Instructions for use

Macrolide-R/MG ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

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IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
06–R	Update of paragraphs "Validation of Sample results" and "Procedure limitations"	23/09/26
05–R	Compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements. Expansion of the use of the product in association with ELITe InGenius® and ELITe BeGenius® instruments, with cervical-vaginal swab matrices. Replacement of the abbreviation MG with MYG Change the name of the component by removing -48 Replacement of 2mL tube 953-217 and white cap 953-223 with 2mL tube 953-065 related to PCR Mix component tubes. Update of the paragraph “References”. Update of the packaging of the PCR Mix tube (paragraph “Materials provided in the product”) Update of the paragraph “Materials Required But Not Provided In The Product” Update of the paragraph “Other products required” Update of the paragraph “Notice to the users” Update of the paragraph “Notice to purchaser: limited license” Update of the paragraph “Symbols” with the symbol “Consult instructions for use”	27/11/25
04	Addition of UDI information Product Description: typo correction related to the alignment between targets and dyes	23/04/24
00–03	new product development and succeeding changes	-

NOTE

The product batches identified by the following LOT numbers are still placed on the market as per IVDD till to their expiration dates, according to Article 110 of IVDR. If you have those product batches, please contact ELITechGroup staff to request the related previous revision of IFUs.

PRODUCT REF	Lot number	Expiry date
RTS401ING	U0925-055	31/07/2027

The Positive Control product batches still placed on the market as per IVDD (identified by the LOT numbers reported in the Positive Control IFU) are technically compatible with the new IVDR version of the amplification kit and can be used, until exhausted, in association with the new IVDR version of the amplification kit and in accordance with its intended use.

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1 INTENDED USE

The product **Macrolide-R/MG ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of the DNA of ***Mycoplasma genitalium*** **(MYG)** extracted from clinical specimens and for identification of main Macrolide resistance associated mutations.

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 first void urine collected without preservatives and cervical-vaginal swab.

The product is intended for use as an aid in the diagnosis of urogenital tract infections in patients suspected of having *Mycoplasma genitalium* infection.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

2 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting Mycoplasma genitalium and main Macrolide resistance associated mutations DNA isolated from specimens and amplified using the assay reagent R/MG PCR Mix, that contains primers and ELITe MGB technology probes.

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 **Macrolide-R/MG ELITe MGB Kit** provides the assay reagent **R/MG PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- 23S rRNA gene of M. genitalium, detected in Channel **MYG**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor® 525 (AP525) 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 **R/MG PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

The **Macrolide-R/MG ELITe MGB Kit** contains sufficient reagents for **48 tests** on the **ELITe InGenius** and **ELITe BeGenius (12 tests each tube)**, with 20 µL used per reaction.

The **Macrolide-R/MG 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
R/MG PCR Mix ref. RTS401ING-48	Mixture of reagents for Real-Time PCR tube with NATURAL cap	**4 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, ref. 72.694.005).
- 0.5 mL sterile screw capped tubes (Sarstedt, ref. 72.730.005)
- 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 1.4.0 (or later). **R_MG ELITe_PC**, Assay Protocol with parameters for Positive Control analysis. **R_MG ELITe_NC**, Assay Protocol with parameters for Negative Control analysis. **R_MG ELITe_U_200_100,** Assay Protocol with parameters for first void urine specimen analysis. **R_MG ELITe_CS_200_100,** Assay Protocol with parameters for cervical-vaginal swab specimen analysis.	**Macrolide-R/MG - ELITe Positive Control** (EG SpA, ref. CTR401ING). **CPE - Internal Control** (EG SpA, ref. CTRCPE). **ELITe InGenius SP200** (EG SpA, ref. INT032SP200). **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for use) **eSWAB ®** (COPAN Italia S.p.A., ref. 480CE), or an equivalent device, for cervical-vaginal swab specimens
ELITe BeGenius (EG SpA, ref. INT040). **ELITe BeGenius Software** version 2.3.0 (or later). **R_MG ELITe _Be_PC**, Assay Protocol with parameters for Positive Control analysis. **R_MG ELITe _Be_NC**, Assay Protocol with parameters for Negative Control analysis. **R_MG ELITe_Be_U_200_100,** Assay Protocol with parameters for first void urine specimen analysis. **R_MG ELITe_Be_CS_200_100,** Assay Protocol with parameters for cervical-vaginal swab specimen analysis.	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

- Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological

samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121°C before disposal.

- Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).
- Wear suitable protective clothes and gloves and protect eyes and face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Carefully wash hands after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with the regulations in force.
- Carefully read all the instructions provided before running the assay.
- While running the assay, follow the product instructions provided.
- Do not use the product after the indicated expiry date.
- Only use 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.

7.2 Warnings and precautions for molecular biology

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

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

The samples must be suitable and, if possible, dedicated to 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)
R/MG 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 and Assay Protocols

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

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)*	+2° / +8°C	-20 ± 10 °C	-70 ± 15 °C
First void urine	collected without preservatives	≤ 1 day	≤ 2 days	≤ 1 month	≤ 1 month
Cervical-vaginal swabs	eSwab® (COPAN) or an equivalent reagent (optional)	≤ 2 days	≤ 2 days	≤ 1 month	≤ 1 month

The first void urine can be used "as is" or 10 folds concentrated by centrifugation at ~1,000 RCF for 10 minutes.

Even if longer storage periods at -70 ° C are possible, as extensively reported by scientific literature, their application should be evaluated internally by the end-users of this product. 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 Macrolide-R/MG ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
First void urine collected without preserva-tives	**ELITe InGenius**	**R_MG 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	**R_MG ELITe _Be_U_ 200_100**		
Cervical-vaginal swabs	**ELITe InGenius**	**R_MG ELITe_CS_200_ 100**		
	ELITe BeGenius	**R_MG ELITe_Be_CS_ 200_100**		

NOTE

Verify if the primary tube and the volume of the sample are compatible with ELITe InGenius or ELITe BeGenius, following the Instruction for use of the extraction kit **ELITeInGeniusSP200** (EG SpA, ref. INT032SP200).

The volume of the sample in a primary tube varies according to the type of the tube loaded. Refer to the instructions for use of the extraction kit for more information on how to set up and perform the extraction procedure.

If required, 200 µL of sample must be transferred into an Extraction tube (for ELITe InGenius) or 200 µL of sample must be transferred into a 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 “7 WARNINGS AND PRECAUTIONS page 5” section.

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

Refer to “Potentially Interfering Substances” in the 11 PERFORMANCE CHARACTERISTICS page 17 section to check data concerning interfering substances.

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

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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 **Macrolide-R/MG ELITe MGB Kit** through the following procedure:

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

9.4 Validation of amplification Positive Control and Negative Control results

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

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

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

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

NOTE

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

NOTE

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

9.5 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the target (channel **MYG**) and the Internal Control (channel **IC**) with the **R_MG ELITe_U_200_100** and **R_MG ELITe_CS_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**
R/MG Positive Control	APPROVED
2) Negative Control	**Status**
R/MG 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
MYG:DNA Detected Macrolide Resistance Positive	***Mycoplasma genitalium*** **DNA was detected** in the sample. A mutation was detected in the tested gene region. The sample could be **resistant to Macrolide**.
MYG:DNA Detected Macrolide Resistance Negative	***Mycoplasma genitalium*** **DNA was detected** in the sample. No mutation was detected in the tested gene region. The sample could be **sensitive to Macrolide**.
MYG:DNA Detected - Typing not feasible-Retest Sample	***Mycoplasma genitalium*** **DNA was detected** in the sample but is not sufficient to proceed with analysis for Macrolide resistance. The test should be repeated.
MYG:DNA Detected Typing not determined	***Mycoplasma genitalium*** **DNA was detected** in the sample but the analysis for Macrolide resistance was not feasible.
MYG:DNA Detected Macrolide Resistance Positive Macrolide Resistance Negative	***Mycoplasma genitalium*** **DNA was detected** in the sample. Both target sequences associated with Macrolide resistance and sensitivity were detected in the sample. The sample could be **resistant to Macrolide.** Possible presence of co-infections between *Mycoplasma genitalium* strain with and without mutations associated to resistance to Macrolides.
MYG:DNA Not detected or below the LoD	***Mycoplasma genitalium*** **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 (incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as “MYG: DNA detected - Typing not feasible-Retest Sample” are not suitable for Macrolide resistance analysis. In this case, the *Mycoplasma genitalium* DNA was detected but the DNA is not sufficient to obtain correct results for Macrolide resistance in a reproducible way. This is due to low *Mycoplasma genitalium* 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).The test needs to be repeated.

NOTE

When a sample is reported as “MYG:DNA Detected - Typing not feasible-Retest Sample”, the result cannot be approved, but the Tm value can be checked by the operator. If the Tm value is lower than 63.0 °C (wildtype Tm lower limit) the sample is “MYG:DNA Detected. Macrolide Resistance Positive”.

Samples reported as “MYG: DNA detected. Typing not determined” without any indication about the Macrolide resistance status are not suitable for genotyping. In this case, the target DNA was detected in the sample, but it was not possible to calculate a Tm or the calculated Tm value was out of the Tm intervals for typing. In this last case could be due to mutations different from the intended ones, samples non collected properly or to problems in the extraction step (inhibitor carry-over in the eluate).

Samples reported as: "MYG: DNA not detected or below the LoD" are suitable for analysis but it was not possible to detect *Mycoplasma genitalium* DNA. In this case it cannot be excluded that MYG DNA is present at a concentration below the limit of detection of the assay (see “11 PERFORMANCE CHARACTERISTICS page 17”).

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

NOTE

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

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

9.6 Sample result reporting

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

The “Sample Report” shows the results details by selected sample (SID).

The “Track Report” shows the results details by selected Track.

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

10 ELITe BeGenius PROCEDURE

The procedure to use the **Macrolide-R/MG 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 **Macrolide-R/MG 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**”.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
6	**Load the samples** into the "Sample Rack". When secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack".	**Load the samples** into the "Elution Rack".	**Load the Positive Control and Negative Control** tubes into the "Elution Rack".
7	**Insert** the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert the "Sample ID" (SID) for each "Position" used (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3). If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
8	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
9	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL	Not applicable	Not applicable
10	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
11	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
	Note: When more than 12 samples are processed, repeat the procedure from point 6.		Not applicable
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable
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.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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 **Macrolide-R/MG 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

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined for **ELITe InGenius** instrument by testing first void urine samples without preservatives spiked with reference material of *Mycoplasma genitalium* (Qnostics, UK, code MG1803023B).

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

The results are reported in the following table.

Table 12 Limit of Detection (organisms / mL) for first void urine samples and ELITe InGenius system

LoD (organism/mL)	95% confidence interval limits	
	Lower bound	Upper bound
247	155	634

The calculated LoD value was verified by testing on **ELITe InGenius** and **ELITe BeGenius** first void urine and concentrated urine and cervical-vaginal swabs spiked by Mycoplasma genitalium reference material (DSMZ, DSM 19775) at the claimed concentration.

The results obtained confirmed the claimed concentration for the target of Macrolide-R/MG ELITe MGB Kit with the three matrices on both ELITe BeGenius and ELITe InGenius.

11.2 Detection of Macrolide resistance

The detection of resistance to Macrolide was evaluated for the assay by testing on ELITe InGenius certified samples (provided by an external laboratory) and plasmid DNAs carrying the amplified region of 23S rRNA gene with the main mutations related to antibiotic resistance listed in the table below:

Table 13

Mutation
A2058G
A2058C
A2058T
A2059G

Table 13 (continued)

Mutation
A2059C
A2062G
A2062T

NOTE

Other mutations in the same region of 23S rRNA gene can be detected by the assay, such as the A2062C.However, this mutation does not appear to be associated with resistance to Macrolides.

The results of the test are reported in the next section about Efficiency of detection.All tested isolates and plasmid DNAs were detected as Mycoplasma genitalium positive and correctly typed as possible resistant to Macrolide by the product Macrolide-R/MG ELITe MGB Kit.

11.3 Inclusivity: Efficiency of detection

The Inclusivity of the assay, as efficiency of detection for *Mycoplasma genitalium*, including the azithromycin resistant variants, was evaluated by *in silico* analysis.

The analysis showed sequences conservation and absence of significant mutations.So, an efficient detection for 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 including mutations for azithromycin resistance.

The results are reported in the following table.

Table 14

Sample	Pos. / Rep.	Mut. / Rep.
M. genitalium wt	3/3	0/3
pMG A2058G	3/3	3/3
pMG A2058C	3/3	3/3
pMG A2058T	3/3	3/3
pMG A2059G	3/3	3/3
pMG A2059C	3/3	3/3
pMG A2062G	12/12	12/12
pMG A2062T	12/12	12/12
MG 410 (A2058G)	1/1	1/1
MG 426 (A2058G)	1/1	1/1
MG 539 (A2059G)	1/1	1/1
MG 540 (A2058G)	1/1	1/1

11.4 Potential interfering organism

The potential cross-reactivity of unintended organisms that may be found in clinical specimens was evaluated for the assay by *in silico* analysis.

The analysis for *Mycoplasma genitalium* showed no significant homologies with other unintended organisms (viruses and bacteria) therefore no potential interference is expected.

The absence of cross-reactivity with potential interfering organisms was also verified through the analysis of a panel of unintended organisms (ATCC and Vircell Microbiologist).

The results are reported in the following table.

Table 15 Potential cross-reactivity

Organism	Strain	Outcome
Chlamydia trachomatis	LGV II	No cross-reactivity
Neisseria gonorrhoeae	DSM 9188	No cross-reactivity
Trichomonas vaginalis	Clinical isolate	No cross-reactivity
Mycoplasma hominis	PG21	No cross-reactivity
Ureaplasma urealyticum	NCTC10177	No cross-reactivity
Escherichia coli	CFT073	No cross-reactivity
Ureaplasma parvum	NCTC 11736	No cross-reactivity
Treponema pallidum	Nichols	No cross-reactivity
Gardnerella vaginalis	594	No cross-reactivity
Mobiluncus mulieris	BV 64-5	No cross-reactivity
Bacteroides fragilis	NCTC 9343	No cross-reactivity
Peptostreptococcus anaerobius	MSHD	No cross-reactivity
Candida albicans	3147	No cross-reactivity
Lactobacillus acidophilus	Pak	No cross-reactivity
HSV1	McIntyre	No cross-reactivity
HSV2	G	No cross-reactivity

All potentially interfering organisms tested showed no cross-reactivity of the target amplification using Macrolide-R/MG ELITe MGB Kit.

The potential inhibition of unintended organisms that may be found in clinical specimens was evaluated for the assay through the analysis of a panel of unintended organisms (Vircell Microbiologist) spiked with the genomic DNA of *Mycoplasma genitalium* at low concentration.

The results are reported in the following table.

Table 16 Potential interference

Organism	Strain	Outcome
Chlamydia trachomatis	LGV II	No interference
Neisseria gonorrhoeae	DSM 9188	No interference
Trichomonas vaginalis	Clinical isolate	No interference
Mycoplasma hominis	PG21	No interference

Table 16 Potential interference (continued)

Organism	Strain	Outcome
Ureaplasma urealyticum	NCTC10177	No interference
Escherichia coli	CFT073	No interference
Ureaplasma parvum	NCTC 11736	No interference
Treponema pallidum	Nichols	No interference
Gardnerella vaginalis	594	No interference
Mobiluncus mulieris	BV 64-5	No interference
Bacteroides fragilis	NCTC 9343	No interference
Peptostreptococcus anaerobius	MSHD	No interference
Candida albicans	3147	No interference
Lactobacillus acidophilus	Pak	No interference
HSV1	McIntyre	No interference
HSV2	G	No interference

All potentially interfering organisms tested showed no inhibition of the target amplification using the Macrolide-R/MG ELITe MGB Kit.

11.5 Interfering substances

The inhibition by potentially interfering substances (endogenous and exogenous) that might be found in clinical specimens was evaluated for the assay by analysis of a panel of substances at relevant concentrations.

The results are reported in the following table.

Table 17 %CV Ct (Ref. + test)

Substance	Pos % Agreement	Outcome
Whole blood	100%	No interference
Sperm	100%	No interference
Mucin	100%	No interference
Azithromycin	100%	No interference
Alkaline Urine	100%	No interference
Acid Urine	100%	No interference
Antiviral	100%	No interference
Antibiotic	100%	No interference
Antifungal	100%	No interference
Lubricant	100%	No interference
Spermicide	100%	No interference

The test showed that all the tested substances do not cross-react with the targets using the Macrolide-R/MG ELITe MGB Kit.

11.6 Repeatability

The Repeatability of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of first void urine specimens negative or spiked with certified reference material of *Mycoplasma genitalium* (DSMZ, DSM 19775).

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

Table 18 Intra - Session Repeatability on ELITe BeGenius

Sample	Pos. / Neg.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	% CV	
Negative	0 / 8	NA	NA	NA	100%
3X LoD	8 / 8	34.59	0.69	1.98	100%
10X LoD	8 / 8	32.51	0.41	1.25	100%

Table 19 Intra - Session Repeatability on ELITe InGenius

Sample	Pos. / Neg.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	% CV	
Negative	0 / 8	NA	NA	NA	100%
3X LoD	8 / 8	35.97	0.64	1.77	100%
10X LoD	8 / 8	32.66	0.30	0.93	100%

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

Table 20 Inter - Session Repeatability on ELITe BeGenius

Sample	Pos. / Neg.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	% CV	
Negative	0 / 16	NA	NA	NA	100%
3X LoD	16 / 16	35.18	0.98	2.78	100%
10X LoD	16 / 16	32.79	0.45	1.37	100%

Table 21 Inter - Session Repeatability on ELITe InGenius

Sample	Pos. / Neg.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	% CV	
Negative	0 / 16	NA	NA	NA	100%
3X LoD	16 / 16	36.13	0.80	2.21	100%
10X LoD	16/ 16	32.91	0.57	1.73	100%

In the Repeatability test, the Macrolide-R/MG ELITe MGB Kit detected all the samples as expected and showed a variability of target Ct values as Coefficient of Percentage Variation %CV lower than 5%.

11.7 Reproducibility

The Reproducibility of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of first void urine specimens negative or spiked with certified reference material of *Mycoplasma genitalium* (DSMZ, DSM 19775).

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

Table 22 Inter-Batch Reproducibility on ELITe BeGenius

Sample	Pos. / Rep.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	%CV	
Negative	0 / 8	-	-	-	100%
3 X LoD	8 / 8	35.75	0.27	0.75	100%
10 X LoD	8 / 8	33.16	0.74	2.23	100%

Table 23 Inter-Batch Reproducibility on ELITe InGenius

Sample	Pos. / Rep.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	%CV	
Negative	0 / 8	-	-	-	100%
3 X LoD	8 / 8	35.79	0.83	2.31	100%
10 X LoD	8 / 8	33.65	0.44	1.29	100%

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

Table 24 Inter-Instrument Reproducibility on ELITe BeGenius

Sample	Pos. / Rep.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	%CV	
Negative	0 / 16	-	-	-	100%
3 X LoD	16 / 16	35.59	0.79	2.22	100%
10 X LoD	16 / 16	33.19	0.46	1.39	100%

Table 25 Inter-Instrument Reproducibility on ELITe InGenius

Sample	Pos. / Rep.	Mycoplasma genitalium			%Agreement
		Mean Ct	SD	%CV	
Negative	0 / 16	-	-	-	100%
3 X LoD	16 / 16	35.67	0.05	1.94	100%
10 X LoD	16/ 16	33.25	0.23	0.69	100%

In the Reproducibility test, the Macrolide-R/MG ELITe MGB Kit detected all the samples as expected and showed a variability of target Ct values as Coefficient of Variation %CV lower than 5%.

11.8 Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of clinical samples positive for *Mycoplasma Genitalium* (MYG), was evaluated in association with **ELITe InGenius** by analyzing clinical samples of first void urine collected without preservatives, concentrated first void urine and cervical vaginal swabs, certified positive or spiked with reference materials.

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

The results are summarized in the following table.

Table 26

Samples	N	positive	negative	% Diagnostic Sensitivity
First void urine positive for MYG	66	65	1	98.5%
Concentrated first void urine positive for MYG	53	53	0	100%
Cervical-vaginal swab positive for MYG	29	29	0	100%
Cervical-vaginal swab spiked for MYG	60	60	0	

Regarding the identification of Macrolide resistance, the results obtained with the assay by Tm analysis ("Macrolide Resistance Negative" (WT) or "Macrolide Resistance Positive" (MUT)) were compared to the data obtained with the reference methods. The results obtained for the different matrices are reported below.

Table 27

Samples	Reference Methods	WT	MUT	WT & MUT	Typing **Agreement**
First void **urine**	WT	13	1	0	**98.4%**
	MUT	0	47	0	
	WT & MUT	0	0	1	
Concentrated first void **urine**	WT	9	1	0	**98.1%**
	MUT	0	42	0	
	WT & MUT	0	0	1	
Cervical-vaginal **swab**	WT	49	0	0	**100%**
	MUT	0	37	0	
	WT & MUT	0	0	0	

11.9 Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of clinical samples negative for *Mycoplasma Genitalium* (MYG), was evaluated in association with **ELITe InGenius** by analyzing clinical samples of first void urine collected without preservatives, concentrated first void urine and cervical vaginal swabs certified negative.

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

The results are summarized in the following table.

Table 28

Samples	N	positive	negative	% Diagnostic Specificity
First void urine negative for MYG	55	0	55	100%
Concentrated first void urine negative for MYG	54	0	54	100%
Cervical-vaginal swab negative for MYG	104	0	104	100%

The IC Ct cut-off value is set at 32 for all matrices.

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File " Macrolide-R/MG ELITe MGB Kit Kit", FTP401ING.

12 REFERENCES

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13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: first void urine and cervical-vaginal swab.

Currently there are no data available concerning product performance with other clinical samples.

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

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

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

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 people.

This product requires the use of personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

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

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 11 PERFORMANCE CHARACTERISTICS page 17).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 amplification or extraction, which can lead to a delay in obtaining final results (see 14 TROUBLESHOOTING page 25).

Results obtained with this product about possible resistance to Macrolides of the *Mycoplasma genitalium* are limited to the detection of main mutations as indicated in the “Performance Characteristics” Section. Other mutations not detected by this product can be associated to resistance to Macrolides. On the other hand, silent mutations can be detected by this product, but they are not associated to resistance to Macrolides. Therefore, a phenotypic antimicrobial susceptibility test is required to confirm the susceptibility or resistance to macrolides.

This product can identify possible co-infection or co-colonization of *Mycoplasma genitalium* strains with and without mutations associated to resistance to Macrolides. However in case of unbalanced co-infections or co-colonizations, test results provided with this product could identify only the predominant population when it’s clearly prevalent.

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 29

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 30

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 31

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). Protect the **PCR Mix** from light while thawing. 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 32

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 33

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 34

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 0123	Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.
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.

17 NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Thermo Fisher Scientific and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Thermo Fisher Scientific. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Thermo Fisher Scientific. Email: outlicensing@thermofisher.com.

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

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

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

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eSwab® is registered trademark of COPAN Italia S.p.A.

Appendix A Macrolide-R/MG 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 **Macrolide-R/MG ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of the DNA of ***Mycoplasma genitalium*** **(MYG)** extracted from clinical specimens and for identification of main Macrolide resistance associated mutations.

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 first void urine collected without preservatives and cervical-vaginal swab.

The product is intended for use as an aid in the diagnosis of urogenital tract infections in patients suspected of having *Mycoplasma genitalium* infection.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

Amplified sequence

Table 35

Sequence	Gene	Fluorophore	Channel
Target 1	23S rRNA	AP525	MYG
Internal Control	IC2	AP680	IC

Validated matrix

Table 36

› first void urine collected without preservatives
› cervical-vaginal swab

Kit content and related products

Table 37

Macrolide-R/MG ELITe MGB Kit (RTS401ING-48)	Macrolide-R/MG – ELITe Positive Control (CTR401ING)
 X 4	 X 3
R/MG PCR Mix 4 tubes of 280 µL 12 reactions per tube 48 reactions per kit 7 freeze-thaw cycles per tube	**R/MG Positive Control** 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles

Table 37 (continued)

Macrolide-R/MG ELITe MGB Kit (RTS401ING-48)		Macrolide-R/MG – ELITe Positive Control (CTR401ING)	
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

Table 38

› ELITe InGenius instrument: INT030. › ELITe BeGenius instrument: INT040. › ELITe InGenius SP 200: INT032SP200.	› CPE - Internal Control: CTRCPE. **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use) **eSWAB ®** (COPAN Italia S.p.A., ref. 480CE), or an equivalent device, for cervical-vaginal swab specimens

ELITe InGenius and ELITe BeGenius protocol

› Sample volume › CPE volume › Total elution volume	200 µL(InGenius and BeGenius) or 1000 µL (InGenius only) 10 µL 100 µL	› Eluate PCR input volume › Q—PCR Mix volume › Frequency of controls	20 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Table 39

Matrix	Target	Limit of Detection	Sensitivity	Specificity
first void urine	MYG	**247 organisms / mL**	98.5% **(65/66)**	100% **(55/55)**
concentrated first void urine	MYG	**247 organisms / mL**	100% **(53/53)**	100% **(54/54)**
cervical-vaginal swab	MYG	**247 organisms / mL**	100% **(89/89)**	100% **(104/104)**

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 40

Sample type	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 ° C**	**-20 ±10 ° C**	**-70 ±15 ° C**
first void urine	collected without preservatives	≤ 1 day	≤ 2 days	≤ 1 month	≤ 1 month
Cervical-vaginal swabs	eSwab® (COPAN) or equivalent device, for cervical-vaginal swab specimens	≤ 2 days	≤ 2 days	≤ 1 month	≤ 1 month

ELITe InGenius Procedures

The user is guided step-by-step by the Graphic User Interface 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

Table 41

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

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

Table 42

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: R_MG ELITe_U_200_100 or R_MG ELITe_CS_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)

Table 43

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: R_MG ELITe_U_200_100 or R_MG ELITe_CS_200_100 or R_MG ELITe_PC or R_MG ELITe_NC	**5.** Select the method "PCR Only" and the sample position "Elution Tube"	**6.** Load the PCR Mix in the Inventory Block
7. Load: PCR Cassette 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 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

Table 44

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

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

Table 45

1. Select "Perform Run" on the touch screen and then click on the run mode «Extract and 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: R_MG ELITe_Be_U_200_100 or R_MG ELITe_Be_CS_200_100 **Note**: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load "PCR Rack" with "PCR Cassette" and the "Extraction Rack" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

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

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

Table 46

1. Select "Perform Run" on the touch screen and then click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit"	**3.** For Controls: for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions). For eluates: for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).
4. Select the "Assay Protocol" of interest: R_MG ELITe_Be_U_200_100 or R_MG ELITe_Be_CS_200_100 or R_MG ELITe_Be_PC or R_MG ELITe_Be_NC	**5.** Load the PCR-Mix in the Reagent/Elution Rack and insert it in the Cooler Unit	**6.** Load "PCR Rack" with "PCR Cassette"
7. Close the door. Start the run	**8.** View, approve and store the results	

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