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NOTICE of CHANGE dated 16/09/2021

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«MDR/MTB ELITe MGB Kit»
Ref. RTS120ING

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

- *Correction of Tm value of katG probe for INH negative resistance.*

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
	DIESE FASSUNG DER GEBRAUCHSANLEITUNG IST KOMPATIBEL MIT DER VORHERIGEN VERSION DES TESTKITS

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MDR/MTB ELITe MGB® Kit

reagent for DNA Real Time amplification

REF **RTS120ING** CE **IVD** -20°C

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INTENDED USE

The **«MDR/MTB ELITe MGB® Kit»** is part of a qualitative nucleic acid amplification assay to detect *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canettii*, *M. microti, M. caprae*) DNA and to identify the main mutations associated with resistance to Rifampicin and/or Isoniazid.

The assay has to be carried out with the **ELITe InGenius®** system, starting from sputum samples, bronchial aspirates (BA), bronchoalveolar lavages (BAL), urine, cavity fluids, biopsies and gastric aspirates previously liquefied, decontaminated and inactivated.

The product may be used for two different purposes:

- as an aid in the diagnosis of tuberculosis from *Mycobacterium tuberculosis* complex, in association with the patient's clinical data and other laboratory test results, in particular the culture methods for mycobacterium,
- as an aid in the diagnosis of tuberculosis and genotypic resistance of *Mycobacterium tuberculosis* complex, in association with the patient's clinical data and other laboratory test results, in particular phenotypic testing for antimicrobial susceptibility.

ASSAY PRINCIPLES

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

For the sole purpose of detecting tuberculosis and identifying genotypic resistance of the MTB complex, starting from DNA extracted from each sample in analysis, in the same analytical session two amplification reactions are carried out using the TB1 PCR Mix and TB2 PCR Mix in two PCR Cassettes.

The **TB1 PCR Mix** test tube amplifies the following targets:
- a region of the repeated sequence IS6110, detected by a specific probe (MTB Channel), to identify MTB complex,
- the 81 bp hot spot region of the rpoB gene, detected by three specific probes (rpoB2, rpoB3, rpoB4 Channels), to identify genotypic resistance to Rifampicin.

The **TB2 PCR Mix** test tube amplifies the following targets:
- the 81 bp hot spot region of the rpoB gene, detected by a specific probe (rpoB1 Channel), to identify genotypic resistance to Rifampicin,
- the region of codon 315 of the katG gene, detected by a specific probe (katG Channel), to identify genotypic resistance to Isoniazid,
- the -15 / -8 promoter region of the inhA gene, detected by a specific probe (inhA Channel), to identify genotypic resistance to Isoniazid.

Furthermore, the exogenous Internal Control is amplified in the TB1 PCR Mix and TB2 PCR Mix. The Internal control is based on an artificial sequence (IC2) and it is detected by a specific probe (IC Channel).

The probes with ELITe MGB® technology are activated when they hybridize with the specific product of the amplification reaction. Fluorescence emission is measured and recorded by the instrument.

At the end of the amplification cycle, the ELITe InGenius instrument automatically analyzes:
- fluorescence curves in order to calculate the "threshold cycles" (Ct) for detecting the MTB complex,
- dissociation curves in order to calculate the melting temperatures (Tm) that allow identifying the presence of normal and/or mutated target genes (rpoB, katG and inhA).

The assay was validated with the **ELITe InGenius** system, starting from sputum samples, bronchoalveolar lavages (BAL), bronchial aspirates (BA), urine, cavity fluids, biopsies and gastric aspirates previously fluidized, decontaminated and inactivated.

PRODUCT DESCRIPTION

The **«MDR/MTB ELITe MGB® Kit»** product supplies two complete and **ready-to-use** mixtures for real-time amplification, TB1 PCR Mix and TB2 PCR Mix, **each aliquoted into four test tubes**. Each tube contains **280 µL** of solution, sufficient for **12 tests** in optimal reagent consumption conditions (at least 2 samples per session) when used with the **ELITe InGenius** instrument.

The **TB1 PCR Mix** mixture contains the specific primers and probes for:
- the **IS6110 MTB complex** repeated sequence. The probe (MTB) is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the 81 bp hot-spot region of **rpoB** gene. The probes (rpoB2, rpoB3 and rpoB4) are labelled with AP639, AP525 and AP593 fluorophores, respectively, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **IC2** artificial sequence of internal control. The probe (IC) is labelled with AP680 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety.

The **TB2 PCR Mix** contains the specific primers and probe for:
- the 81 bp hot-spot region of **rpoB** gene. The probe (rpoB1) is labelled with AP639 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the codon 315 region of **katG** gene. The probe (katG) is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the -15 / -8 region of **inhA** gene promoter. The probe (inhA) is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **IC2** artificial sequence of internal control. The probe (IC) is labelled with AP680 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety.

Both TB1 and TB2 PCR Mixes contain buffer, magnesium chloride, nucleotide triphosphates, stabilizers and the enzyme DNA polymerase with thermic activation (hot start).

The product is sufficient for **48 tests in association with ELITe InGenius**, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Risk classification
TB1 PCR Mix	Complete reaction mixture RED cap	**4 x 280 µL**	**-**
TB2 PCR Mix	Complete reaction mixture WHITE cap	**4 x 280 µL**	**-**

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar flow 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 (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 PCR and result interpretation of samples, the **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) instrument and the following specific Assay protocols are required:

- parameters for the amplification positive control **"MDR-MTB ELITe_PC"**,
- parameters for the amplification negative control **"MDR-MTB ELITe_NC"**,
- parameters for samples to be analyzed **"MDR-MTB ELITe_SP_200_100"**, **"MDR-MTB ELITe_BAL_200_100"**, **"MDR-MTB ELITe_U_200_100"**, **"MDR-MTB ELITe_CL_200_100"**, **"MDR-MTB ELITe_B_200_100"**, **"MDR-MTB ELITe_GA_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 Filter tips Axygen»** (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 plasmid DNAs and phage genomic RNA.

As template of amplification positive control, the specific product **«MDR/MTB - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR120ING), is required. This is a stabilised solution of plasmid DNAs.

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WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Clinical samples from patient with suspect tuberculosis must be handled according to the state or local regulations about safety practice (working environment and personnel training).

Clinical samples from patient with suspect tuberculosis must be inactivated before the use in association with ELITe InGenius.

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 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.

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

The samples must be suitable and, if possible, dedicated for this type of analysis. Inactivated 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 **TB1 and TB2 PCR Mixes** must be stored at -20 °C in the dark.
The **TB1 and TB2 PCR Mixes** 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

This product must be used with the following clinical samples:

Sputum samples

The Sputum samples for DNA extraction must be collected and identified according to mycobacteriology laboratory guidelines, transported and stored at +2 / +8 °C for a maximum of two days. Samples must be liquefied with a solution of N-Acetil L-Cysteine and decontaminated with sodium hydroxide solution (Mycobacteriology Laboratory Manual, Global Laboratory Initiative). The liquefied and decontaminated sample must be then inactivated at 95°C for 30 minutes. For the analysis with this product 0.2 mL of inactivated sample has to be transferred into the "**Sonication tube**" provided with **«ELITe InGenius SP 200 Consumable Set»**.

The liquefied and decontaminated Sputum samples can be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. Avoid freeze / thaw cycles.

Note: to carry out the DNA extraction from Sputum samples by the **ELITe InGenius** and **ELITe InGenius Software** version **1.3** (or later versions), use the Assay Protocol **MDR-MTB ELITe_SP_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.

Bronchoalveolar lavage (BAL), bronchial aspirates (BA)

The BAL / BA samples for DNA extraction must be collected and identified according to mycobacteriology laboratory guidelines, transported and stored at +2 / +8°C for a maximum of two days. Samples must be liquefied with a solution of N-Acetil L-Cysteine and decontaminated with sodium hydroxide solution (Mycobacteriology Laboratory Manual, Global Laboratory Initiative). The liquefied and decontaminated sample must be then inactivated at 95°C for 30 minutes. For the analysis with this product 0.2 mL of inactivated sample has to be transferred into the "**Sonication tube**" provided with **«ELITe InGenius SP 200 Consumable Set»**.

The liquefied and decontaminated BAL / BA samples can be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. Avoid freeze / thaw cycles.

Note: to carry out the DNA extraction from BAL/ BA samples by the **ELITe InGenius** and **ELITe InGenius Software** version **1.3** (or later versions), use the Assay Protocol **MDR-MTB ELITe_BAL_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.

Urine

The urine samples for DNA extraction must be collected and identified according to mycobacteriology laboratory guidelines, transported and stored at +2 / +8°C for a maximum of two days. Samples must be concentrated and decontaminated with sodium hydroxide solution (Mycobacteriology Laboratory Manual, Global Laboratory Initiative) The concentrated and decontaminated sample must be then inactivated at 95°C for 30 minutes. For the analysis with this product 0.2 mL of inactivated sample has to be transferred into the "**Sonication tube**" provided with **«ELITe InGenius SP 200 Consumable Set».**

The concentrated and decontaminated urine samples can be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. Avoid freeze / thaw cycles.

Note: to carry out the DNA extraction from urine samples by the **ELITe InGenius** and **ELITe InGenius Software** version **1.3** (or later versions), use the Assay Protocol **MDR-MTB ELITe_U_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.

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Cavity fluids

The cavity fluid samples for DNA extraction must be collected and identified according to mycobacteriology laboratory guidelines, transported and stored at +2 / +8°C for a maximum of two days. Samples must be concentrated and decontaminated with sodium hydroxide solution (Mycobacteriology Laboratory Manual, Global Laboratory Initiative) The concentrated and decontaminated sample must be then inactivated at 95°C for 30 minutes. For the analysis with this product 0.2 mL of inactivated sample has to be transferred into the "**Sonication tube**" provided with **«ELITe InGenius SP 200 Consumable Set».**

The concentrated and decontaminated cavity fluid samples can be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. Avoid freeze / thaw cycles.

Note: to carry out the DNA extraction from cavity fluid samples by the **ELITe InGenius** and **ELITe InGenius Software** version **1.3** (or later versions), use the Assay Protocol **MDR-MTB ELITe_CL_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.

Biopsies

The biopsy samples for DNA extraction must be collected and identified according to mycobacteriology laboratory guidelines, transported and stored at +2 / +8°C for a maximum of two days. Break down the samples according to laboratory procedures and decontaminate them with a sodium hydroxide solution (Mycobacteriology Laboratory Manual, Global Laboratory Initiative). The decontaminated sample must be then inactivated at 95°C for 30 minutes. For the analysis with this product 0.2 mL of inactivated sample has to be transferred into the "**Sonication tube**" provided with **«ELITe InGenius SP 200 Consumable Set».**

The decontaminated biopsy samples can be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. Avoid freeze / thaw cycles.

Note: to carry out the DNA extraction from biopsy samples by the **ELITe InGenius** and **ELITe InGenius Software** version **1.3** (or later versions), use the Assay Protocol **MDR-MTB ELITe_B_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.

Gastric aspirates

The gastric aspirate samples for DNA extraction must be collected and identified according to mycobacteriology laboratory guidelines, transported and stored at +2 / +8°C for a maximum of two days. Samples must be liquefied with a solution of N-Acetil L-Cysteine and decontaminated with sodium hydroxide solution (Mycobacteriology Laboratory Manual, Global Laboratory Initiative). The liquefied and decontaminated sample must be then inactivated at 95°C for 30 minutes. For the analysis with this product 0.2 mL of inactivated sample has to be transferred into the "**Sonication tube**" provided with **«ELITe InGenius SP 200 Consumable Set»**.

The liquefied and decontaminated gastric aspirate samples can be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. Avoid freeze / thaw cycles.

Note: to carry out the DNA extraction from gastric aspirate samples by the **ELITe InGenius** and **ELITe InGenius Software** version **1.3** (or later versions), use the Assay Protocol **MDR-MTB ELITe_GA_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.

Interfering substances

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

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 **TB Positive Control** reagent (not provided with this kit) in association with protocol **MDR-MTB ELITe_PC**,
- as amplification Negative Control (**TB Negative Control**), use molecular biology grade water (not provided with this kit) in association with protocol **MDR-MTB 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.
Furthermore, the amplification controls must be re-run when:
- a new lot of amplification reagents is started,
- the results of quality controls (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius** instrument.

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used.

PROCEDURE

The procedure to use the **MDR/MTB 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** instrument and select the login mode "**CLOSED**",
- verify that the amplification controls (Controls, TB Positive Control, TB Negative Control) were run in association with the amplification reagent lot to be used and the results are approved and valid (Status). If there are not amplification control results approved or valid, generate 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 Protocols available with the product **MDR/MTB ELITe MGB® Kit** are described in the table below:

Assay Protocol for the MDR/MTB ELITe MGB® Kit
product for the detection of tuberculosis and identification of genotypic resistance of MTB complex

Name	Matrix	Unit ratio	Features
MDR-MTB ELITe_SP_200_100	Sputum	Positive / negative / resistance positive / resistance negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
MDR-MTB ELITe_BAL_200_100	BAL/BA	Positive / negative / resistance positive / resistance negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL I Sample PCR input volume: 20 µL

MDR-MTB ELITe_U_200_100	Urine	Positive / negative / resistance positive / resistance negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
MDR-MTB ELITe_CL_200_100	Cavity fluids	Positive / negative / resistance positive / resistance negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
MDR-MTB ELITe_B_200_100	Biopsies	Positive / negative / resistance positive / resistance negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
MDR-MTB ELITe_GA_200_100	Gastric aspirates	Positive / negative / resistance positive / resistance negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

Note: In assays for the detection of tuberculosis and the identification of genotypic resistance of MTB complex **TB1 PCR Mix** and **TB2 PCR Mix** are required.

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

Setup of the session

The product **MDR/MTB 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.

Note: The **ELITe InGenius** system can be linked to the "Laboratory 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 with sample extraction and amplification, carry out the following steps as per the GUI:

1. Thaw the test tubes containing the TB1 PCR Mix and TB2 PCR Mix. Each test tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 samples per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw the test tubes containing the TB1 PCR Mix and TB2 PCR Mix in the dark because these reagents are sensitive to the light.

2. Thaw the CPE test tubes for the session. Each test tube is sufficient for 12 extractions. Gently mix, centrifuge the test tubes for 5 seconds.
3. Select “Perform Run” from the screen “Home”.
4. Ensure that “Extraction Input Volume” is set at 200 µL and that “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 (e.g. MDR-MTB ELITe_SP_200_100).
7. Ensure that the “Protocol” displayed is: “Extract + PCR”.
8. Select the sample loading position in the “Sample Position” column and select “Extraction Tube”. Click “Next” to continue the setup.
9. Load CPE and TB1 PCR Mix and the TB2 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 Mixes can be kept on board in the refrigerated block up to 7 work sessions 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 the test tubes containing the TB1 PCR Mix and TB2 PCR Mix. Each test tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 samples per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw the test tubes containing the TB1 PCR Mix and TB2 PCR Mix in the dark because these reagents are 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 use in the “Assay” column (e.g. MDR-MTB ELITe_SP_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 the TB1 PCR Mix and the TB2 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 Acids 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 mixes can be kept on board in the refrigerated block up to 7 work sessions 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 the test tubes containing the TB1 PCR Mix and TB2 PCR Mix. Each test tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 samples per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw the test tubes containing the TB1 PCR Mix and TB2 PCR Mix in the dark because these reagents are sensitive to the light.

2. Thaw the TB Positive Control tube for the session. Each tube is sufficient for 2 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 100 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 MDR-MTB ELITe_PC in the "Assay" column and fill in the lot number and expiry date of TB Positive Control.
8. For the Negative Control, select MDR-MTB ELITe_NC in the "Assay" column and fill in the lot number and expiry date of the molecular biology grade water.
9. Click "Next" to continue the setup.
10. Load the TB1 PCR Mix and the TB2 PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load and 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 TB Positive Control tube and the Negative Control tube 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 mixes can be kept on board in the refrigerated block up to 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.

Note: 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.

Detection of tuberculosis and identification of genotypic resistance of MTB complex

The **ELITe InGenius** system generates the results with the product **MDR/MTB 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 target probes (MTB, rpoB1, rpoB2, rpoB3, rpoB4, katG and inhA) in the Positive Control and Negative Control amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols "MDR-MTB ELITe_PC" and "MDR-MTB 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.

Note: The Positive Control and Negative Control are processed in association with both TB1 PCR Mix and TB2 PCR Mix and so the control results have to be approved for both the PCR Mixes. By default the GUI shows the control results for TB1 PCR Mix (Controls). By clicking on the assay field, the assay window is opened and the TB2 PCR Mix can be selected in order to approve the related control results.

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

The results of Positive Control and Negative Control amplification runs are used by the instrument software to setup the "Control Charts". At least four Positive Control and Negative Control results from four different runs are requested. After that, the results of Positive Control and Negative Control 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 as well.

B. Validation of sample results

The fluorescence signals emitted by the target probes (MTB, rpoB1, rpoB2, rpoB3, rpoB4, katG, inhA) and by the Internal Control probe (IC) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols MDR-MTB ELITe_SP_200_100, MDR-MTB ELITe_BAL_200_100, MDR-MTB ELITe_U_200_100, MDR-MTB ELITe_CL_200_100, MDR-MTB ELITe_B_200_100, MDR-MTB ELITe_GA_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**
TB Positive Control	APPROVED
2) Negative control	**Status**
TB Negative Control	APPROVED

For each sample, the assay result is automatically interpreted by the system starting from the Ct values (for MTB complex and IC) and Tm values (for MTB and resistance analysis) as established by the **ELITe InGenius software** algorithm and the Assay protocol parameters.

The table below shows the possible messages for the result of the MTB complex detection.

Result of a session on the sample	Interpretation
MTB:DNA detected Typing as follow:	The **MTB complex DNA was detected** in the sample in sufficient quantity to run the antibiotic resistance analysis (see following table).
MTB:DNA detected Typing not feasible	The **MTB complex DNA was detected** in the sample but in insufficient quantity to run the antibiotic resistance analysis
MTB:DNA not detected or below the LoD	The **MTB complex DNA was not detected** in the sample. The sample is **valid negative** or the target concentration is lower than the limit of detection (LoD) of the assay.
Invalid - Retest Sample	**Assay result invalid** due to problems with the Internal Control (incorrect extraction or carry-over of inhibitors). The test must be repeated.
Inconclusive – Retest Sample	**Assay result not determined** due to problems detecting MTB complex. The test must be repeated.

The possible result messages about the analysis for antibiotic resistance of the sample are listed the table below.

Result of a session on the sample	Interpretation
RIF:Resistance Negative	No mutations in the rpoB gene region analyzed. The sample could be **sensitive to Rifampicin**.
RIF:Resistance Positive: Probe/s	Mutations present in the rpoB gene region analyzed. The sample could be **resistant to Rifampicin**. The probes that detected the mutations are indicated in the results.
INH:Resistance Negative	No mutations in the katG and inhA gene regions analyzed. The sample could be **sensitive to Isoniazid**.
INH:Resistance Positive: Probe/s	Mutations present in the katG and inhA gene regions analyzed. The sample could be **resistant to Isoniazid**. The probes that detected the mutations are indicated in the results.
RIF:Typing Invalid - Retest Sample INH:Typing Invalid - Retest Sample	**Assay result invalid** due to problems in the antibiotic resistance analysis. The test must be repeated.

When the DNA of MTB complex is detected in the sample and the analysis for antibiotic resistance is performed, result messages can occur in different combinations (e.g. MTB DNA Detected. Typing as follow: RIF Resistance Negative, INH Resistance Positive: katG, inhA).

Samples reported as “MTB DNA Detected. Typing not feasible” by the **ELITe InGenius software** are not suitable for analysis for antibiotic resistance. In this case, the MTB DNA was detected but the DNA is not sufficient (MTB Ct > 31) to obtain correct results in a reproducible way. This is due to low MTB concentration in the sample or to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitors carry-over in the eluate).

Note: When a sample is reported as “MTB DNA Detected. Typing not feasible” the Tm of the target probes (rpoB1, rpoB2, rpoB3, rpoB4, katG, inhA) can be checked by operator. If all Tm values fall within the limits reported in the table below for wild type genes, the sample is “RIF Resistance Negative” and “INH Resistance Negative”.

Probe	Limits of Wild type Tm	Outcome
rpoB1	66.0 ≤ Tm ≤ 80.0	RIF Resistance Negative
rpoB2	70.0 ≤ Tm ≤ 80.0	
rpoB3	68.0 ≤ Tm ≤ 80.0	
rpoB4	63.5 ≤ Tm ≤ 80.0	
katG	70.0 ≤ Tm ≤ 80.0	INH Resistance Negative
inhA	66.0 ≤ Tm ≤ 80.0	

Samples reported as “Invalid - Retest Sample” by the **ELITe InGenius software** are not suitable for result interpretation. 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.

Samples reported as “Inconclusive - Retest Sample” by the **ELITe InGenius software** are not suitable for result interpretation. In this case, an error occurred in the MTB complex DNA detection due to problems in the extraction or amplification step (inhibitors carry-over in the eluate, mix-up of PCR Mixes), which may cause incorrect results.

Samples reported as “RIF Typing Invalid - Retest Sample" and “INH Typing Invalid - Retest Sample” by the **ELITe InGenius software** are not suitable for the antibiotic resistance analysis. In this case, an error occurred during the antibiotic resistance detection due to problems in the extraction or amplification step (inhibitors carry-over in the eluate, issue during PCR setup), which may cause incorrect results. However, **the samples are positive for MTB complex DNA**.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode “as is” or diluted 1:3 in molecular biology grade water. 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 reported as "MTB DNA detected Typing as follows: RIF Resistance Positive: rpoB2, rpoB3, rpoB4, rpoB1” by the **ELITe InGenius software** are not suitable for analysis for antibiotic resistance as it is not correct detecting mutations for all rpoB probes. In this case, the MTB DNA was detected but the rpoB gene amplification was inhibited, which cause incorrect results. However, **the samples are positive for MTB complex DNA**.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode “as is” or diluted 1:10 in molecular biology grade water. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode.

Note: Please, when a retest is needed, take into account that the assay will be carried out with both TB1 PCR Mix and TB2 PCR Mix and so the eluate volume must be sufficient for two reactions (at least 50 µL).

Samples reported as: "MTB DNA Not Detected or below the LoD" are suitable for analysis but it was not possible to detect MTB complex DNA. In this case it cannot be excluded that MTB complex 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 account all the clinical data and the other laboratory test outcomes concerning the patient, particularly phenotypic testing for antimicrobial susceptibility.

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 sorted 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 the MDR/MTB ELITe MGB® Kit product was determined in association with fluidized, decontaminated and deactivated sputum samples and with the ELITe InGenius system.

The LoD was calculated by testing a panel of sputum samples certified MTB-negative and spiked by *Mycobacterium tuberculosis* (MTB) reference material, strain H37Ra (ATCC), quantified by real time amplification. Nine dilution levels of MTB were prepared, from 320 CFU/mL to 1 CFU/mL. Each dilution level was tested in 12 replicates with the ELITe InGenius system in "Extract + PCR" mode.

The LoD was calculated using Probit regression as the concentration that has a 95% probability of being positive. The estimated LoD was verified by analysis of 20 replicates of sputum samples spiked by MTB reference material at the claimed concentration. Furthermore, the same LoD was also verified for the BAL / BA matrix by analysis of 20 replicates BAL / BA samples spiked by MTB reference material at the claimed concentration. The results obtained support the claimed LoD.

The LoD in association with Cavitary Fluid, Urine and Biopsy samples was verified by analysis of 20 replicates of each matrix spiked by MTB reference material at a concentration of 20 CFU/mL. The results obtained support the claimed LoD.

The final results are shown in the following table.

Limit of Detection with the ELITe InGenius system			
Matrix	LoD (CFU/mL)	Confidence interval 95% (CFU/ml)	
		lower limit	upper limit
Sputum	6	4	15
BAL / BA	6	-	-
Cavitary Fluid	20	-	-
Urine	20	-	-
Biopsy	20	-	-

Detection of resistance to Rifampicin and/or Isoniazid

The detection of resistance to Rifampicin and/or Isoniazid using the MDR/MTB ELITe MGB® Kit product in association with the ELITe InGenius system was evaluated by analyzing a few certified samples of genome DNA from antibiotic-resistant MTB complex isolates (provided by an external laboratory) and characterized by the mutations given in the table below.

Mutations in the 81 bp hot-spot region of the rpoB gene (numbering of *E. coli* codons)
Q510L, L511P, L511R, Q513L, Q513P, M515I, D516V, D516Y, D516G, Q517P, S522L, S522P, H526L, H526Y, H526D, H526N, H526R, H526C, H526P, S531L, S531W, A532V, L533P
Mutations in the region of codon 315 of the katG gene
S315N, S315T
Mutations in the promoter region of the inhA gene
-15T, -8A, -8C, -7A

The samples of extracted DNA were diluted and analyzed in association with the ELITe InGenius system in "PCR Only" mode.

All isolates tested resulted positive for MTB and were correctly typed as resistant to Rifampicin and/or Isoniazid by the MDR/MTB ELITe MGB® Kit product.

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Detection efficiency (inclusivity)

The detection efficiency of the mycobacteria species included in the *Mycobacterium tuberculosis* complex of the MDR/MTB ELITe MGB® Kit product was evaluated by *in silico* analysis of sequences available in the EBI ENA database.

The regions selected for hybridization of the primers and fluorescent probes were verified with respect to the alignment of the sequences of the targets MTB (IS6110), rpoB, katG and inhA. The hybridization regions highlighted sequence conservation and the absence of significant mutations in *M. tuberculosis, M. bovis, M. africanum, M. canettii, M. microti, M. caprae*.

The efficiency of detection of the mycobacteria species included in the *Mycobacterium tuberculosis* complex was also verified by analysis of a panel of certified genome DNA of *M. tuberculosis, M. bovis, M. africanum, M. canettii, M. microti, M. caprae*.

The samples of extracted DNA were diluted and analyzed in association with the ELITe InGenius system in "PCR Only" mode.

All isolates tested were detected as positive for MTB by the MDR/MTB ELITe MGB® Kit product.

Potentially interfering markers

The potential cross-reactivity with other targets of the MDR/MTB ELITe MGB® Kit product was evaluated by *in silico* analysis of sequences available in the EBI ENA database.

The regions selected for hybridization of the primers and fluorescent probes were verified with respect to the alignment of prokaryotic sequences, including nontuberculous mycobacteria (NTM) and other organisms that could be present in clinical samples. The hybridization regions highlighted the absence of significant homologies and have not indicated potential interferences.

The absence of cross-reactivity with NTM was also verified by analysis of a panel of certified genome DNA of *M. avium, M. gordonae, M. abscessus, M. intracellulare, M. fortuitum, M. kansasii, M. xenopi, M. chelonae*. Additionally, a panel of certified genome DNA of other organisms potentially present in sputum samples was also analyzed: *Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila, Streptococcus pneumoniae* and *Haemophilus influenzae*.

The samples of extracted DNA were analyzed in association with the ELITe InGenius system in "PCR Only" mode.

All isolates were negative for MTB when analyzed with the MDR/MTB ELITe MGB® Kit product.

Interfering substances

A panel of potentially interfering substances at the maximum relevant concentration was tested with the MDR/MTB ELITe MGB® Kit product. The substances tested were antibiotics (Rifampicin and Isoniazid) and sputum components (mucin, whole human blood).

The substances were individually added to MTB-negative sputum samples positivized with *Mycobacterium tuberculosis* reference material at a concentration of approx. 2500 CFU/mL.

The samples were tested in 3 replicates with the ELITe InGenius system in "Extract + PCR" mode.

The results are shown in the following table.

Substance	Concentration	Corrected results
Rifampicin	25 µg/ml	3/3
Isoniazid	50 µg/ml	3/3
Porcine mucin	2% w/v (20 mg/ml)	3/3
Whole blood in EDTA	5% v/v	3/3

None of the substances tested at the maximum relevant concentration was shown to interfere with the MDR/MTB ELITe MGB® Kit product.

Repeatability

The repeatability of the results obtained with the MDR/MTB ELITe MGB® Kit product in association with the ELITe InGenius system was verified by analyzing MTB-positive and MTB-negative samples.

A sample of MTB-negative sputum positivized with *Mycobacterium tuberculosis* reference material at the concentration of approx. 2500 CFU / mL and a sample of MTB-negative sputum were analyzed in three replicates in two sessions per day with a same batch of product (intra-session repeatability). Three different product batches were analyzed on three different days (inter-batch repeatability) with the same instrument and by the same operator.

The samples were processed on the ELITe InGenius system in "Extract + PCR" mode.

The Ct values of the MTB complex target (IS6110) and of the Internal Control target (IC2), as well as the Tm values of all pathogen targets (IS6110, rpoB, katG and inhA) were used to calculate the Percent Coefficient of Variation (%CV) in order to assess repeatability, understood as imprecision.

Below is a summary of the results.

	Intra-session repeatability		Inter-batch repeatability	
Target	**mean Ct**	**%CV Ct**	**mean Ct**	**%CV Ct**
MTB	30.29	0.92	30.43	1.09
IC	29.11	1.91	29.89	2.54
Target	**mean Tm**	**%CV Tm**	**mean Tm**	**%CV Tm**
MTB	68.3	0.08	68.4	0.11
rpoB1	67.0	0.12	67.0	0.20
rpoB2	71.4	0.11	71.5	0.15
rpoB3	69.9	0.37	70.0	0.26
rpoB4	66.3	0.71	66.4	0.53
katG	70.8	0.08	70.8	0.13
inhA	67.7	0.24	67.0	0.20

The repeatability of the MDR/MTB ELITe MGB® Kit product for each target has shown a %CV that did not exceed 3%.

Reproducibility

The reproducibility of the results obtained with the MDR/MTB ELITe MGB® Kit product in association with the ELITe InGenius system was verified by analyzing MTB-positive and MTB-negative samples.

A sample of MTB-negative sputum positivized with *Mycobacterium tuberculosis* reference material at the concentration of approx. 2500 CFU / mL and a sample of MTB-negative sputum were analyzed in three replicates in two sessions per day. Three different product batches were analyzed on three different days, on three different instruments and by three different operators.

The samples were processed on the ELITe InGenius system in "Extract + PCR" mode.

The Ct values of the MTB target (IS6110) and of the internal control target (IC2), as well as the Tm values of the pathogen targets (IS6110, rpoB, katG and inhA) were used to calculate the percent coefficient of variation (%CV) in order to assess reproducibility as imprecision.

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Below is a summary of the results.

	Reproducibility	
Target	**mean Ct**	**%CV Ct**
MTB	30.71	1.04
IC	30.88	2.39
Target	**mean Tm**	**%CV Tm**
MTB	68.4	0.23
rpoB1	66.9	0.19
rpoB2	71.5	0.21
rpoB3	70.0	0.30
rpoB4	66.4	0.51
katG	70.9	0.16
inhA	67.2	0.61

The Reproducibility of the MDR/MTB ELITe MGB® Kit product for each target has shown a %CV that did not exceed 3%.

Sputum: Diagnostic Sensitivity and Specificity

The assay's Diagnostic Sensitivity, as confirmation of positive clinical samples, was evaluated by analyzing 50 clinical samples of MTB-positive sputum tested by culture.

The assay's Diagnostic Specificity, as confirmation of negative clinical samples, was evaluated by using 50 clinical samples of MTB-negative sputum tested by culture.

The sputum samples were collected, pretreated (see "Samples and controls), placed in culture and certified as MTB-positive or MTB-negative by an external laboratory. The samples were then inactivated and tested with the MDR/MTB ELITe MGB® Kit product and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

		Culture		
		Pos.	**Neg.**	**Total**
MDR/MTB ELITe MGB Kit	**Pos.**	50	1	51
	Neg.	0	47	47
	Total	50	48	98

All MTB-positive samples resulted positive with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's Diagnostic Sensitivity is 100%.

Among the MTB-negative samples, two samples resulted invalid and were excluded from the analysis. Of the 48 valid samples, 47 resulted negative with the MDR/MTB ELITe MGB® Kit product. The discordant positive result was confirmed as positive by re-testing the sputum sample. In this test, the assay's diagnostic specificity is 98%.

Furthermore, the same MTB-positive and MTB-negative samples were also tested by another CE-IVD marked molecular diagnostic assay and by AFB Smear Microscopy.

The clinical samples analyzed by another EC-IVD marked molecular diagnostic assay resulted 50 MTB-positive and 50 MTB-negative as when tested by culture. The table below summarizes the comparison of the results with the MDR/MTB ELITe MGB® Kit product.

		MDx CE-IVD Assay		
		Pos.	**Neg.**	**Total**
MDR/MTB ELITe MGB Kit	**Pos.**	50	1	51
	Neg.	0	47	47
	Total	50	48	98

All MTB-positive samples resulted positive with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's Diagnostic Sensitivity is 100%.

Among the MTB-negative samples, two samples resulted invalid and were excluded from the analysis. Of the 48 valid samples, 47 resulted negative with the MDR/MTB ELITe MGB® Kit product. The discordant positive result was confirmed as positive by re-testing the sputum sample. In these tests, the assay's Diagnostic Specificity is 98%.

The clinical samples tested by AFB Smear Microscopy resulted 44 MTB-positive and 56 MTB-negative. The table below summarizes the comparison of the results with the MDR/MTB ELITe MGB® Kit product.

		AFB Smear Microscopy		
		Pos.	Neg.	Total
MDR/MTB ELITe MGB Kit	Pos.	44	7	51
	Neg.	0	47	47
	Total	44	54	98

All MTB-positive samples resulted positive with the MDR/MTB ELITe MGB® Kit product.

Among the MTB-negative samples, two samples resulted invalid and were excluded from the analysis. Of the 54 valid samples, 47 resulted negative with the MDR/MTB ELITe MGB® Kit product. Six of 7 discrepant samples were confirmed as positive by two other reference methods (culture and CE-IVD marked molecular diagnostic assay). The last discordant positive result was confirmed positive by re-testing the sample of sputum alone with the MDR/MTB ELITe MGB® Kit product.

Sputum: Confirmation of antibiotic-resistant MTB-positive samples

The Diagnostic Sensitivity of the antibiotic resistance assay, as confirmation of antibiotic-resistant MTB-positive samples, was evaluated by analyzing the following samples:

- 20 clinical sputum samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation),
- 20 clinical sputum samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation),
- 20 clinical sputum samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation).

Clinical samples of MTB-negative sputum were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Res. (rpoB)	INH Res. (katG)	INH Res. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	20	20	0	0	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	20	0	17	0	3
MTB-positive samples, resistant to Isoniazid (inhA mutation)	20	0	0	18	2

All samples resulted MTB-positive.

All samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation) were correctly typed as possible resistant to Rifampicin.

Seventeen out of 20 samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation) were correctly typed as possible resistant to Isoniazid. It was not possible to type 3 samples due to the low MTB signal and were therefore excluded from the analysis.

Eighteen out of 20 samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation) were correctly typed as possible resistant to Isoniazid. It was not possible to type 2 samples due to the low MTB signal and were therefore excluded from the analysis.

In these tests, the Diagnostic Sensitivity of the antibiotic resistance assay is 100%.

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Sputum: Confirmation of antibiotic-sensitive MTB-positive samples

The Diagnostic Specificity of the antibiotic resistance assay, as confirmation of antibiotic-sensitive MTB-positive samples, was evaluated by analyzing 50 clinical samples of sputum positive for antibiotic-sensitive MTB.

The sputum samples were collected, pretreated (see "Samples and controls), placed in culture and certified as MTB-positive and sensitive to Rifampicin and Isoniazid by an external laboratory using an antimicrobial susceptibility test. The samples were then inactivated and tested with the MDR/MTB ELITe MGB® Kit product and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Resistance negative + INH Resistance negative	Typing not feasible
MTB-positive samples, sensitive to antibiotics	50	47	3

All samples resulted MTB-positive, and 47 out of 50 were correctly typed as possible sensitive to Rifampicin and Isoniazid. It was not possible to type 3 samples due to the low MTB signal and were therefore excluded from the analysis.

In this test, the Diagnostic Specificity of the antibiotic resistance assay is 100%.

BAL / BA: Diagnostic Sensitivity and Specificity

The assay's Diagnostic Sensitivity, as confirmation of positive clinical samples, was evaluated by analyzing 6 clinical samples of MTB-positive BAL / BA tested by culture and 40 positivized clinical samples of BAL / BA.

The assay's Diagnostic Specificity, as confirmation of negative clinical samples, was evaluated by using 40 clinical samples of MTB-negative BAL / BA tested by culture.

The BAL / BA samples were collected, pretreated (see "Samples and controls), placed in culture and certified as MTB-positive or MTB-negative by an external laboratory. Positivized samples were added with MTB isolates sensible to antibiotics at the final concentration of approx. 20 CFU/mL. The samples were then inactivated and tested with the MDR/MTB ELITe MGB® Kit product and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

		Culture		
		Pos.	Neg.	Total
MDR/MTB ELITe MGB Kit	Pos.	42	1	43
	Neg.	4	39	43
	Total	46	40	86

Among the MTB-positive or positivized samples, 42 out of 46 samples resulted with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's Diagnostic Sensitivity is 91.3 %.

Among the MTB-negative samples, 39 out of 40 samples resulted negative with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's diagnostic specificity is 97.5 %.

BAL / BA: Confirmation of antibiotic-resistant MTB-positive samples

The Diagnostic Sensitivity of the antibiotic resistance assay, as confirmation of antibiotic-resistant MTB-positive samples, was evaluated by analyzing the following samples:

- 2 clinical BAL / BA samples MTB-positive tested by culture and resistant to Rifampicin and Isoniazid
- 40 clinical BAL / BA samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation),
- 40 clinical BAL / BA samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation),
- 40 clinical BAL / BA samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation).

Clinical samples of MTB-negative BAL / BA were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Res. (rpoB)	INH Res. (katG)	INH Res. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin and Isoniazid	2	0	0	0	2
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	40	40	0	0	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	40	0	40	0	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	40	0	0	39	1

All samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation) were correctly typed as possible resistant to Rifampicin.

All samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation) were correctly typed as possible resistant to Isoniazid.

All samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation) were correctly typed as possible resistant to Isoniazid. It was not possible to type 1 sample due to the low MTB signal and were therefore excluded from the analysis.

In these tests, the Diagnostic Sensitivity of the antibiotic resistance assay is 100%.

BAL / BA: Confirmation of absence of mutation in MTB-positive samples

The Diagnostic Specificity of the antibiotic resistance assay, as confirmation of antibiotic-sensitive MTB-positive samples, was evaluated by analyzing 80 clinical samples of BAL / BA positivized with an MTB isolate bearer of a mutation but negative for the other two.

Clinical samples of MTB-negative BAL / BA were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Sens. (rpoB)	INH Sens. (katG)	INH Sens. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	40	0	40	40	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	40	40	0	40	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	40	39	39	0	1

All samples positivized with an MTB isolate with a mutation of rpoB gene were correctly typed as not mutated for katG and inhA genes.

All samples positivized with an MTB isolate with a mutation of katG gene were correctly typed as not mutated for rpoB and inhA genes.

All samples positivized with an MTB isolate with a mutation of inhA gene were correctly typed as not mutated for rpoB and katG genes. It was not possible to type 1 sample due to the low MTB signal and were therefore excluded from the analysis.

In this test, the Diagnostic Specificity of the antibiotic resistance assay is 100%.

Urine: Diagnostic Sensitivity and Specificity

The assay’s Diagnostic Sensitivity, as confirmation of positive clinical samples, was evaluated by analyzing 12 clinical samples of MTB-positive urine tested by culture and 8 positivized clinical samples of urine.

The assay’s Diagnostic Specificity, as confirmation of negative clinical samples, was evaluated by using 20 clinical samples of MTB-negative urine tested by culture.

The urine samples were collected, pretreated (see “Samples and controls), placed in culture and certified as MTB-positive or MTB-negative by an external laboratory. Positivized samples were added with MTB isolates sensible to antibiotics at the final concentration of approx. 20 CFU/mL. The samples were then inactivated and tested with the MDR/MTB ELITe MGB® Kit product and the ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

		Culture		
		Pos.	Neg.	Total
MDR/MTB ELITe MGB Kit	Pos.	16	0	16
	Neg.	4	20	24
	Total	20	20	40

Among the MTB-positive or positivized samples, 16 out of 20 samples resulted with the MDR/MTB ELITe MGB® Kit product. In this test, the assay’s Diagnostic Sensitivity is 80 %.

Among the MTB-negative samples, all samples resulted negative with the MDR/MTB ELITe MGB® Kit product. In this test, the assay’s diagnostic specificity is 100 %.

Urine: Confirmation of antibiotic-resistant MTB-positive samples

The Diagnostic Sensitivity of the antibiotic resistance assay, as confirmation of antibiotic-resistant MTB-positive samples, was evaluated by analyzing the following samples:

- 20 clinical urine samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation),
- 20 clinical urine samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation),
- 20 clinical urine samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation).

Clinical samples of MTB-negative urine were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Res. (rpoB)	INH Res. (katG)	INH Res. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	20	20	0	0	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	20	0	20	0	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	20	0	0	20	0

All samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation) were correctly typed as possible resistant to Rifampicin.

All samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation) were correctly typed as possible resistant to Isoniazid.

All samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation) were correctly typed as possible resistant to Isoniazid.

In these tests, the Diagnostic Sensitivity of the antibiotic resistance assay is 100%.

Urine: Confirmation of absence of mutation in MTB-positive samples

The Diagnostic Specificity of the antibiotic resistance assay, as confirmation of antibiotic-sensitive MTB-positive samples, was evaluated by analyzing 40 clinical samples of urine positivized with an MTB isolate bearer of a mutation but negative for the other two.

Clinical samples of MTB-negative urine were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Sens. (rpoB)	INH Sens. (katG)	INH Sens. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	20	0	20	20	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	20	20	0	20	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	20	20	20	0	0

All samples positivized with an MTB isolate with a mutation of rpoB gene were correctly typed as not mutated for katG and inhA genes.

All samples positivized with an MTB isolate with a mutation of katG gene were correctly typed as not mutated for rpoB and inhA genes.

All samples positivized with an MTB isolate with a mutation of inhA gene were correctly typed as not mutated for rpoB and katG genes.

In this test, the Diagnostic Specificity of the antibiotic resistance assay is 100%.

Biopsy: Diagnostic Sensitivity and Specificity

The assay's Diagnostic Sensitivity, as confirmation of positive clinical samples, was evaluated by analyzing 22 clinical samples of MTB-positive biopsy tested by culture and 20 positivized clinical samples of biopsy.

The assay's Diagnostic Specificity, as confirmation of negative clinical samples, was evaluated by using 40 clinical samples of MTB-negative biopsy tested by culture.

The biopsy samples were collected, pretreated (see "Samples and controls), placed in culture and certified as MTB-positive or MTB-negative by an external laboratory. Positivized samples were added with MTB isolates sensible to antibiotics at the final concentration of approx. 20 CFU/mL. The samples were then inactivated and tested with the MDR/MTB ELITe MGB® Kit product and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

		Culture		
		Pos.	Neg.	Total
MDR/MTB ELITe MGB Kit	Pos.	38	0	38
	Neg.	4	40	44
	Total	42	40	82

Among the MTB-positive or positivized samples, 38 out of 42 samples resulted with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's Diagnostic Sensitivity is 90.5 %.

Among the MTB-negative samples, all samples resulted negative with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's diagnostic specificity is 100 %.

Biopsy: Confirmation of antibiotic-resistant MTB-positive samples

The Diagnostic Sensitivity of the antibiotic resistance assay, as confirmation of antibiotic-resistant MTB-positive samples, was evaluated by analyzing the following samples:

- 40 clinical biopsy samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation),
- 40 clinical biopsy samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation),
- 40 clinical biopsy samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation).

Clinical samples of MTB-negative biopsy were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in "Extract + PCR" mode.

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The results are summarized in the following table.

Samples	MTB positive	RIF Res. (rpoB)	INH Res. (katG)	INH Res. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	40	40	0	0	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	40	0	40	0	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	40	0	0	40	0

All samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation) were correctly typed as possible resistant to Rifampicin.

All samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation) were correctly typed as possible resistant to Isoniazid.

All samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation) were correctly typed as possible resistant to Isoniazid.

In these tests, the Diagnostic Sensitivity of the antibiotic resistance assay is 100%.

Biopsy: Confirmation of absence of mutation in MTB-positive samples

The Diagnostic Specificity of the antibiotic resistance assay, as confirmation of antibiotic-sensitive MTB-positive samples, was evaluated by analyzing 80 clinical samples of biopsy positivized with an MTB isolate bearer of a mutation but negative for the other two.

Clinical samples of MTB-negative biopsy were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Sens. (rpoB)	INH Sens. (katG)	INH Sens. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	40	0	40	40	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	40	40	0	40	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	40	40	40	0	0

All samples positivized with an MTB isolate with a mutation of rpoB gene were correctly typed as not mutated for katG and inhA genes.

All samples positivized with an MTB isolate with a mutation of katG gene were correctly typed as not mutated for rpoB and inhA genes.

All samples positivized with an MTB isolate with a mutation of inhA gene were correctly typed as not mutated for rpoB and katG genes.

In this test, the Diagnostic Specificity of the antibiotic resistance assay is 100%.

Cavitary Fluids: Diagnostic Sensitivity and Specificity

The assay's Diagnostic Sensitivity, as confirmation of positive clinical samples, was evaluated by analyzing 20 clinical samples of MTB-positive cavitary Fluids tested by culture and 20 positivized clinical samples of cavitary Fluids.

The assay's Diagnostic Specificity, as confirmation of negative clinical samples, was evaluated by using 40 clinical samples of MTB-negative cavitary Fluids tested by culture.

The cavitary Fluids samples were collected, pretreated (see "Samples and controls), placed in culture and certified as MTB-positive or MTB-negative by an external laboratory. Positivized samples were added with MTB isolates sensible to antibiotics at the final concentration of approx. 20 CFU/mL. The samples were then inactivated and tested with the MDR/MTB ELITe MGB® Kit product and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

		Culture		
		Pos.	Neg.	Total
MDR/MTB ELITe MGB Kit	Pos.	39	0	39
	Neg.	1	40	41
	Total	40	40	80

Among the MTB-positive or positivized samples, 39 out of 40 samples resulted with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's Diagnostic Sensitivity is 97.5 %.

Among the MTB-negative samples, all samples resulted negative with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's diagnostic specificity is 100 %.

Cavitary Fluids: Confirmation of antibiotic-resistant MTB-positive samples

The Diagnostic Sensitivity of the antibiotic resistance assay, as confirmation of antibiotic-resistant MTB-positive samples, was evaluated by analyzing the following samples:

- 40 clinical cavitary Fluids samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation),
- 40 clinical cavitary Fluids samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation),
- 40 clinical cavitary Fluids samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation).

Clinical samples of MTB-negative cavitary Fluids were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Res. (rpoB)	INH Res. (katG)	INH Res. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	40	40	0	0	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	40	0	40	0	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	40	0	0	40	0

All samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation) were correctly typed as possible resistant to Rifampicin.

All samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation) were correctly typed as possible resistant to Isoniazid.

All samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation) were correctly typed as possible resistant to Isoniazid.

In these tests, the Diagnostic Sensitivity of the antibiotic resistance assay is 100%.

Cavitary Fluids: Confirmation of absence of mutation in MTB-positive samples

The Diagnostic Specificity of the antibiotic resistance assay, as confirmation of antibiotic-sensitive MTB-positive samples, was evaluated by analyzing 80 clinical samples of cavitary Fluids positivized with an MTB isolate bearer of a mutation but negative for the other two.

Clinical samples of MTB-negative cavitary Fluids were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in "Extract + PCR" mode.

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The results are summarized in the following table.

Samples	MTB positive	RIF Sens. (rpoB)	INH Sens. (katG)	INH Sens. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	40	0	40	40	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	40	40	0	40	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	40	40	40	0	0

All samples positivized with an MTB isolate with a mutation of rpoB gene were correctly typed as not mutated for katG and inhA genes.

All samples positivized with an MTB isolate with a mutation of katG gene were correctly typed as not mutated for rpoB and inhA genes.

All samples positivized with an MTB isolate with a mutation of inhA gene were correctly typed as not mutated for rpoB and katG genes.

In this test, the Diagnostic Specificity of the antibiotic resistance assay is 100%.

Gastric Aspirate: Diagnostic Sensitivity and Specificity

The assay's Diagnostic Sensitivity, as confirmation of positive clinical samples, was evaluated by analyzing 22 clinical samples of MTB-positive gastric aspirate tested by culture.

The assay's Diagnostic Specificity, as confirmation of negative clinical samples, was evaluated by using 20 clinical samples of MTB-negative gastric aspirate tested by culture.

The gastric aspirate samples were collected, pretreated (see "Samples and controls), placed in culture and certified as MTB-positive or MTB-negative by an external laboratory. The samples were then inactivated and tested with the MDR/MTB ELITe MGB® Kit product and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

		Culture		
		Pos.	Neg.	Total
MDR/MTB ELITe MGB Kit	Pos.	18	0	18
	Neg.	4	20	24
	Total	22	20	42

Among the MTB-positive or positivized samples, 18 out of 22 samples resulted with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's Diagnostic Sensitivity is 81.8 %.

Among the MTB-negative samples, all samples resulted negative with the MDR/MTB ELITe MGB® Kit product. In this test, the assay's diagnostic specificity is 100 %.

Gastric Aspirate: Confirmation of antibiotic-resistant MTB-positive samples

The Diagnostic Sensitivity of the antibiotic resistance assay, as confirmation of antibiotic-resistant MTB-positive samples, was evaluated by analyzing the following samples:

- 20 clinical gastric aspirate samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation),
- 20 clinical gastric aspirate samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation),
- 20 clinical gastric aspirate samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation).

Clinical samples of MTB-negative gastric aspirate were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Res. (rpoB)	INH Res. (katG)	INH Res. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	20	20	0	0	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	20	0	20	0	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	20	0	0	20	0

All samples positivized with an MTB isolate resistant to Rifampicin (rpoB gene mutation) were correctly typed as possible resistant to Rifampicin.

All samples positivized with an MTB isolate resistant to Isoniazid (katG gene mutation) were correctly typed as possible resistant to Isoniazid.

All samples positivized with an MTB isolate resistant to Isoniazid (inhA gene mutation) were correctly typed as possible resistant to Isoniazid.

In these tests, the Diagnostic Sensitivity of the antibiotic resistance assay is 100%.

Gastric Aspirate: Confirmation of absence of mutation in MTB-positive samples

The Diagnostic Specificity of the antibiotic resistance assay, as confirmation of antibiotic-sensitive MTB-positive samples, was evaluated by analyzing 40 clinical samples of gastric aspirate positivized with an MTB isolate bearer of a mutation but negative for the other two.

Clinical samples of MTB-negative gastric aspirate were positivized at the final concentration of approx. 5000 CFU/mL with MTB isolates certified by an external laboratory for mutations in one of the three target genes using a CE-IVD marked molecular diagnostic assay. The samples were tested with MDR/MTB ELITe MGB® Kit and the ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	MTB positive	RIF Sens. (rpoB)	INH Sens. (katG)	INH Sens. (inhA)	Typing not feasible
MTB-positive samples, resistant to Rifampicin (rpoB mutation)	20	0	20	20	0
MTB-positive samples, resistant to Isoniazid (katG mutation)	20	20	0	20	0
MTB-positive samples, resistant to Isoniazid (inhA mutation)	20	20	20	0	0

All samples positivized with an MTB isolate with a mutation of rpoB gene were correctly typed as not mutated for katG and inhA genes.

All samples positivized with an MTB isolate with a mutation of katG gene were correctly typed as not mutated for rpoB and inhA genes.

All samples positivized with an MTB isolate with a mutation of inhA gene were correctly typed as not mutated for rpoB and katG genes.

In this test, the Diagnostic Specificity of the antibiotic resistance assay is 100%.

Please note: The complete data and results from the tests carried out to evaluate the product’s performance features with matrices and instruments are recorded in the Product Technical File for the "MDR/MTB ELITe MGB Kit", FTP 120ING.

REFERENCES

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PROCEDURE LIMITATIONS

Use this product only with clinical samples of sputum, bronchoalveolar lavages (BAL), bronchial aspirates (BA), urine, cavity fluids, biopsies and gastric aspirates liquefied, decontaminated and inactivated.

Do not use this product with samples containing mucine at concentration higher than 2%: mucine inhibits the amplification reaction of nucleic acids and can cause invalid results.

At the moment there are no data available concerning product performance with the following clinical samples: cerebrospinal fluid (CSF), necrotic materials, pus, stool, whole blood.

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. 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.

Results obtained with this product about possible resistance to Rifampicin and/or Isoniazid of the MTB are limited to the detection of main mutations as indicated in the “Assay Principles” Section. Other mutations not detected by this product can be associated to resistance to Rifampicin and/or Isoniazid. On the other hand, silent mutations can be detected by this product, but they are not associated to resistance to Rifampicin and/or Isoniazid. Phenotypic Antimicrobial Susceptibility Test is then required to confirm MTB Rifampicin and/or Isoniazid susceptibility.

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**
Session setup error.	Check position of the PCR Mix and positive control. Check volumes of the 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 Assistance.

Invalid Negative Control reaction	
Possible causes	**Solutions**
Session setup error.	Check position of the PCR Mix and negative control. Check volumes of the 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 new aliquots of PCR Mixes.
Contamination of the extraction area, of racks or of inventory blocks.	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Assistance.

Invalid Sample reaction **Inconclusive results / Typing not feasible / Typing invalid**	
Possible causes	**Solutions**
Session setup error.	Check position of the PCR Mix and sample. Check volumes of the PCR Mix and sample.
Internal Control degradation.	Use new aliquots of internal control.
Inhibition due to sample interfering substances.	Repeat the amplification with a 1:3 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction and amplification of the sample with a 1:2 dilution in molecular biology grade water of the primary sample in a session in “Extract + PCR” mode.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Assistance.

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 required: - 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 primary sample in an “Extract + PCR” session.

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SYMBOLS

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

NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by da Thermo Fisher Scientific and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Life Technologies Corporation. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, da Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008. Phone: +1(760)603-7200. Fax: +1(760)602-6500. Email: outlicensing@thermofisher.com.

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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MDR/MTB ELITe MGB® kit used with ELITe InGenius®

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This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com
This document is available only in English.

A. Intended use

The «MDR/MTB ELITe MGB® Kit» is part of a qualitative nucleic acid amplification assay to detect *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. africanum, M. bovis, M. canettii, M. microti, M. caprae*) DNA and to identify the main mutations associated with resistance to Rifampicin and/or Isoniazid.

The product may be used for two different purposes:

- as an aid in the diagnosis of tuberculosis from Mycobacterium tuberculosis complex, in association with the patient's clinical data and other laboratory test results, in particular the culture methods for mycobacterium,

- as an aid in the diagnosis of tuberculosis and genotypic resistance of Mycobacterium tuberculosis complex, in association with the patient's clinical data and other laboratory test results, in particular phenotypic testing for antimicrobial susceptibility.

The assay is CE-IVD validated in combination with the instrument **ELITe InGenius®**.

B. Amplified sequence

Mix	Target	Gene	Fluorophore
TB1	**MTB Complex**	IS6110	FAM
	Rifampicin resistance	rpoB gene (rpoB2, rpoB3, rpoB4)	AP639; AP525; AP593
TB2	**Rifampicin resistance**	rpoB gene (rpoB1)	AP639
	Isoniazid resistance	katG	FAM
		inhA	AP593
TB1/TB2	**Internal Control**	Artificial sequence	AP680

C. Validated matrix

› **Sputum, bronchial aspirates (BA), bronchoalveolar lavages (BAL), urine, biopsies, cavity fluids and gastric aspirates** previously liquefied, decontaminated and inactivated.

D. Kit content

TB1 PCR Mix	TB2 PCR Mix
Ready-to-use PCR Master Mix 4 tubes of 280 µL 7 freeze-thaw cycles per tube	Ready-to-use PCR master Mix 4 tubes of 280 µL 7 freeze-thaw cycles per tube

› 48 reactions per kit › Storage Temperature: -20°C › Maximum shelf-life: 24 months

E. Material required not provided in the kit

› **ELITe InGenius** instrument: INT030
› **ELITe InGenius SP 200** extraction cartridges: INT032SP200
› **ELITe InGenius PCR Cassette** amplification cartridges: INT035PCR
› **ELITe InGenius SP 200 Consumable Set** consumables for extraction: INT032CS
› **MDR/MTB- ELITe Positive Control :** CTR120ING
› **CPE- Internal Control :** CTRCPE
› **ELITe InGenius Waste Box:** F2102-000
› **300 µL Filter Tips Axygen:** TF-350-L-R-S

F. ELITe InGenius protocol

› Sample volume	200 µL
› Internal Control volume	10 µL
› Total eluate volume	100 µL
› PCR eluate input volume for each mix	20 µL
› Q-PCR Mix volume for each mix	20 µL
› Unit of qualitative result	CFU/mL
› Frequency of controls	15 days

G. Performance

Sample	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
MTB Complex	Sputum	**6 CFU/mL**	**100%** 50/50*	**98%** 47/48*
Rifampicin and Isoniazid resistance	Sputum	-	**100%** 55/55*	**100%** 47/47*
MTB Complex	BAL/BA	**6 CFU/mL**	**91.3%** 42/46*	**97.5%** 39/40*
Rifampicin and Isoniazid resistance	BAL/BA	-	**100%** 119/119*	**100%** 119/119*
MTB Complex	Urine	**20 CFU/mL**	**80%** 16/20*	**100%** 20/20*
Rifampicin and Isoniazid resistance	Urine	-	**100%** 60/60*	**100%** 60/60*
MTB Complex	Biopsy	**20 CFU/mL**	**90.5%** 38/42*	**100%** 40/40*
Rifampicin and Isoniazid resistance	Biopsy	-	**100%** 120/120*	**100%** 120/120*
MTB Complex	Cavitary Liquid	**20 CFU/mL**	**97.5%** 39/40*	**100%** 40/40*
Rifampicin and Isoniazid resistance	Cavitary Liquid	-	**100%** 120/120*	**100%** 120/120*
MTB Complex	Gastric aspirates	**20 CFU/mL**	**81.8%** 18//22*	**100%** 20/20*
Rifampicin and Isoniazid resistance	Gastric aspirates	-	**100%** 60/60*	**100%** 60/60*

**confirmed samples/ tested samples*

H. Procedures

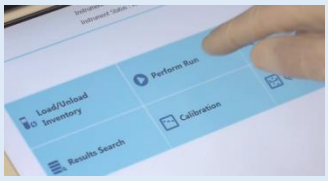
The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed. Three operational mode are available: complete run, or extraction only, or PCR only.

Before analysis

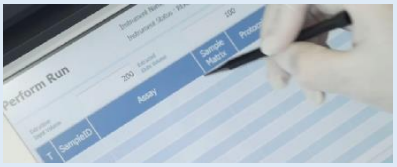
1. Switch on ELITe InGenius Identification with username and password Select the mode "Closed"	**2.** Verify controls: TB pos. and neg. controls in the "Control menu" *NB:* Both have been run, approved and not expired	**3.** Thaw TB1 and TB2 PCR Mixes and the Internal Control tubes Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

1. Select “Perform Run” on the touch screen



2. Verify the extraction volumes:
Input: “200 µL”, eluate: “100 µL”



3. Scan the sample barcodes with hand-held barcode reader or type the sample ID

4. Select the “Assay protocol” of interest

5. Select the sample position:
“Extraction tube”

6. Load the PCR Mixes and the Internal Control in the inventory block

7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, Extraction tube racks

8. Close the door
Start the run

9. View, approve and store the results

Procedure 2 - PCR only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “PCR only” and set the sample position “Extra tube”	**6.** Load the extracted nucleic acid tubes in the rack n°4
7. Load the PCR cassette rack Load the PCR Mixes in the inventory block	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” and set the sample position: Extraction tube	**6.** Load the Internal Control in the inventory block
7. Load: Extraction cartridge, Elution tube, Tip cassette, Extraction tube racks	**8.** Close the door Start the run	**9.** Archive the eluate sample

**confirmed samples/ tested samples*