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MOLECULAR DIAGNOSTICS

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NOTICE of CHANGE dated 30/07/18

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«ESBL ELITe MGB® Kit»
Ref. RTS201ING

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

- *Formal corrections in "Samples and Controls" section.*

Composition, use and performance of the product remain unchanged.

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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ESBL ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS201ING CE -20°C

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INTENDED USE

The «**ESBL ELITe MGB® Kit**» product is part of a qualitative nucleic acids amplification real-time multiplex assay for the detection of the DNA of Extended Spectrum Beta-Lactamase **CTX-M-1**, **CTX-M-9**, **CTX-M-14** and **CTX-M-15*** genes of *Enterobacteriaceae* in DNA samples extracted from rectal swabs and blood culture.

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

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

* 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 with an automated integrated system for extraction, Real Time amplification and results interpretation.

Starting from DNA extracted from the samples being tested, four amplification reactions specific for the following Extended Spectrum Beta-Lactamase genes are performed in the cartridge:

- genes of CTX-M-1 and CTX-M-15 family, detected by a specific probe labelled with AP593 fluorophore,
- genes of CTX-M-9 and CTX-M-14 family, detected by a specific probe labelled with AP593 fluorophore,

Furthermore, the extraction and inhibition Internal Control is also amplified in the cartridge. The Internal Control is based on an artificial sequence and detected by a specific probe labelled with AP525 fluorophore.

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 Extended Spectrum Beta-Lactamase genes in the starting sample.

The assay has been validated with **ELITe InGenius®**, automated integrated system for extraction, amplification and detection of nucleic acids.

PRODUCT DESCRIPTION

The «**ESBL ELITe MGB® Kit**» product supplies the ESBL 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** systems.

The ESBL PCR Mix content:

- the specific primers and probe for the **CTX-M-1** and **CTX-M-15** genes family. The probe is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the specific primers and probe for the **CTX-M-9** and **CTX-M-14** gene family. The probe is labelled with AP593 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 is labelled with AP525 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the buffer, the magnesium chloride, the nucleotide triphosphates, the stabilizers and the enzyme Taq DNA polymerase with thermic activation (hot start).

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

The product is sufficient for **96 tests in association with ELITe InGenius**, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
ESBL 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 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 are required:

- parameters for the amplification positive control **«ESBL ELITe_PC»** (ELITechGroup S.p.A.),
- parameters for the amplification negative control **«ESBL ELITe_NC»** (ELITechGroup S.p.A.),
- parameters for samples to be analyzed **«ESBL ELITe _RcS_200_100»** and **«ESBL ELITe_BC_200_100»** (ELITechGroup S.p.A.).

For automatic sample analysis with the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) 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 Filter Tips»** (Axygen BioScience Inc., CA, 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 **«ESBL - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR201ING), is required. This is a stabilised solution of plasmid DNAs.

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

- 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.

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

SAMPLES AND CONTROLS

Samples

T This product is validated for use with the following clinical samples:

Rectal swabs collected in eNAT™ kit (COPAN Italia S.p.A., ref. 606CS01R, 2 mL)

The rectal swabs for DNA extraction must 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 analysis with this product 0.2 mL of sample in eNAT™ medium has to be transferred into the sonication tube provided with **«ELITe InGenius SP 200 Consumable Set»**.

Rectal swabs collected in FecalSwab™ (COPAN Italia S.p.A., ref. 470CE, 2 mL)

The rectal swabs for DNA extraction must 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 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.

N.B.: when the DNA extraction from rectal swabs is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.1 (or later equivalent versions), use the Assay protocol **ESBL 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 for nucleic acid extraction must be collected and 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 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».

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

N.B.: 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 **ESBL 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:

Cultural 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".

N.B.: when nucleic acid extraction from Cultural isolates is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.2 (or later equivalent versions), use the extraction protocols **ESBL 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.

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Interfering substances

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

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

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 a Positive amplification Control, use the **ESBL - ELITe Positive Control** product (not provided with this kit) in association with protocol **ESBL ELITe_PC**,

as a Negative amplification Control, use molecular grade water (not provided with this kit) in association with protocol **ESBL ELITe_NC**.

N.B.: 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.
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 **ESBL ELITe MGB® Kit** with the system **ELITe InGenius** 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, ESBL Positive Control, ESBL 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 **ESBL ELITe MGB® Kit** is described in the table below.

Assay protocol for ESBL ELITe MGB® kit			
Name	**Matrix**	**Report**	**Characteristics**
ESBL 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
ESBL 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 **ESBL 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 Positive Control and Negative Control run (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.

N.B.: the ELITe InGenius system can be linked to the "Location Information Server" (LIS) through which it is possible to send 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, carry out the following steps as per the GUI:

1. Thaw ESBL 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.
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. ESBL ELITe_RS_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 "Sonicator Tube".

   Click "Next" to continue the setup.

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9. Load CPE and ESBL 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.

N. B.: 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.

N. B.: 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.

N. B.: The PCR Mix can be kept on board in the refrigerated block up to **21 hours** (7 work session of 3 hours each).

B. Amplification run

To set up the amplification run carry out the following steps as per GUI:

1. Thaw ESBL 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.
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. ESBL ELITe_RS_200_100).
6. Select "PCR Only" in the "Protocol" column.
7. Ensure the sample loading position in the "Sample Position" column is "ExtraTube (bottom row)". Click "Next" to continue the setup.
8. Load ESBL 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.

N. B.: 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.

N. B.: 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.

N. B.: The PCR mix can be kept on board in the refrigerated block up to **21 hours** (7 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 ESBL 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.
2. Thaw the ESBL 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. In the Track of interest, select the Assay protocol to be used in the "Assay" column.
6. For the positive control, select ESBL ELITe_PC in the "Assay" column and fill in the lot number and expiry date of ESBL Positive Control.
7. For the negative control, select ESBL ELITe_NC in the "Assay" column and fill in the lot number and expiry date of the molecular biology grade water.
8. Click "Next" to continue the setup.
9. Load ESBL PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load the "PCR Cassettes", the ESBL Positive Control tube and the negative control tube 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.

N.B.: The results of Positive Control and Negative Control amplification runs are used by the instrument software to set up the "Control Charts". Four Positive Control and Negative Control results from four different runs are requested to set up the control chart. After that, the results of Positive control and Negative Controls are used for monitoring the amplification step performances. Refer to the instrument user's manual for more details.

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

N.B.: 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.

N.B.: The PCR mix can be kept on board in the refrigerated block up to **21 hours** (7 work sessions of 3 hours each).

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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.

N.B.: the ELITe InGenius system can be linked to the "Location Information Server" (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 **ESBL 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 Extended Spectrum Beta-Lactamase genes (CTX-M-1-9-14-15) in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocols "ESBL ELITe_PC" and "ESBL 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**.

Before analysing any sample it is absolutely mandatory to verify that amplification Positive Control and Negative Control were run with the lot of amplification reagent to be used and results are approved and valid. The availability of "Approved" (Status) amplification Positive Control and Negative Control results is shown in the "Controls" window of the GUI. If the amplification Positive Control and Negative Control result are missing, generate them as described above.

N.B.: When the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the "not passed" message is shown on the "Controls" screen and it is not possible to approve it. In this case the amplification Positive Control or Negative Control reaction has to be repeated.

N.B.: When 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 Extended Spectrum Beta-Lactamase genes (CTX-M-1-9-14-15) and by the probe of Internal Control (IC) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocol ESBL ELITe _RcS_200_100.

N.B.: Before analysing any sample, verify that amplification controls were run with the lot of amplification reagent to be used and results are approved and valid. The availability of "Approved" (Status) amplification control results is shown in the "Controls" window of the GUI. If the amplification control results are missing, generate them as described above.

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
ESBL Positive Control	APPROVED
2) Negative Control	**Status**
ESBL 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 of a sample are listed the table below.

Result of sample run	Interpretation
CTX-M-1-9-14-15: DNA Detected.	**CTX-M-1, CTX-M-15, CTX-M-9 or CTX-M-14 gene DNA was detected** in the sample.
CTX-M-1-9-14-15: DNA Not Detected or below LoD.	**CTX-M-1, CTX-M-15, CTX-M-9 and CTX-M-14 gene DNA was not detected** in the sample. The sample is negative for these genes or their concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Not valid assay result** due to Internal Control failure (Incorrect extraction or inhibitor carry-over)

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 cause incorrect results and false negative.

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 Extended Spectrum Beta-Lactamase CTX-M-1, CTX-M-15, CTX-M-9 and CTX-M-14 genes DNA are reported as: " CTX-M-1-9-14-15: DNA Not Detected or below LoD". In this case it cannot be excluded that the Extended Spectrum Beta-Lactamase CTX-M-1, CTX-M-15, CTX-M-9 and CTX-M-14 genes DNA is present at a concentration below the limit of detection of the assay (see “Performance characteristics”).

N.B.: 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.

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PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) of the ESBL ELITe MGB® Kit used in association to resuspended rectal swabs samples and ELITe InGenius system was verified by testing 4 ESBL strains, one of each of the following gene types: CTX-M-1, CTX-M-9, CTX-M-14 e CTX-M-15. The ESBL organisms were grown and quantitated by plating and colony counting. Six levels of dilutions of each strain, starting with the concentration above the expected LoD, were prepared in negative rectal matrix. Each dilution level was processed in 12 replicates on ELITe InGenius system in “Extraction + PCR” mode. The LoD for each of the ESBL strains was estimated by probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call. The 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 System (CFU / mL)				
Gene	Bacterial Isolate	LoD (CFU / mL)	95% confidence interval (CFU / mL)	
			lower bound	upper bound
CTX-M-1	*E. coli*, DICON-091	55	43	79
CTX-M-9	*E. coli*, DICON-055	29	21	46
CTX-M-14	*E. coli*, DICON-045	273	220	384
CTX-M-15	*E. coli*, NCTC13400	36	28	55

Efficiency of detection (inclusivity)

The efficiency of detection on different variants of Extended Spectrum Beta-Lactamase genes (inclusivity) was evaluated by comparison of sequences with nucleotide database.
The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probes in the alignment of the sequences available in the database for the Extended Spectrum Beta-Lactamase genes showed their conservation and absence of significant mutations for the variants reported in the following table.

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

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

The final results are reported in the following table.

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

Organism	Isolate	Gene	Concentration (CFU/mL)	Result
E. coli	DICON-091	CTX-M-1	165	Inclusive
E. coli	DICON-211	CTX-M-1	165	Inclusive
K. pneumoniae	DICON-126	CTX-M-1	165	Inclusive
K. pneumoniae	DICON-001	CTX-M-1	165	Inclusive
E. coli	DICON-003	CTX-M-1	165	Inclusive
E. coli	DICON-055	CTX-M-9	87	Inclusive
E. coli	DICON-098	CTX-M-9	87	Inclusive
E. coli	DICON-085	CTX-M-9	87	Inclusive
E. coli	DICON-045	CTX-M-14	819	Inclusive
K. pneumoniae	DICON-060	CTX-M-14	819	Inclusive
E. coli	DICON-054	CTX-M-14	819	Inclusive
E. coli	NCTC13400	CTX-M-15	108	Inclusive
E. coli	NCTC13451	CTX-M-15	108	Inclusive
E. coli	NCTC13450	CTX-M-15	108	Inclusive

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

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

The results are summarized in the following table.

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

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

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.

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

Potential interfering markers of the product ESBL ELITe MGB® Kit

Organism	Isolate	Concentration (CFU/mL)	Result
K. pneumoniae	ATCC 700603	10^6	No cross-reactivity
E. coli	ATCC BAA-201	10^6	No cross-reactivity
S. marcescens	UCLA 14-13-A11	10^6	No cross-reactivity
A. baumannii	NCTC 13301	10^6	No cross-reactivity
A. lwoffii	ATCC 15309	10^6	No cross-reactivity
B. adolescentis	ATCC 15703	10^6	No cross-reactivity
B. longum	ATCC 15707	10^6	No cross-reactivity
C. jejuni	ATCC 33292	10^6	No cross-reactivity
C. albicans	Zeptometrix Z006	10^6	No cross-reactivity
C. freundii	ATCC 8090	10^6	No cross-reactivity
C. difficile	ATCC 43593	10^6	No cross-reactivity
C. perfringens	ATCC 13124	10^6	No cross-reactivity
P. mirabilis	ATCC 12453	10^6	No cross-reactivity
P. aeruginosa	ATCC 27853	10^6	No cross-reactivity
S. enterica	ATCC 700720	10^6	No cross-reactivity

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

Interfering substances

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

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

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

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

Repeatability

The Repeatability of the ESBL ELITe MGB® Kit in association with the ELITe InGenius system was tested by performing 3 Runs/day per 2 Days with a panel in 3 Replicates/Run. The panel included three positive samples (CTX-M-1, CTX-M-9 and CTX-M-15 ESBL strains at 3x LoD) and a negative sample.

The results as Ct values for each target gene and internal control (IC) were analysed as percentage Coefficient of Variability (%CV), by obtaining the repeatability as imprecision intra-run and imprecision inter-run.

The Repeatability of the ESBL ELITe MGB® Kit for each target gene, as intra-run and inter-run %CV, did not exceed 2.5%.

A summary of results is shown below.

Intra-run Repeatability of the product ESBL ELITe MGB® Kit

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

Inter-run Repeatability of the product ESBL ELITe MGB® Kit

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

Reproducibility

The Reproducibility as “Batch to batch” variability of the ESBL ELITe MGB® Kit in association with the ELITe InGenius system was tested by 1 Operator by performing 3 Runs/day for 3 Days with 3 Batches of product and with a panel in 3 Replicates/Run. The panel included three positive samples (CTX-M-1, CTX-M-9 and CTX-M-15 ESBL strains at 3x LoD) and a negative sample.

The Reproducibility as “Batches, Instruments and Operators” variability of the ESBL ELITe MGB® Kit in association with the ELITe InGenius system was tested by 3 Operators by performing 3 Runs/day for 3 Days on 3 instruments with 3 Batches of product and with a panel in 3 Replicates/Run. The panel included three positive samples (CTX-M-1, CTX-M-9 and CTX-M-15 ESBL strains at 3x LoD) and a negative sample.

The results as Ct values for each target gene and internal control (IC) were analysed as percentage Coefficient of Variability (%CV), by obtaining the reproducibility as imprecision inter-batches and imprecision inter-site.

The reproducibility of the ESBL ELITe MGB® Kit for each target gene, as “Batch to batch” and “Batches, Instruments and Operators” %CV, did not exceed 2 %.

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A summary of results is shown below.

Inter-batches Reproducibility of ESBL ELITe MGB® Kit

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

Inter-site Reproducibility of ESBL ELITe MGB® Kit

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

Absence of cross-contamination

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

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

Whole system failure

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

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

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

Diagnostic sensitivity: confirmation of positive samples

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

The 51 positive rectal swab samples were identified by culture method (chromID® ESBL, bioMérieux) and characterized by a validated “home-brew” Real-Time PCR assay.

The other 96 rectal swabs samples were identified as negative by culture method and then spiked with 4 ESBL strains for each of the following gene types: CTX-M-1, CTX-M-9, CTX-M-14 e CTX-M-15. For each strain, 24 samples were analysed.

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

The results are summarized in the following table.

Samples	N	positive	negative	invalid
CTX-M-1 or CTX-M-15 positive Rectal Swab	38	38	0	0
CTX-M-9 or CTX-M-14 positive Rectal Swab	9	9	0	0
CTX-M-1 or M-15 and CTX-M-9 or M-14 positive Rectal Swab	4	3	1	0
CTX-M-1-spiked Rectal Swab (isolate DICON-091)	24	24	0	0
CTX-M-9-spiked Rectal Swab (isolate DICON-055)	24	24	0	0
CTX-M-14-spiked Rectal Swab (isolate DICON-045)	24	24	0	0
CTX-M-15-spiked Rectal Swab (isolate NCTC13400)	24	23	0	1

All but two of the tested samples resulted positive with the ESBL ELITe MGB® Kit. One sample (CTX-M-1-15 and CTX-M-9-14 positive) gave a negative result and one sample (CTX-M-15 spiked) gave an invalid result. Both samples showed a high turbidity. The invalid sample was not included in the analysis.

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was also evaluated by analyzing some ESBL-positive blood culture samples.

51 blood culture samples were identified by culture method.

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

The results are summarized in the following table.

Samples	N	positive	negative	invalid
CTX-M positive blood culture	51	51	0	0

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

In these tests (blood culture and rectal swab testing), the assay sensitivity was equal to 99.5 %.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated by analyzing some ESBL-negative rectal swab samples and blood culture samples.

49 negative rectal swab samples were identified by culture method while other 5 samples were identified by a validated “home-brew” Real-Time PCR assay.

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

37 negative blood culture samples were identified by culture method.

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

The results are summarized in the following table.

Samples	N	positive	negative	invalid
ESBL negative Rectal Swab	54	0	54	0
ESBL negative Blood Culture	37	2	35	0

Two blood culture samples gave positive results with the ESBL ELITe MGB® Kit.

In this test the assay specificity was equal to 97,8%.

N. B.: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File "ESBL ELITe MGB Kit", FTP RTS201ING.

REFERENCES

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

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PROCEDURE LIMITATIONS

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

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

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

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.

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.
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 in fresh eNAT™ medium of the sample in an “Extract + PCR” session.
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: - repeat the amplification of eluted sample in molecular biology grade water, in a “PCR only” session or - repeat the extraction with a dilution of the primary sample in fresh eNAT™ medium, in an “Extract + PCR” session.

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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

TaqMan™ 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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