ELITechGroup
MOLECULAR DIAGNOSTICS

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NOTICE of CHANGE dated 14/11/18

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«CRE ELITe MGB® Kit»
Ref. RTS200ING

This new revision of the Instruction for Use (IFU) contains the following changes:

- *Change of the probe used ofr OXA-48-like genes detection..*

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIE REVIEW VON DIESER IFU IST KOMPATIBLE MIT DER VORIGE VERSION VON DEM TEST-KIT

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CRE ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS200ING CE -20°C

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INTENDED USE

The **«CRE ELITe MGB® Kit»** product is part of a qualitative nucleic acids amplification assay for the detection of the DNA of carbapenem-resistance genes **KPC, NDM, VIM, IMP, OXA-48-like*** of *Enterobacteriaceae* in DNA samples extracted from rectal swabs and blood culture.

The product is intended for use in the diagnosis and screening of infections of *Enterobacteriaceae* positive for carbapenem-resistance genes, together with the patient's clinical data and other laboratory test results.

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

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

ASSAY PRINCIPLES

The assay consists of a multiplex real time amplification reaction performed in association with **ELITe InGenius®**, an automated integrated system for extraction, amplification, detection and results interpretation.

Starting from DNA extracted from each sample under test, five different amplification reactions are performed by the **CRE PCR mix** in the PCR Cassette in order to amplify the following genes which confer resistance to carbapenem antibiotics:

- genes of KPC family, detected by the specific probe KPC (Channel 1),
- genes of NDM family, detected by the specific probe NDM (Channel 4),
- genes of VIM family, detected by the specific probe VIM (Channel 4),
- genes of IMP family, detected by the specific probe IMP (Channel 4),
- genes of OXA-48-like family, detected by the specific probe OXA (Channel 5).

Furthermore, the extraction and inhibition Internal Control is also amplified in the cartridge. The Internal Control is based on an artificial sequence (IC2) detected by the specific probe IC (Channel 2).

The probes with TaqMan™ MGB technology are activated when they hybridize with the specific product of the amplification reaction and they are hydrolyzed by the Taq DNA polymerase enzyme. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. Data processing allows detection of any DNA of the carbapenem-resistance genes in the starting sample.

The assay has been validated with **ELITe InGenius**.

PRODUCT DESCRIPTION

The **«CRE ELITe MGB® Kit»** product supplies the CRE PCR Mix, a **ready to use** complete mixture for Real Time amplification, **aliquoted into eight test tubes**. Each tube contains **280 µL** of solution, sufficient for **12 tests** in optimal reagent consumption conditions (at least 2 tests per session) when used with **ELITe InGenius** system.

The CRE PCR Mix contains the specific primers and probe for:

- the specific primers and probe for the **KPC** gene family. The probe KPC (Channel 1) is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the specific primers and probe for the **NDM** gene family. The probe NDM (Channel 4) is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the specific primers and probe for the **VIM** gene family. The probe VIM (Channel 4) is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the specific primers and probe for the **IMP** gene family. The probe IMP (Channel 4) is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the specific primers and probe for the **OXA-48-like** gene family. The probe OXA (Channel 5) is labelled with AP693 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the specific primers and probe for the **IC2** synthetic sequence of internal control. The probe IC (Channel 2) is labelled with AP525 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,

The CRE PCR Mix contains buffer, magnesium chloride, nucleotide triphosphates, stabilizers and the enzyme 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 product is sufficient for **96 tests in association with ELITe InGenius**, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
CRE PCR Mix	Complete reaction mixture	**8 x 280 µL**	**-**

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of DNA from the samples to be analyzed, the extraction and inhibition internal control, the amplification positive control and the consumables are **not** included in this product.

For automatic DNA extraction, Real Time amplification and result interpretation of samples to be analyzed, the **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) instrument and the following specific Assay protocols (ELITechGroup S.p.A.) are required:

- parameters for the amplification positive control **«CRE ELITe_PC»**,
- parameters for the amplification negative control **«CRE ELITe_NC»**,
- parameters for samples to be analyzed **«CRE ELITe_RcS_200_100»** and **«CRE ELITe_BC_200_100»**.

With the **«ELITe InGenius»** instrument the following generic products are required:

- extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200),
- consumables for extraction and amplification **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS),
- amplification cartridges **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR),
- tips **«300 µL Universal Fit Filter Tips»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S),
- boxes **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000).

As template of extraction and inhibition internal control, the generic product **«CPE - Internal Control»** (ELITechGroup S.p.A., ref. CTRCPE), is required. This is a stabilised solution containing two plasmid DNAs and the genomic RNA of MS2 phage.

As template of amplification positive control, the specifc product **«CRE - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR200ING), is required. This is a stabilised solution of plasmid DNAs.

As collection device for rectal swab samples, the following generic products are recommended:

- eNAT™ kit (COPAN Italia S.p.A., ref. 606CS01R), swab and vial with 2 mL of medium,
- FecalSwab™ (COPAN Italia S.p.A., ref. 470CE), swab and vial with 2 mL of medium.

CRE ELITe MGB® Kit
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WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite 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 able to transmit infective agents. 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 neutralised before disposal.

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 with the product before running the assay.
While running the assay, follow the instructions provided with the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided with the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

Lab 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 PCR Cassettes must be handled in such a way to reduce as much as possible amplification product diffusion into the environment in order to avoid sample and reagent contamination.

Warnings and precautions specific for the components

The **CRE PCR Mix** must be stored at -20 °C in the dark.
The **CRE PCR Mix** can be frozen and thawed for no more than **four times**: further freezing / thawing cycles may cause a loss of product performance.

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Rectal swabs collected in eNAT™ kit

The rectal swabs for DNA extraction is recommended to be collected in eNAT™ kit and identified according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of 4 weeks, otherwise they must be frozen and stored at -20 °C for a maximum of six months or at -70 °C for longer periods. Before the analysis with this product 0.2 mL of sample in eNAT™ medium has to be transferred in the sonication tube provided with «ELITe InGenius® SP 200 Consumable Set».

Rectal swabs collected in FecalSwab™ kit

The rectal swabs for DNA extraction is recommended to be collected in FecalSwab™ and identified according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days. Before the analysis with this product 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 +2 / +8 °C for a maximum of 4 weeks or frozen and stored at -20 °C for a maximum of six months or at -70 °C for longer periods. 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.

Note: when the DNA extraction from rectal swabs is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.2 (or later equivalent versions), use the Assay protocol **CRE ELITe_RcS_200_100**. This protocol processes 200 µL of sample, adds the **CPE**-Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Blood culture

The blood culture samples must be identified according to laboratory guidelines. The samples must be transported and stored at room temperature for a maximum of 24 hours.

Before the analysis with this product dilute the sample 1:1000 in molecular grade 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 in a sonicator tube provided with «ELITe InGenius® SP 200 Consumable Set».

Note: when nucleic acid extraction from blood culture is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.2 (or later equivalent versions), use the extraction protocol **CRE ELITe_BC_200_100**. This protocol processes 200 µL of sample, adds the CPE at 10 µL / extraction and elutes the nucleic acids in 100 µL.

This product is compatible for use with the following clinical samples:

Coltural isolates

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 the sonication tube provided with «ELITe InGenius SP 200 Consumable Set».

Note: when nucleic acid extraction from coltural isolates is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.2 (or later equivalent versions), use the extraction protocols **CRE ELITe_BC_200_100**. This protocol processes 200 µL of sample, adds the CPE at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in paragraph “Interfering substances” of chapter “Performance characteristics”.

Note: High content of faecal matrix collected with the rectal swab (sample with high turbidity) can inhibit the assay.

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Amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the amplification controls for the amplification reagent lot that will be used in testing:

as amplification Positive Control, use the **CRE - ELITe Positive Control** product (not provided with this kit) in association with the Assay Protocol **CRE ELITe_PC**,

as amplification Negative Control, use molecular grade water (not provided with this kit) in association with the Assay Protocol **CRE ELITe_NC**.

Note: The **ELITe InGenius** system requires approved and valid results of amplification controls for each amplification reagent lot stored in its database.
The amplification control results, approved and stored in the database, will expire **after 15 days**. At the expiration date it is necessary to re-run the positive and negative controls in association with the amplification reagent lot in use.
Furthermore the amplification controls must be re-run when:

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

Quality controls

It is recommended to validate the whole analysis procedure, extraction and amplification, by testing as process controls a negative tested sample and a positive tested sample or a reference material.

PROCEDURE

The procedure to use the **CRE ELITe MGB® Kit** with the **ELITe InGenius** system consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and approval of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the **ELITe InGenius** and select the login mode “**CLOSED**”,
- verify that the amplification controls (Controls, CRE Positive Control, CRE Negative Control) are run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification controls approved or valid, run them as described in the following paragraphs,
- 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 ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB® kits, the ELITe InGenius instrument and the cited matrix.

The Assay protocol available for sample testing with the product **CRE ELITe MGB® Kit** is described in the table below.

Assay protocol for CRE ELITe MGB® Kit

Name	Matrix	Report	Characteristics
CRE ELITe_RcS_200_100	Rectal swab	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
CRE ELITe_BC_200_100	Blood Culture	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Setup of the session

The product **CRE ELITe MGB® Kit** can be used with the **ELITe InGenius** system in order to perform:

A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Amplification run for Positive Control and Negative Control (PCR only),

All the parameters needed for the session are included in the Assay protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

Note: the ELITe InGenius system can be linked to the "Laboratory Information Server" (LIS) through which it is possible to load the work session information. Refer to the instrument user's manual for more details.

The main steps for the setup of the three types of run are described here below.

A. Integrated run

To setup an integrated run with sample extraction and amplification, carry out the following steps as per the GUI:

1. Thaw CRE PCR Mix tubes for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw CRE PCR Mix in the dark because the reagent is sensitive to the light.

2. Thaw the CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home" screen.
4. Ensure that the "Extraction Input Volume" is 200 µL and the Extracted Elute Volume is 100 µL.
5. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
6. Select the Assay protocol to be used in the "Assay" column (i.e. CRE ELITe_RcS_200_100).
7. Ensure that the "Protocol" displayed is: "Extract + PCR".
8. Select the sample loading position in the "Sample Position" column:
   - if a primary tube is used, select "Primary Tube",
   - if a secondary tube is used, select "Sonication Tube".

   Click "Next" to continue the setup.
9. Load CPE and CRE PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" button to continue the setup.
10. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
11. Load the "PCR Cassettes", the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the samples to be extracted, following the GUI instruction. Click "Next" to continue the setup.
12. Close the instrument door.
13. Press "Start" to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to 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 °C. Avoid spilling the extracted sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: The PCR Mix can be kept on board in the refrigerated block up to **4 work session** of 3 hours each.

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B. Amplification run

To set up an amplification run starting from extracted Nucleic Acids, carry out the following steps as per GUI:

1. Thaw CRE PCR Mix tubes for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw CRE PCR Mix in the dark because the reagent is sensitive to the light.

2. Select "Perform Run" from the "Home" screen.
3. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
4. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
5. Select the Assay protocol to be used in the "Assay" column (i.e. CRE ELITe_RcS_200_100).
6. Select "PCR Only" in the "Protocol" column.
7. Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Click "Next" to continue the setup.
8. Load CRE PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" to continue the setup.
9. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load the "PCR Cassettes" and the extracted Nucleic Acid samples following the GUI instruction. Click "Next" to continue the setup.
11. Close the instrument door.
12. Press "Start" to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to 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 and stored at -20 °C. Avoid the spilling of the extracted sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR Mix can be kept on board in the refrigerated block up to **4 work session** of 3 hours each.

C. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, carry out the following steps as per GUI:

1. Thaw CRE PCR Mix tubes for the session. Each tube is sufficient for preparing 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw CRE PCR Mix in the dark because the reagent is sensitive to the light.

2. Thaw the CRE - Positive Control tube for the session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
4. Select "Perform Run" from the "Home" screen.
5. Even if no extraction will be carried out, ensure that the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL

6. In the Track of interest, select the Assay protocol to be used in the “Assay” column.
7. For the Positive Control, select CRE ELITe_PC in the “Assay” column and fill in the lot number and expiry date of CRE - Positive Control,
8. For the Negative Control, select CRE ELITe_NC and fill in the lot number and expiry date of the molecular biology grade water.
9. Click “Next” to continue the setup.
10. Load CRE PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
12. Load the “PCR Cassettes”, the CRE - Positive Control tube and the tube with water for molecular biology (CRE Negative Control) following the GUI instruction. Click “Next” to continue the setup.
13. Close the instrument door.
14. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Positive Control must be removed from the instrument, capped and stored at -20 °C. Avoid spilling the Positive Control. The remaining Negative Control must be disposed.

Note: At the end of the run the PCR Cassettes with the reaction products and consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: The PCR Mix can be kept on board in the refrigerated block up to **4 work session** of 3 hours each.

Review and approval of results

At the end of the run, the “Results Display” screen is automatically shown. In this screen the sample / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports (“Sample Report” or “Track Report”). Refer to the instrument user’s manual for more details.

Note: the ELITe InGenius system can be linked to the “Laboratory Information System” (LIS) through which it is possible send the work session results to the laboratory data center. Refer to the instrument user’s manual for more details.

The **ELITe InGenius** system generates the results with the product **CRE ELITe MGB® Kit** through the following procedure:

A. Validation of amplification Positive Control and Negative Control results,
B. Validation of sample results,
C. Sample result reporting.

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes of resistance genes (channels **KPC**, **NDM VIM IMP** e **OXA**) in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the ELITe InGenius software with the parameters included in the Assay protocols “CRE ELITe_PC” and “CRE ELITe_NC”.

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

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire **after 15 days**.

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The results of Positive Control and Negative Control amplification runs are used by the instrument software to setup the “Control Charts” and are used for monitoring the amplification step performances. Refer to the instrument user’s manual for more details.

Note: If the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen and it is not possible to approve it. In this case the Positive Control or Negative Control amplification reactions have to be repeated.

Note: If the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

B. Validation of Sample results

The fluorescence signals emitted by the probes of resistance genes (channels **KPC**, **NDM VIM IMP** e **OXA**) and by the probe of Internal Control (channel IC) in the sample amplification reactions are analysed automatically and interpreted by the ELITe InGenius software with the parameters included in the Assay protocol CRE ELITe _RcS_200_100.

Results are shown in the reports generated by the instrument ("Result Display").
The sample run can be approved when the two conditions reported in the table below are met.

1) Positive Control	**Status**
CRE Positive Control	APPROVED
2) Negative Control	**Status**
CRE Negative Control	APPROVED

For each sample, the assay result is automatically interpreted by the system as established by the ELITe InGenius software algorithm and the Assay protocol parameters.

The possible result messages are listed in the table below. For each valid sample the system reports a combination of three messages specifying if the CRE genes are either detected or not detected.

Result of sample run	Interpretation
KPC DNA Detected.	**KPC gene DNA was detected** in the sample.
NDM, VIM or 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 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 and IMP DNA Not Detected or below 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 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 not suitable for result interpretation are reported as “Invalid - Retest Sample” by the **ELITe InGenius software**. In this case, the Internal Control DNA was not efficiently detected due to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitors carry-over in the eluate), which may lead to false negative call.

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

Samples suitable for analysis but in which it was not possible to detect resistance gene DNA are reported as: “KPC, NDM, VIM, IMP or OXA DNA Not Detected or below LoD". In this case it cannot be excluded that the resistance gene DNA is present at a concentration below the limit of detection of the assay (see “Performance characteristics).

Note: The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes concerning the patient.

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

C. Sample result reporting

The sample results are stored in the database and can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the details of a work session sorted by selected sample (SID).

The “Track Report” shows the details of a work session by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) of CRE ELITe MGB® Kit used in association to resuspended rectal swabs samples and ELITe InGenius® system was verified by testing 6 CRE strains, one of each of the following gene types: KPC, IMP, VIM, NDM, OXA-48 and OXA-181. The CRE organisms were grown and quantitated by plating and colony counting. At least 6 levels of dilutions of each strain, starting with the concentration above the expected LoD, were prepared in negative rectal matrix. Replicates of each dilution level were processed on ELITe InGenius® system in Extraction + PCR mode. LoD for each of the CRE strains was estimated by binary logistic regression analysis of the data as the concentration corresponding to 95% probability of a positive call. The estimated LoD was confirmed by analysis of 20 replicates of dilutions of each organism at the corresponding concentration.

The final results are reported in the following table.

Limit of Detection for resuspended rectal swab samples and ELITe InGenius® (CFU / mL)

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

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.

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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.

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® system 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.

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
VIM- positive cultural isolates	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.

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.

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.

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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 InGenius system.

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.

Repeatability

The Repeatability, as intra-run imprecision, of the CRE ELITe MGB® Kit in association with the ELITe InGenius system was tested by performing 3 Runs/day per 5 Days with two 4 member panels in 3 Replicates/Run. The first panel included three positive samples (KPC + NDM + OXA, VIM and IMP CRE strains at low concentration) and a negative sample. The second panel of the same organisms at moderate concentration and a negative sample was tested analogously.

The results as Ct values for each CRE target gene (and internal control) and each concentration individually were analysed using ANOVA procedure.

The Intra-run imprecision was found to be the main component of within laboratory imprecision. However the variability of the CRE ELITe MGB® Kit for each CRE target gene did not exceed a percentage Coefficient of Variability (%CV) of 2.0%.

A summary of results is shown below.

Repeatability of the CRE ELITe MGB® Kit

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%
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%

Reproducibility

The Reproducibility, as "Batch to batch" and "Instrument to Instrument" variability, of the CRE ELITe MGB® Kit in association with the ELITe InGenius system was tested by 2 Operators by performing 2 Runs/day for 8 Days on 3 Instruments with 3 Batches of product and with two 3 member panels in 2 Replicates/Run. The first panel included three positive samples (KPC + NDM + OXA, VIM and IMP CRE strains at low concentration). The second panel of the same organisms at moderate concentration was tested analogously.

A second study was performed by 2 Operators by performing 3 Runs/day for 1 Day on 3 Instruments with 3 Batches of product and with a negative sample in 4 Replicates/Run.

The results as Ct values for each CRE target gene (and internal control) and each concentration individually were analysed using ANOVA procedure.

The effects of Instrument and product Batch were found to be consistently statistically significant. However the variability of the CRE ELITe MGB® Kit for each CRE target gene did not exceed a %CV of 2.3%.

A summary of results is shown below.

Reproducibility of the CRE ELITe MGB® Kit						
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%

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.

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.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing some CRE-positive rectal swab samples and some rectal swab samples spiked with CRE isolates, given the difficulty of finding a significant number of positive clinical samples for each CRE target genes.

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The 30 positive rectal swab samples were identified by culture method (Carba Smart medium, bioMérieux) and characterized by a validated home-made MALDI-TOF assay and Real-Time PCR assay.

The other 120 rectal swabs samples were identified as negative by culture method and then spiked with 10 CRE strains, two of each of the following gene types: KPC, IMP, VIM, NDM, OXA-48-like. For each strain, 12 samples were analysed.

The samples were collected in FecalSWAB™, diluted in eNAT™ kit and then tested with CRE ELITe MGB® Kit and ELITe InGenius system in Extraction + PCR mode.

The results are summarized in the following table.

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

All but two of the tested samples resulted positive with the CRE ELITe MGB® Kit. The two samples (CAR.50 and CAR.84) gave an invalid result and showed a high turbidity. Re-test of diluted samples gave a negative result for CAR.50 (spiked with isolate B1 KPC-2) and positive result for CAR.84 (spiked with isolate VIM-4). In this test the sensitivity was equal to 99.3%.

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated also by analyzing some CRE-positive blood culture samples and some blood culture samples spiked with CRE isolates, given the difficulty of finding a significant number of positive clinical samples for each CRE target genes.

The 29 blood culture samples were identified by microscope and culture method and characterized by MALDI-TOF assay.

The other 20 blood culture samples were identified as negative by culture method and then spiked with 4 CRE strains of the following gene types: OXA-48, NDM, VIM e IMP. For each strain, 5 samples were analysed.

The samples were diluted in ultrapure water and then tested with CRE ELITe MGB® Kit and ELITe InGenius system in Extraction + PCR mode.

The results are summarized in the following table.

Samples	N	positive	negative	invalid
KPC-positive blood culture	16	16	0	0
OXA-48-positive blood culture	5	5	0	0
NDM- positive blood culture	4	4	0	0
IMP- positive blood culture	2	2	0	0
VIM- positive blood culture	2	2	0	0
OXA-48 spiked blood culture	5	5	0	0
NDM spiked blood culture	5	5	0	0
VIM spiked blood culture	5	5	0	0
IMP spiked blood culture	5	5	0	0

All samples resulted positive with the CRE ELITe MGB® Kit. In this test the sensitivity was equal to 100%.

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.

52 negative rectal swab samples were identified by culture method (Carba Smart medium, bioMérieux).

The 45 blood cultures were negative for growth after 36 hours.

The rectal swab samples were collected in FecalSWAB™, diluted in eNAT™ kit and then tested with CRE ELITe MGB® Kit and ELITe InGenius system in Extraction + PCR mode.

The blood culture samples were diluted in ultrapure water and then tested with CRE ELITe MGB® Kit and ELITe InGenius system in Extraction + PCR mode.

The results are summarized in the following table.

Samples	N	positive	negative	invalid
CRE negative Rectal Swab	52	0	52	0
CRE negative blood culture	45	2	43	0

In the first analysis, 51 samples of rectal swabs were negative with the CRE ELITe MGB® Kit. One sample was found to be invalid (CAR.31) and had high turbidity. This diluted and retested sample was negative. In this test the specificity of the assay was equal to 100%.

All blood culture samples tested were valid with the CRE ELITe MGB® Kit. Forty-three (43) out of 45 samples were confirmed negative, two samples resulted positive with a Ct close to the limit of detection of the metod. In this test the specificity was equal to 96%.

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 "CRE ELITe MGB® Kit", FTP RTS200ING.

REFERENCES

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

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PROCEDURE LIMITATIONS

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

Do not use samples with high turbidity with this product: the faecal matrix inhibits the amplification reaction of the nucleic acids and may cause invalid results.

There are no data available concerning product performance with DNA extracted from the following clinical samples: blood culture, faecal supernatant.

The results obtained with this product depend on an adequate 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 products for nucleic acid extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the positive samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations. However, cross-contaminations can be avoided only 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 work clothes 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 special clothing and instruments dedicated to work session setup to avoid false positive results.

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

A 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). In this case the result could be a false negative.

In case of co-infection, the sensitivity for a target can be influenced by the amplification of a second target.

Results obtained with this product may sometimes be invalid due to failure of internal control. In this case the sample shall be retested, starting from extraction, which can lead to a delay in obtaining final results.

Possible polymorphisms within the region of the target DNA covered by the product primers and probes may impair detection of target DNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, false positive and false negative results obtained 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.

TROUBLESHOOTING

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Positive Control. Check the volumes of PCR Mix and Positive Control.
Positive control degradation.	Use a new aliquot of Positive Control.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Contamination of the negative control	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, of Racks or of Inventory Block.	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and sample. Check the volumes of PCR Mix and sample.
PCR Mix degradation.	Use a new aliquot of PCR Mix and Internal Control.
Inhibition due to sample interfering substances.	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 of the clinical sample in fresh eNAT™ medium in a “Extract + PCR” session.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - selected the track related to the sample and approve manually the result. If a Ct value is require: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of sample in an “Extract + PCR” session.

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Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination during pre-analytical steps	Avoid any contact between micropipette and tube wall. Clean the micropipette with fresh 3% sodium hypochlorite solution 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 ELITe InGenius 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 or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix and / or CPE.

SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
CE	Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests.
	Attention, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

NOTICE TO PURCHASER: LIMITED LICENSE

ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800, 9,169,256 and EP patent numbers 1068358, 1144429, 1232157, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

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