Instructions for use

MTB EXTRA ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS121ING

UDI 08033891487263

CE IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
03	Additional testing on negative sputum samples.	02/09/25
02	Expanded use on the automated and integrated instrument ELITe BeGenius with Bronchoalveolar lavage / bronchial aspirates samples, Urine, Cavitary Fluids, Biopsies and Gastric aspirate matrices. New graphics and content setting of the IFU.	29/11/24
01	Expanded use on the automated and integrated instrument ELITe BeGenius and sputum matrix.	22/02/24
00	new product development	—

NOTE

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

TABLE OF CONTENT

1 INTENDED USE

The product **MTB EXTRA ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of *Mycobacterium tuberculosis* (MTB) complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canettii*, *M. microti, M. caprae*) DNA.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of sputum, bronchial aspirates (BA), bronchoalveolar lavages (BAL), urine, cavity fluids, biopsies and gastric aspirates previously liquefied, decontaminated and inactivated.

The product is intended for use as an aid in the diagnosis of infections from *Mycobacterium tuberculosis* complex, in combination with all relevant clinical observations and laboratory outcomes, in particular the culture methods for *Mycobacterium*.

2 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting *Mycobacterium tuberculosis* (MTB) complex isolated from specimens and amplified using the assay reagent **MTB EXTRA PCR Mix**, that contains primers and probes with ELITe MGB technology.

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

In the ELITe MGB probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore. Note the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

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

- the **IS6110 MTB complex** repeated sequence, detected in Channel **MTB**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye.
- Internal control (**IC**), specific for artificial sequence IC2**,** detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor® 680 (AP680) dye.

The **MTB EXTRA PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, the stabilizers and hot start DNA polymerase.

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

The **MTB EXTRA ELITe MGB Kit** can be also used in association with equivalent instruments.

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
MTB EXTRA PCR Mix ref. RTS121ING	Mixture of reagents for Real-Time PCR, in tube with RED cap	**8 x 280 µL**	**-**

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~5,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (volume range: 0.5-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

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

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

Table 2

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA, ref. INT030). **ELITe InGenius Software** version 1.3.0.19 (or later). **MTB EXTRA ELITe_PC**, Assay Protocol with parameters for Positive Control analysis. **MTB EXTRA ELITe_NC**, Assay Protocol with parameters for Negative Control analysis. **MTB EXTRA ELITe_SP_200_100,** Assay Protocol with parameters for Sputum specimen analysis. **MTB EXTRA ELITe_BAL_200_100,** Assay Protocol with parameters for Bronchoalveolar lavage (BAL) and bronchial aspirates (BA) specimen analysis **MTB EXTRA ELITe_U_200_100,** Assay Protocol with parameters for Urine specimen analysis. **MTB EXTRA ELITe_CL_200_100,** Assay Protocol with parameters for cavitary fluid specimen analysis. **MTB EXTRA ELITe_B_200_100,** Assay Protocol with parameters for biopsy specimen analysis. **MTB EXTRA ELITe_GA_200_100,** Assay Protocol with parameters for gastric aspirate specimen analysis.	**MDR/MTB - ELITe Positive Control** (EG SpA, ref.CTR120ING). **ELITe InGenius SP200** (EG SpA, ref. INT032SP200). **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use)
ELITe BeGenius (EG SpA, ref. INT040). **ELITe BeGenius Software** version 2.3.0 (or later). **MTB EXTRA ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis. **MTB EXTRA ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis. **MTB EXTRA ELITe_Be_SP_200_100,** Assay Protocol with parameters for Sputum specimen analysis. **MTB EXTRA ELITe_Be_BAL_200_100,** Assay Protocol with parameters for Bronchoalveolar lavage (BAL) and bronchial aspirates (BA) specimen analysis. **MTB EXTRA ELITe_Be_U_200_100,** Assay Protocol with parameters for Urine specimen analysis. **MTB EXTRA ELITe_Be_CL_200_100,** Assay Protocol with parameters for cavitary fluid specimen analysis. MTB EXTRA ELITe_Be_B_200_100, Assay Protocol with parameters for biopsy specimen analysis. **MTB EXTRA ELITe_Be_GA_200_100,** Assay Protocol with parameters for gastric aspirate specimen analysis.	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 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 and ELITe BeGenius.

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121° C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

Wear suitable protective clothes and gloves and protect eyes and face.

Never pipette solutions by mouth.

Do not eat, drink, smoke or apply cosmetic products in the work areas.

Carefully wash hands after handling samples and reagents.

Dispose of leftover reagents and waste in compliance with the regulations in force.

Carefully read all the instructions provided before running the assay.

While running the assay, follow the product instructions provided.

Do not use the product after the indicated expiry date.

Only use reagents provided with the product and those recommended by the manufacturer.

Do not use reagents from different batches.

Do not use reagents from other manufacturers.

7.2 Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to sample nucleic acid degradation or sample contamination by PCR products.

Laboratory coats, gloves and tools dedicated to work session setup are needed. It is necessary to have available separate areas for the molecular biology test and the microbiological culture test. Never handle the liquid or solid culture into the area designated for extraction / amplification reactions.

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The extraction products must be handled to prevent dispersion into the environment and to avoid contamination of the instrument's working area.

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

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
MTB EXTRA PCR Mix	-20°C or below (protected from light)	one month	up to seven	up to seven separate* sessions of three hours each or up to 7 consecutive hours (2 sessions of 3 hours each and the time needed to start a third session)

** with intermediate freezing*

8 SPECIMENS AND CONTROLS

8.1 Specimens

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

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ± 10 °C**	**-70 ± 15 °C**
Sputum	liquefied with a solution of N- Acetil L-Cysteine and decontaminated with sodium hydroxide solution(2 then inactivated at 95°C for 30 minutes	≤ 1 hour(1	≤ 2 days(2	≤ 1 month(5	≥ 1 month
Bronchoalveolar lavage (BAL) and bronchial aspirates (BA)	liquefied with a solution of N- Acetil L-Cysteine and decontaminated with sodium hydroxide solution(2 then inactivated at 95°C for 30 minutes	≤ 1 hour(1	≤ 2 days(2	≤ 1 month(5	≥ 1 month
Urine	concentrated and decontaminated with sodium hydroxide solution(2, then inactivated at 95°C for 30 minutes	≤ 1 hour(1	≤ 2 days(2	≤ 1 month(5	≥ 1 month
Cavitary fluid	concentrated and decontaminated with sodium hydroxide solution(2, then inactivated at 95°C for 30 minutes	≤ 1 hour(3	≤ 2 days(2	≤ 1 month(5	≥ 1 month
Biopsy	break down and decontaminated with sodium hydroxide solution(2, then inactivated at 95°C for 30 minutes	≤ 1 hour(3	≤ 7 days(4	≤ 1 month(3	≥ 1 month
Gastric aspirate	liquefied with a solution of N- Acetil L-Cysteine and decontaminated with sodium hydroxide solution(2, then inactivated at 95°C for 30 minutes	≤ 1 hour(1	≤ 2 days(2	≤ 7 days(4	≥ 1 month

1) (CLSI MP48 2nd Edition, "Laboratory Detection and Identification of Mycobacteria")

2) (Mycobacteriology Laboratory Manual, Global Laboratory Initiative).

3) (KPNW Specimen Requirements)

4) (Mayo Clinic Laboratories)

5) (ARUP Laboratories)

It is recommended to divide the specimens into aliquots before freezing to prevent repeated freeze / thaw cycles. When using frozen samples, thaw the samples just before the extraction to avoid possible nucleic acid degradation.

To perform samples testing on the **ELITe InGenius** and **ELITe BeGenius**, the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB Kits and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrices.

Table 5 Assay Protocols for MTB EXTRA ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
Sputum	**ELITe InGenius**	**MTB EXTRA ELITe_SP_200_100**	Positive /Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MTB EXTRA ELITe_Be_SP_200_100**		
Bronchoal-veolar lavage (BAL) and bronchial aspirates (BA)	**ELITe InGenius**	**MTB EXTRA ELITe_BAL_200_100**	Positive /Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MTB EXTRA ELITe_Be_BAL_200_100**		
Urine	**ELITe InGenius**	**MTB EXTRA ELITe_U_200_100**	Positive /Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MTB EXTRA ELITe_Be_U_200_100**		
Cavitary fluid	**ELITe InGenius**	**MTB EXTRA ELITe_CF_200_100**	Positive /Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MTB EXTRA ELITe_Be_CF_200_100**		
Biopsy	**ELITe InGenius**	**MTB EXTRA ELITe_B_200_100**	Positive /Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MTB EXTRA ELITe_Be_B_200_100**		

Table 5 Assay Protocols for MTB EXTRA ELITe MGB Kit (continued)

Gastric aspirate	**ELITe InGenius**	**MTB EXTRA ELITe_GA_200_100**	Positive /Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**MTB EXTRA ELITe_Be_GA_200_100**		

For all protocols, 200 µL of inactivated sample must be transferred into Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius).

NOTE

Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the “Warnings and Precautions” section.

Purified nucleic acids can be left at room temperature for 16 hours and stored at -20 °C or below for no longer than one month.

Refer to11.4 Interfering substances page 20 in the 11 PERFORMANCE CHARACTERISTICS page 19 to check data concerning interfering substances.

8.2 PCR controls

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

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

NOTE

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

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

8.3 Quality controls

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

9 ELITe InGenius PROCEDURE

The procedure to use the **MTB EXTRA ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

Table 6

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) Sample result reporting

9.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Controls" menu on the Home page, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "Specimens and Controls")

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

9.2 STEP 2 - Session Setup

The **MTB EXTRA ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe InGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **12 tests** in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the three types of run follow the steps below while referring to the GUI

Table 7

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature. For this assay, 200 µL of sample must be transferred in an Extraction tube previously labelled.	**Thaw Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 4 reactions.
2	**Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	Not applicable	**Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an “Elution tube”, provided with ELITe InGenius SP 200 Consumable Set.
3	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.
4	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
5	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	Not applicable
6	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”)	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”)	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
7	Ensure the “Protocol” displayed is: “Extract + PCR”.	Select “PCR Only” in the “Protocol” column.	Ensure “PCR Only” is selected in the “Protocol” column.
8	Select the sample loading position as “Extraction Tube” in the “Sample Position” column.	Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”.	Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”.
9	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
10	**Load CPE and PCR Mix** on the “Inventory Block” referring to the “Load List” and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.
11	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
12	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
13	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
14	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**Load** PCR Cassette and Elution tubes with samples extracted	**Load** PCR Cassette, Positive Control and Negative Control tubes.
15	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
16	Close the instrument door.	Close the instrument door.	Close the instrument door.
17	Press “Start”.	Press “Start”.	Press “Start”.

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified and stored at -20 ±10 °C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of about 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the **Positive Control**. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run, the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

9.3 STEP 3 - Review and approval of results

The **ELITe InGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen, results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe InGenius** generates results with the **MTB EXTRA ELITe MGB Kit** through the following procedure:

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

9.3.1 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius Software** interprets the PCR results for the targets of the Positive Control and Negative Control reaction with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct and Tm values are used to verify the system (reagents lot and instrument).

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

The Positive Control and Negative Control results expire after **15 days**.

The results of the Positive Control and Negative Control amplification are used by the **ELITe InGenius software** to set up the Control Charts monitoring the amplification step performances. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control runs must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same run, the samples can be approved but their results are not validated. In this case, the failed Control(s) and samples must all be repeated.

9.3.2 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (channel **MTB**) and the Internal Control (channel **IC**) with the **MTB EXTRA ELITe_SP_200_100**, **MTB EXTRA ELITe_BAL_200_100**, **MTB EXTRA ELITe_U_200_100**, **MTB EXTRA ELITe_CL_200_100**, **MTB EXTRA ELITe_B_200_100**, and **MTB EXTRA ELITe ELITe_GA_200_100** Assay Protocol parameters.

Results are shown in “Results Display” screen.

The sample results can be approved when the two conditions in the table below are true.

Table 8

1) Positive Control	**Status**
MTB EXTRA Positive Control	APPROVED
2) Negative Control	**Status**
MTB EXTRA Negative Control	APPROVED

The sample results are automatically interpreted by the **ELITe InGenius software** using Assay Protocol parameters. The possible result messages are listed in the table below.

For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

Table 9

Result of sample run	**Interpretation**
MTB: DNA detected.	The **MTB complex DNA was detected** in the sample.
MTB: DNA not detected or below the LoD.	The **MTB complex DNA was not detected** in the sample. The sample is a **valid negative** or the target concentration is below the assay Limit of Detection.
Invalid-Retest Sample.	**Not valid assay result** caused by Internal Control failure (due to e.g., incorrect extraction, inhibitors carry- over). The test should be repeated.

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, pretreatment, extraction or PCR steps (e. g., incorrect sampling, degradation or loss of DNA during the extraction or inhibitors in the eluate), which may cause incorrect results. If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see “14 TROUBLESHOOTING page 25”).

Samples reported as: "MTB: DNA not detected or below the LoD" are suitable for analysis but the DNA of MTB complex was not detected. In this case the sample may be either negative for the DNA of the MTB complex, or the DNA of the target is present at a concentration below the Limit of Detection of the assay (see “11 PERFORMANCE CHARACTERISTICS page 19”).

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

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

9.3.3 Sample result reporting

- The sample results are stored in the database and reports can be exported as “Sample Report” and “Track Report”.
- The “Sample Report” shows the results details by selected sample (SID).
- The “Track Report” shows the results details by selected Track.
- The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

10 ELITe BeGenius PROCEDURE

The procedure to use the **MTB EXTRA ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 10

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) Sample result reporting

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in “**CLOSED**” mode,
- in the “Controls” menu on the Home page, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see “Specimens and Controls”).

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

10.2 STEP 2 - Session Setup

The **MTB EXTRA ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All the required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe BeGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for 12 tests in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the three types of run follow the steps below while referring to the GUI:

Table 11

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature). For this assay, **200 µL of sample** must be transferred in a 2mL Sarstedt tube previously labelled.	If needed, **thaw the Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes at room temperature for 30 minutes. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 4 reactions.
2	**Thaw** the needed **CPE** tubes at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	Not applicable	Prepare the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
3	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen	Select "**Perform Run**" from the "Home" screen.
4	Remove all the Racks from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
5	Select the "Run mode": "**Extract + PCR".**	Select the "Run mode": "**PCR Only**".	Select the "Run mode": "**PCR Only**".
6	**Load the samples** into the "Sample Rack". When secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack".	**Load the samples** into the "Elution Rack".	**Load the Positive Control and Negative Control** tubes into the "Elution Rack".
7	**Insert** the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert the "Sample ID" (SID) for each "Position" used (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3). If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
8	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
9	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL	Not applicable	Not applicable
10	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).
11	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
	Note: When more than 12 samples are processed, repeat the procedure from point 6.		Not applicable
12	Load the “Elution tubes” into the “Elution Rack” (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable
13	Insert the “Elution Rack” into the “Cooler Unit” starting from “Lane 3” (L3). When more than 12 samples are processed, repeat using “Lane 2” (L2).	Not applicable	Not applicable
14	Click “Next” to continue.	Not applicable	Not applicable
15	**Load CPE and PCR Mix** into the “Reagent/Elution Rack”.	**Load the PCR Mix** into “Reagent/ Elution Rack”.	**Load the PCR Mix** into “Reagent/ Elution Rack”.
16	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent and / or CPE enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
17	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
18	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
19	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
20	Load the “**PCR Rack**” with “PCR Cassette” in the Inventory Area.	Load the “**PCR Rack**” with “PCR Cassette” in the Inventory Area.	Load the **“PCR Rack**” with “PCR Cassette” in the Inventory Area.
21	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
22	Load the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables.	Not applicable	Not applicable
23	Close the instrument door.	Close the instrument door.	Close the instrument door.
24	Press “Start”.	Press “Start”.	Press “Start”.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified and stored at -20 ±10 °C for no longer than one month. Avoid the spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of about 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the Positive Control. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

10.3 STEP 3 - Review and approval of results

The **ELITe BeGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe BeGenius** generates the results with the **MTB EXTRA ELITe MGB Kit** through the following procedure:

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

NOTE

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

11 PERFORMANCE CHARACTERISTICS

NOTE

The performance characteristic studies performed with the ELITe InGenius were carried out using the "**TB1 PCR Mix**" component of the EG SpA **MDR/MTB ELITe MGB Kit** (Ref. RTS120ING), that has the same formulation of the **MTB EXTRA PCR Mix** component of the EG SpA **MTB EXTRA ELITe MGB Kit** (Ref. RTS121ING). The **MTB EXTRA PCR** was used for the studies performed with ELITe BeGenius; same samples and same thermal profile were used on both instruments.

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined with sputum samples on ELITe InGenius using dilution of *Mycobacterium tuberculosis* (MTB), strain H37Ra reference material (ATCC, ref. 25177).

Probit regression analysis was performed on the results, and the LoD defined as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Table 12 Limit of Detection (CFU/mL) for sputum samples and the ELITe InGenius

Matrix	LoD (CFU / mL)	95% confidence interval limits	
		Lower limit	Upper limit
Sputum	6	4	15

The calculated LoD value was verified by testing on ELITe InGenius and ELITe BeGenius, sputum and BAL / BA, spiked by MTB reference material at the claimed concentration (6 CFU/mL), testing 20 replicates.

The Limit of Detection (LoD) of the assay in association to Cavitary Fluid, Urine and Biopsy was verified on the ELITe InGenius and ELITe BeGenius instruments, testing 20 replicates of matrix samples and spiked by MTB certified reference material at concentration of 20 CFU/mL.

The results for each matrix are reported in the following table.

Table 13 Limit of Detection with ELITe InGenius and ELITe BeGenius

Sample	Titer
Sputum	6 CFU / mL
BAL/BA	6 CFU / mL
Urine	20 CFU / mL
Cavitary fluid	20 CFU / mL
Biopsy	20 CFU / mL
Gastric aspirate	20 CFU / mL

11.2 Inclusivity: Efficiency of detection

The inclusivity of the assay, as detection efficiency of the mycobacteria species included in the *Mycobacterium tuberculosis* complex of the product was evaluated by *in silico* analysis of sequences available in nucleotide database.

The analysis showed sequence conservation and absence of significant mutation in the available MTB sequence. So, an efficient detection for the different strains and/or isolates is expected.

The inclusivity was also verified through the analysis of certified genomic DNA from clinical samples (provided by an external laboratory) and plasmid DNAs.

All samples were correctly detected as positive for MTB by the product on ELITe InGenius.

11.3 Potentially interfering markers

The potential cross-reactivity with other unintended organisms of the assay was evaluated by *in silico* analysis of sequences available in the EBI ENA nucleotide database.

The regions chosen for hybridisation of the primers and the fluorescent probes were checked on the alignment of the sequences, of nontuberculous mycobacteria (NTM) and other organisms that could be present in clinical samples. The hybridisation regions showed absence of significant homologies and no potential interference.

The absence of cross-reactivity with NTM was also verified by testing 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*.

All the potential cross-reacting organisms were negative for MTB when tested by the MTB EXTRA ELITe MGB Kit.

11.4 Interfering substances

The cross-reactivity by potentially interfering substances that might be found in samples was evaluated for the assay by analysis of a panel of substances at relevant concentrations. The substances tested were antibiotics (Rifampicin and Isoniazid) and sputum components (mucin, whole human blood).

The results are reported in the following table.

Table 14

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

The test showed that the tested substances do not inhibit the targets detection using the MTB EXTRA ELITe MGB Kit.

11.5 Repeatability

The repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of sputum negative or spiked with *Mycobacterium tuberculosis* reference material (ATCC, ref.

25177).

An example of Intra-Session Repeatability (on one day) results is shown in the tables below.

Table 15 ELITe InGenius Intra-session Repeatability

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	8	-	-	-	100%
3x LoD	8	36.55	0.62	1.71	100%
10x LoD	8	35.86	0.65	1.81	100%

Table 16 ELITe BeGenius Intra-session Repeatability

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	8	-	-	-	100%
3x LoD	8	37.53	0.65	1.74	100%
10x LoD	8	36.54	0.60	1.65	100%

An example of Inter-Session Repeatability (on two days) results is shown in the tables below.

Table 17 ELITe InGenius Inter-session Repeatability

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	16	-	-	-	100%
3x LoD	16	36.55	0.69	1.89	100%
10x LoD	16	35.79	0.59	1.64	100%

Table 18 ELITe BeGenius Inter-session Repeatability

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	16	-	-	-	100%
3x LoD	16	37.49	0.60	1.59	100%
10x LoD	16	36.61	0.69	1.87	100%

In the Repeatability test, the MTB EXTRA ELITe MGB Kit detect all samples as expected and showed a maximum variability of target Ct value as %CV lower than 5%.

11.6 Reproducibility

The reproducibility of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of sputum samples negative or spiked with *Mycobacterium tuberculosis* reference material (ATCC, ref. 25177).

The results of the Inter-Batch Reproducibility (two lots) are shown in the tables below.

Table 19 ELITe InGenius Inter-Batch Reproducibility

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	8	-	-	-	100%
3x LoD	8	36.68	0.71	1.94	100%
10x LoD	8	35.70	0.79	2.21	100%

Table 20 ELITe BeGenius Inter-Batch Reproducibility

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	8	-	-	-	100%
3x LoD	8	37.93	0.71	1.88	100%
10x LoD	8	36.56	0.55	1.50	100%

Inter-Instrument Reproducibility (on two days, two lots and two instruments) are shown in the tables below.

Table 21 ELITe InGenius Inter-Instrument Reproducibility

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	8	-	-	-	100%
3x LoD	8	36.50	0.63	1.71	100%
10x LoD	8	35.91	0.57	1.58	100%

Table 22 ELITe BeGenius Inter-Instrument Reproducibility

Sample	N	MTB			%Agreement
		Mean	SD	%CV	
Negative	8	-	-	-	100%
3x LoD	8	37.52	0.39	1.05	100%
10x LoD	8	36.71	0.43	1.17	100%

In the Reproducibility test, the MTB EXTRA ELITe MGB Kit detect all samples as expected and showed a maximum variability of target Ct value as %CV lower than 5%.

11.7 Diagnostic Specificity: Confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with ELITe InGenius by analysing clinical samples MTB-negative tested by culture.

As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Table 23 Diagnostic Specificity

Sample	N	Positive	Negative	% Diagnostic Specificity
Sputum	52	1	51	98.1%
BAL/BA	40	1	39	97.5%
Urine	20	0	20	100%
Cavitary fluid	40	0	40	100%

Table 23 Diagnostic Specificity (continued)

Sample	N	Positive	Negative	% Diagnostic Specificity
Biopsy	40	0	40	100%
Gastric aspirate	20	0	20	100%

Furthermore, for sputum samples, the MTB - negative samples were also tested by another CE-IVD marked molecular diagnostic assay and by AFB Smear Microscopy; the results are reported in the table below.

Table 24 Diagnostic Specificity of sputum samples in comparison with other assays

Sample	N	Positive	Negative	% Diagnostic Specificity
Sputum tested by CE-IVD marked molecular diagnostic assay	52	1	51	98.1%
Sputum tested by AFB Smear Microscopy	58	7	51	89.47%

The IC Ct cut-off values for each matrix in association to ELITe InGenius and ELITe BeGenius are reported in the following table.

Table 25 IC Ct cut-off values

Sample	IC Ct cut-off values	
	ELITe InGenius	ELITe BeGenius
Sputum	34	35
BAL/BA	35	35
Urine	35	35
Cavitary fluid	34	34
Biopsy	34	35
Gastric aspirate	34	34

11.8 Diagnostic Sensitivity: Confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with ELITe InGenius by analysing clinical samples certified positive tested by culture or spiked with MTB reference material.

As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Table 26 Diagnostic Sensitivity

Sample	N	Positive	Negative	% Diagnostic Sensitivity
Sputum	50	50	0	100%
BAL/BA	46	42	4	91.3%
Urine	20	16	4	80%
Cavitary fluid	40	39	1	97.5%
Biopsy	42	38	4	90.5%
Gastric aspirate	22	18	4	81.8%

Furthermore, for sputum samples, the MTB-positive samples were also tested by another CE-IVD marked molecular diagnostic assay and by AFB Smear Microscopy, the results are reported in the table below.

Table 27 Diagnostic Sensitivity of sputum samples in comparison with other assays

Sample	N	Positive	Negative	% Diagnostic Sensitivity
Sputum tested with CE-IVD marked molecular diagnostic assay	50	50	0	100%
Sputum tested with AFB Smear Microscopy	44	44	0	100%

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File MTB EXTRA ELITe MGB Kit, FTP 121ING.

12 REFERENCES

Thierry D. et al. (1990) Nucleic Acids Res. 18: 188

E. A. Lukhtanov et al. (2007) Nucleic Acids Res. 35: e30

Mycobacteriology laboratory manual (Global Laboratory Initiative, First edition, April 2014).

K. Linnet et al. (2004) Clin. Chem. 50: 732 - 740.

13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: 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.

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

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

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

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

This product requires the use of laboratory clothing and instruments dedicated to work session setup to avoid false positive results.

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

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

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

Possible polymorphisms, insertions or deletions within the region of the DNA targeted 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 in combination with all relevant clinical observations and laboratory results.

As with any other diagnostic medical device, there is a residual risk of obtaining invalid, or erroneous results with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient. However, this residual risk associated to the intended use of the product has been weighed against the potential benefits to the patient and it has been assessed acceptable.

14 TROUBLESHOOTING

Table 28

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Positive Control. Check the volumes of PCR Mix and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Positive Control degradation.	Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use a new aliquot of Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 29

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 30

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control, and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 31

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the positive control.	Check for target Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target with a possible mutation. The target in the sample should be sequenced to confirm mutation.

Table 32

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If significant amplification is observed in PCR plot select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: - repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a “PCR Only” session. - repeat the extraction of the sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 33

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination in preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the GUI. Follow the loading sequence indicated by the software.
Laboratory environmental contamination.	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix and / or CPE.

15 SYMBOLS

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

16 NOTICE TO THE USERS

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and /or the patient is established. To inform ELITechGroup S. p. A., manufacturer of this device, please use the following mail address: egspa. vigilance@elitechgroup.com..

A “Summary of Safety and Performance” will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional. Before the notice of full functionality of Eudamed has been published, the “Summary of Safety and Performance” will be made available to the public upon request by email at emd.support@elitechgroup.com, without undue delay.

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ELITe MGB ® detection reagents are covered by one or more of U. S. Patent numbers 7319022, 7348146, 7541454, 7671218, 7723038, 7767834, 8163910, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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

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Appendix A MTB EXTRA ELITe MGB Kit used in association with Genius series® platforms

CAUTION

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

Intended use

The product **MTB EXTRA ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of *Mycobacterium tuberculosis* (MTB) complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canettii*, *M. microti, M. caprae*) DNA.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of sputum, bronchial aspirates (BA), bronchoalveolar lavages (BAL), urine, cavity fluids, biopsies and gastric aspirates previously liquefied, decontaminated and inactivated.

The product is intended for use as an aid in the diagnosis of infections from *Mycobacterium tuberculosis* complex, in combination with all relevant clinical observations and laboratory outcomes, in particular the culture methods for *Mycobacterium*.

Amplified sequence

Target for Qualitative Application	Gene	Fluorophore	Channel
› Target	› IS6110	› FAM	MTB
› Internal Control	› IC2 Artificial sequence	› AP680	IC

Validated matrix

• sputum • bronchial aspirates (BA) / bronchoalveolar lavages (BAL) • urine • cavity fluids • biopsies • gastric aspirates	previously liquefied, decontaminated and inactivated

Kit content and related products

MTB EXTRA ELITe MGB Kit (RTS121ING)		MDR/MTB - ELITe Positive Control (CTR120ING)	
 X 8		 X 3	
MTB EXTRA PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube		**TB Positive Control** 3 tubes of 160 µL 2 reactions per tube 6 reactions per kit 2 freeze-thaw cycles	
Maximum shelf-life:	**24 months**	Maximum shelf-life	**24 months**
Storage temperature	**≤ -20°C**	Storage temperature	**≤ -20°C**

Other products required not provided in the kit

• ELITe InGenius instrument: INT030. • ELITe BeGenius instrument: INT040. • ELITe InGenius SP 200: INT032SP200. • ELITe InGenius and ELITe BeGenius Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use)	• CPE - Internal Control: CTRCPE

ELITe InGenius and ELITe BeGenius protocol

› Sample volume › CPE volume › Total elution volume	200 µL 10 µL 100 µL	› Eluate PCR input volume › Q—PCR Mix volume › Frequency of controls › Frequency of calibration	20 µL 20 µL 15 days 30 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
Sputum	6 CFU / mL	100% 50/50*	98% 47/48*
BAL/BA	6 CFU / mL	91.3% 42/46*	97.5% 39/40*
Urine	20 CFU / mL	80% 16/20*	100% 20/20*
Cavitary fluid	20 CFU / mL	97.5% 39/40*	100% 40/40*
Biopsy	20 CFU / mL	90.5% 38/42	100% 40/40*
Gastric aspirate	20 CFU / mL	81.8% 18/22*	100% 20/20*

**confirmed samples / tested samples*

Sample preparation

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

Table 34

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Sputum	liquefied with a solution of N- Acetil L-Cysteine and decontaminated with sodium hydroxide solution[2] then inactivated at 95°C for 30 minutes	≤ 1 hour[1]	≤ 2 days[2]	≤ 1 month[5]	≥ 1 month
Bronchoalveolar lavage (BAL) and bronchial aspirates (BA)	liquefied with a solution of N- Acetil L-Cysteine and decontaminated with sodium hydroxide solution[2] then inactivated at 95°C for 30 minutes	≤ 1 hour[1]	≤ 2 days[2]	≤ 1 month[5]	≥ 1 month
Urine	concentrated and decontaminated with sodium hydroxide solution[2], then inactivated at 95°C for 30 minutes	≤ 1 hour[1]	≤ 2 days[2]	≤ 1 month[5]	≥ 1 month
Cavitary fluid	concentrated and decontaminated with sodium hydroxide solution[2], then inactivated at 95°C for 30 minutes	≤ 1 hour[3]	≤ 2 days[2]	≤ 1 month[5]	≥ 1 month
Biopsy	break down and decontaminated with sodium hydroxide solution[2], then inactivated at 95°C for 30 minutes	≤ 1 hour[3]	≤ 7 days[4]	≤ 1 month[3]	≥ 1 month
Gastric aspirate	liquefied with a solution of N- Acetil L-Cysteine and decontaminated with sodium hydroxide solution[2], then inactivated at 95°C for 30 minutes	≤ 1 hour[1]	≤ 2 days[2]	≤ 7 days[4]	≥ 1 month

1) (CLSI MP48 2nd Edition, "Laboratory Detection and Identification of Mycobacteria")

2) (Mycobacteriology Laboratory Manual, Global Laboratory Initiative).

3) (KPNW Specimen Requirements)

4) (Mayo Clinic Laboratories)

5) (ARUP Laboratories)

ELITe InGenius Procedures

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

Before analysis

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

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

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL"	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: MTB EXTRA ELITe_SP_200_100 MTB EXTRA ELITe_BAL_200_100 MTB EXTRA ELITe_U_200_100 MTB EXTRA ELITe_CL_200_100 MTB EXTRA ELITe_B_200_100 MTB EXTRA ELITe_GA_200_100	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix and the Internal Control in the Inventory Block
7. Load: PCR Cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE
If an Extract Only mode is needed, refer to the instrument user's manual for procedure.

Procedure 2: PCR Only (e.g., eluates, controls)

1. Select “Perform Run” on the touch screen	**2.** Verify the extraction volumes: Input: “200 µL”, elution: “100 µL”	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the “Assay Protocol” of interest: MTB EXTRA ELITe_PC or MTB EXTRA ELITe_NC or MTB EXTRA ELITe_SP_200_100 MTB EXTRA ELITe_BAL_200_100 MTB EXTRA ELITe_U_200_100 MTB EXTRA ELITe_CL_200_100 MTB EXTRA ELITe_B_200_100 MTB EXTRA ELITe_GA_200_100	**5.** Select the method “PCR Only” and the sample position “Elution Tube”	**6.** Load the PCR Mix in the Inventory Block
7. Load: PCR Cassette rack and the Elution tube rack with the extracted nucleic acid	**8.** Close the door. Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

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

Before analysis

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

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

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay Protocol” of interest: MTB EXTRA ELITe_Be_SP_200_100 MTB EXTRA ELITe_Be_BAL_200_100 MTB EXTRA ELITe_Be_U_200_100 MTB EXTRA ELITe_Be_CL_200_100 MTB EXTRA ELITe_Be_B_200_100 MTB EXTRA ELITe_Be_GA_200_100 **Note:** If a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Basket” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE
If an Extract Only mode is needed, refer to the instrument user’s manual for procedure.

Procedure 2: PCR Only (e.g., eluates, controls)

1. Select “Perform Run” on the touch screen and then click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay Protocol” of interest: MTB EXTRA ELITe_Be_PC or MTB EXTRA ELITe_Be_NC or: MTB EXTRA ELITe_Be_SP_200_100 MTB EXTRA ELITe_Be_BAL_200_100 MTB EXTRA ELITe_Be_U_200_100 MTB EXTRA ELITe_Be_CL_200_100 MTB EXTRA ELITe_Be_B_200_100 MTB EXTRA ELITe_Be_GA_200_100	**5.** Load the PCR-Mix in the Reagent/ Elution Rack and insert it in the Cooler Unit	**6.** Load “PCR Rack” with “PCR Cassette”
7. Close the door. Start the run	**8.** View, approve and store the results	

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