Reproducibility on ELITe BeGenius

Sample	N	NDM VIM IMP			KPC			OXA			% Agreement
		Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	Mean Ct	SD Ct	%CV	
Negative	9	-	-	-	-	-	-	-		-	**100%**
NDM + OXA-48	12	34.23	0.31	0.91	-	-	-	33.49	0.48	1.43	**100%**
VIM + KPC	12	31.24	0.21	0.68	30.13	0.25	0.83	-	-		**100%**
IMP + OXA-181	12	34.12	1.28	3.74	-	-	-	34.89	1.18	3.37	**100%**

In the Reproducibility test, the CRE 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 by analyzing some CRE-negative rectal swab and blood culture samples.

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 23 Diagnostic specificity

Samples	N	positive	negative	% Diagnostic Specificity
CRE negative Rectal Swab	52	0	52	100%
CRE negative Blood Culture	45	2	43	95.5%

The IC Ct cut-off value is set at 34 for rectal swabs and blood culture samples when tested with ELITe InGenius and ELITe BeGenius

11.10 Diagnostic Sensitivity: confirmation of negative samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing rectal swab and blood culture samples, positive for CRE or spiked with CRE isolates.

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 24 Diagnostic Sensitivity

Samples	N	positive	negative	% Diagnostic Sensitivity
KPC-positive Rectal Swab	25	25	0	100%
VIM-positive Rectal Swab	4	4	0	
OXA-48-positive Rectal Swab	1	1	0	
KPC-spiked Rectal Swab (isolate 207-1 KPC- 3)	12	12	0	
KPC-spiked Rectal Swab (isolate B1 KPC-2)	12	11	1	
NDM-spiked Rectal Swab (isolate NDM-1)	12	12	0	
NDM-spiked Rectal Swab (isolate NDM-5)	12	12	0	
VIM-spiked Rectal Swab (isolate VIM-1)	12	12	0	
VIM-spiked Rectal Swab (isolate VIM-4)	12	12	0	
IMP-spiked Rectal Swab (isolate *K. pneumoniae* AR-Bank 0034)	12	12	0	
IMP-spiked Rectal Swab (isolate E. *coli* NCTC 13476)	12	12	0	
OXA-48-like spiked Rectal Swab (isolate OXA-48)	12	12	0	
OXA-48 spiked Rectal Swab (isolate OXA- 232)	12	12	0	
KPC-positive blood culture	16	16	0	
OXA-48-positive blood culture	5	5	0	
NDM- positive blood culture	4	4	0	
IMP- positive blood culture	2	2	0	
VIM- positive blood culture	2	2	0	
OXA-48 spiked blood culture	5	5	0	
NDM spiked blood culture	5	5	0	
VIM spiked blood culture	5	5	0	
IMP spiked blood culture	5	5	0	

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 Section 7 of the Product Technical File "CRE ELITe MGB® Kit", FTP RTS200ING.

12 REFERENCES

L. S. Tzouvelekis et al. (2012) *Clin. Microbiol. Rev.* 25(4): 682 - 707.

13 PROCEDURE LIMITATIONS

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

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 25

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 4 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 26

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 27

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 4 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 27 (continued)

Invalid Sample reaction	
Possible Causes	**Solutions**
Inhibition due to interfering substances in the sample.	Repeat the amplification “as is” or 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 28

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 29

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

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

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

Appendix A CRE 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 **CRE ELITe MGB® Kit** 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 carbapenem-resistance genes* (KPC, NDM, VIM, IMP, OXA-48-like) of *Enterobacteriaceae.*

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 as an aid in the diagnosis and screening of infections of *Enterobacteriaceae* positive for carbapenem-resistance genes, in association with the patient's clinical data and other laboratory test results.

The product is also compatible for the characterization of Enterobacteriaceae positive for carbapenem-resistance genes in DNA samples extracted from cultural isolate.

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 11 PERFORMANCE CHARACTERISTICS page 17

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	KPC gene family	FAM	KPC
Target 2	NDM, VIM, IMP gene family	AP593	NDM VIM IMP
Target 3	OXA-48-like gene family	AP639	OXA
Internal Control	artificial sequence IC2	AP525	IC

Validated matrix

› Rectal Swab
› Blood Culture

Kit content and related products

CRE ELITe MGB Kit (RTS200ING)		CRE- ELITe Positive Control (CTR200ING)	
 X 8		 X 3	
CRE PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 4 freeze-thaw cycles per tube		CRE 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 and ELITe BeGenius Consumables (see ELITe InGenius and ELITe BeGenius Instruction for use)	› 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	KPC	99 CFU / mL	100%	100%
	NDM	144 CFU / mL		
	VIM	399 CFU / mL		
	IMP	273 CFU / mL		
	OXA-48	300 CFU / mL		
	OXA-181	179 CFU / mL		
Blood Culture	KPC	N.A.	100 %	95.5%
	NDM	N.A.		
	VIM	N.A.		
	IMP	N.A.		
	OXA-48	N.A.		
	OXA-181	N.A.		

N.A. = not applicable

Sample preparation

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

Sample type	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	Long periods
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.

ELITe InGenius Procedures

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

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL"	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: CRE ELITe_RcS_200_100 or CRE 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: CRE ELITe_RcS_200_100 or CREn ELITe_BC_200_100 or CRE ELITe_PC or CRE 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 (GUI) of ELITe BeGenius software to setup the run. All the steps, extraction, Real-Time PCR and result interpretation, are automatically performed. Two operational modes are available: complete run (Extract + PCR), or PCR Only.

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode “**CLOSED**”.	**2.** Verify controls: **Positive Control** and **Negative Control** in the “Controls” menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay Protocol” of interest: CRE ELITe_Be_RcS_200_100 or CRE 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.** For Controls: for each “Position”enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions). For eluates: for each “Position enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).
4. Select the “Assay Protocol” of interest: CRE ELITe_Be_RcS_200_100 or CRE ELITe_Be_BC_200_100 or CRE ELITe_Be_PC or CRE 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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