Instructions for use

STI PLUS ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS400ING

UDI 08033891486525

CE 0123

IVD

CHANGE HISTORY

<table>
<tr><th>Rev.</th><th>Notice of change</th><th>Date (dd/mm/yy)</th></tr>
<tr><td>07–R</td><td>Update of the paragraph “Validation of sample results”: alignment to the new interpretation phrases defined in the Assay Protocol</td><td>06/12/24</td></tr>
<tr><td>06–R</td><td>Compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements.
NOTE
The following product batches are still placed on the market as per IVDD till to their expiration dates, according to Article 110 of IVDR. If you have these product batches, please contact ELITechGroup staff to request the related previous version of IFUs
<table>
<tr><th>PRODUCT REF.</th><th>Lot Number</th><th>Expiry date</th></tr>
<tr><td>RTS400ING</td><td>U0824-004</td><td>31/01/26</td></tr>
<tr><td>RTS400ING</td><td>U1024-071</td><td>27/02/26</td></tr>
</table>
Change in PCR-Mix formulation to enhance fluorescence signals of MG and TV targets, to use a new buffer without Triton X-100, to implement Tm analysis to discriminate N.gonorrhoeae from other Neisseria spp., to solve issue of not detected CT target signals in presence of coinfection with high titles of TV.
New evaluation studies have been performed: upgrade of the analytical and diagnostic performances in PERFORMANCE CHARACTERISTICS paragraph (Clinical sample stability; Limit of detection; Linear Measuring Range; Cross reactivity; Inhibition organisms and substances; Repeatability and Reproducibility, Diagnostic Specificity and Diagnostic Sensitivity)
Update of the Intended use: Validation of the products in association with ELITe InGenius (REF INT030) and ELITe BeGenius (REF INT040) instruments with urine and cervical swab matrices.
New graphics and content setting of the IFU</td><td>06/11/24</td></tr>
<tr><td>05</td><td>Expansion of use of the product in association with «ELITe BeGenius» instrument (REF INT040)</td><td>26/09/22</td></tr>
<tr><td>00 — 04</td><td>new product development and succeeding changes</td><td>-</td></tr>
</table>

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1 INTENDED USE

The product **STI PLUS ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids Real-Time PCR assay **for the detection and identification of the DNA of *Chlamydia trachomatis***, ***Neisseria gonorrhoeae***, ***Mycoplasma genitalium*** and ***Trichomonas vaginalis*** extracted from clinical specimens.

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 swabs.

The product is intended for use as an aid in the diagnosis of urogenital tract infections in patients suspected of having *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium* or *Trichomonas vaginalis* infections.

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

2 ASSAY PRINCIPLES

The assay is a qualitative Real-Time PCR detecting *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium* and *Trichomonas vaginalis* DNA isolated from specimens and amplified using the assay reagent **STI PLUS 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).

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.

The fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

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

- Chlamydia trachomatis **dnaB**-like gene (endogenous plasmid), detected in Channel **CT**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 525 (AP525) dye.
- Chlamydia trachomatis **ompA** chromosomal gene, detected in Channel **CT** ; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 525 (AP525) dye.
- Neisseria gonorrhoeae **pivNG** gene, detected in Channel **NG**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 593 (AP593) dye.
- Mycoplasma genitalium **23S rRNA** gene, detected in Channel **MG**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 639 (AP639) dye.
- Trichomonas vaginalis repeated sequence **L23861**, detected in Channel **TV**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye.

- Internal Control (**IC**), specific for the human **beta globin** gene sequence, detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 559 (AP559) dye.

The **STI PLUS PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

The Internal Control monitors the extraction process and PCR efficiency (in vaginal cervical swab samples it also allows us to monitor the cellularity of the sample).

The assay test can be performed in two different ways:

- testing the first void urine, using as exogenous template for Internal Control (**CPE – Internal Control**) which is added by the instrument **ELITe InGenius** or **ELITe BeGenius** to monitor the extraction process and the PCR efficiency,

- testing cervical-vaginal swab samples, using as endogenous template of Internal Control the human genomic DNA in the sample, to monitor the sample cellularity, the extraction process and the PCR efficiency.

The **STI PLUS ELITe MGB Kit** contains sufficient reagents for **96 tests** on the **ELITe InGenius** and **ELITe BeGenius (12 tests each tube)**, with 20 µL used per reaction.

The **STI PLUS 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
STI PLUS PCR Mix **ref. RTS400ING**	Mixture of reagents for Real-Time PCR in tube with **NATURAL 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 (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 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 control and the consumables are **not** included in 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) **STI PLUS ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **STI PLUS ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **STI PLUS ELITe_U_200_100**, Assay Protocol with parameters for Urine specimen analysis **STI PLUS ELITe_CS_200_100**, Assay Protocol with parameters for Cervical-vaginal swabs specimen analysis	**ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS) **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR), **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000) **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only **CPE - Internal Control** (EG SpA, ref. CTRCPE) **STI PLUS - ELITe Positive Control** (EG SpA,ref. CTR400ING) **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 2.1.0 (or later) **STI PLUS ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **STI PLUS ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **STI PLUS ELITe_Be_U_200_100**, Assay Protocol with parameters for Urine specimen analysis **STI PLUS ELITe_Be_CS_200_100**, Assay Protocol with parameters for Cervical-vaginal swabs 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 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 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 in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination.

The PCR Cassette must be handled carefully and never opened to avoid PCR product diffusion into the environment and sample and reagent 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)
STI PLUS PCR Mix	-20°C or below (protected from light)	one month	up to seven	up to seven separate sessions* of about three hours each or up to 7 consecutive hours (2 sessions of about 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
First void Urine	collected without preservatives	≤ 1 day	≤ 2 days	≤ 1 month	≤ 1 month
Cervical-vaginal swabs	eSwab® (COPAN)	≤ 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 STI PLUS ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
First void Urine	**ELITe InGenius**	**STI PLUS 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	**STI PLUS ELITe_Be_U_200_100**	Positive / Negative	
Cervical-vaginal swabs	**ELITe InGenius**	**STI PLUS ELITe_CS_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: N.A. Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**STI PLUS ELITe_Be_CS_200_100**	Positive / Negative	

For all protocols, 200 µL of 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 to “Potentially Interfering Substances” in the Performance Characteristics 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 **STI PLUS - ELITe Positive Control** (not provided with this kit) with the **STI PLUS ELITe_PC** and **STI PLUS ELITe_Be_PC** Assay Protocols.
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **STI PLUS ELITe_NC** and **STI PLUS 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 **STI PLUS 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 **STI PLUS 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	If required **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** (if needed) **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.

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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 **STI PLUS 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 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 (Channels **CT, NG**, **MG**, **TV**) and the Internal Control (Channel **IC**) with the **STI PLUS ELITe_U_200_100** and **STI PLUS 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**
STI PLUS Positive Control	APPROVED
2) Negative Control	**Status**
STI PLUS 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
CT: DNA Detected	***C. trachomatis* DNA was detected** in the sample.
NG: DNA Detected Neisseria gonorrhoeae	***N. gonorrhoeae* DNA was detected** in the sample.
NG: DNA Determined, Species not determined	**Other related species DNA was detected** in the sample. Possible Low copies of *N. gonorrhoeae* not detected
NG: DNA Determined, Species not determined DNA Detected Neisseria gonorrhoeae	***N. gonorrhoeae* DNA was detected** in the sample, presence of DNA from other related species detected.
MG: DNA Detected	***M. genitalium* DNA was detected** in the sample.
TV: DNA Detected	***T. vaginalis* DNA was detected** in the sample.
CT: DNA Not detected or below the LoD	***C. trachomatis* DNA was not detected** in the sample. The sample is negative for the *C. trachomatis* DNA, or its concentration is below the assay Limit of Detection.
NG: DNA Not detected or below the LoD	***N. gonorrhoeae* DNA was not detected** in the sample. The sample is negative for the *N. gonorrhoeae* DNA, or its concentration is below the assay Limit of Detection.
MG: DNA Not detected or below the LoD	***M. genitalium* DNA was not detected** in the sample. The sample is negative for the *M. genitalium* DNA, or its concentration is below the assay Limit of Detection.
TV: DNA Not detected or below the LoD	***T. vaginalis* DNA was not detected** in the sample. The sample is negative for the *T. vaginalis* DNA or its 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, 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 30).

NOTE

When the endogenous Internal Control is used with cervical-vaginal swabs, take into account that the number of cells in the sample could be not sufficient due to an incorrect sampling.

NOTE

When a sample is reported as “NG: DNA Determined, Species not determined”, the sample must be tested with other methods of analysis.

Samples reported as “XX: DNA Not detected or below the LoD", are suitable for analysis but the DNA of the targets was not detected. In this case, the sample may be either negative for the DNA of the targets or the DNA of the targets is present at a concentration below the Limit of Detection of the assay (see 11 PERFORMANCE CHARACTERISTICS page 18).

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 **STI PLUS 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 **STI PLUS 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. Each tube is sufficient for 4 reactions. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.
2	If required **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).
8	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
9	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL.
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** (if needed) **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 **STI PLUS 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 BeGenius and ELITe InGenius instruments, by testing a panel of first void urine spiked with reference materials of *C. trachomatis* (ZeptoMetrix, USA)*, N. gonorrhoeae* (ZeptoMetrix, USA)*, M. genitalium* (DSMZ, DE) and *T. vaginalis* (ZeptoMetrix, USA).

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 for first void urine samples and ELITe InGenius

Pathogen	LoD (organisms / mL)	95% confidence interval	
		Lower limit	Upper limit
C. trachomatis	21	11	73
N. gonorrhoeae	59	32	276
M. genitalium	244	160	647
T. vaginalis	17	8	73

The calculated LoD value was verified by testing on ELITe BeGenius and ELITe InGenius first void urine, concentrated first void urine and Cervical-vaginal Swabs spiked with the *C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis* certified reference material at the claimed concentration.

The results obtained confirmed the claimed concentration for all the targets of STI PLUS ELITe MGB Kit with the three matrices on both ELITe BeGenius and ELITe InGenius.

11.2 Inclusivity: Efficiency of detection on different strain or isolates

The Inclusivity of the assay, as efficiency of detection for different strain or isolates of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T.vaginalis*, was evaluated by *in silico* analysis.

The analysis showed sequence conservation and absence of significant mutations. So, an efficient detection for the different strains or isolates is expected.

The Inclusivity was also verified through the analysis of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* reference materials (Zeptometrix and DSMZ).

The results are reported in the following table.

Table 13

Sample	Strain	Pos. / Rep.	Outcome
C. trachomatis	Z054	12/12	CT detected
N. gonorrhoeae	Z001	12/12	NG detected
M. genitalium	ID 23-412	12/12	MG detected
T. vaginalis	Z159	12/12	TV detected

All samples were correctly detected by the STI PLUS ELITe MGB Kit.

11.3 Interference among targets

The potential interference among targets of the assay was evaluated by a test of co-amplification of *C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis*.

For each target, the lower concentration detectable in all replicates is reported in the following table.

Table 14 Interference among targets

Target in test (low copies)	Interfering target at ~10^5 copies / reaction			
	C. trachomatis	***N. gonorrhoeae***	***M. genitalium***	***T. vaginalis***
C. trachomatis	-	10 c. / rxn	10 c. / rxn	10 c. / rxn
N. gonorrhoeae	10 c. / rxn	-	10 c. / rxn	10 c. / rxn
M. genitalium	50 c. / rxn	500 c. / rxn	-	2,500 c. / rxn
T. vaginalis	10 c. / rxn	10 c. / rxn	10 c. / rxn	-

11.4 Potential interfering organisms: cross-reactivity

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 *C. trachomatis* (CT), *M. genitalium* (MG) and *T. vaginalis* (TV) showed no significant homology with other unintended organisms (viruses, bacteria, protozoa and fungi), therefore no cross-reactivity is expected for them. The analysis for *N. gonorrhoeae* (NG) showed significant homology only with some strains of *Neisseria lactamica* (e.g., NCTC10617), therefore cross-reactivity is expected.

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

The final results are reported in the following table.

Table 15

Organism	Positive / Replicates					Outcome
	TV	CT	IC	NG	MG	
Mycoplasma hominis	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Ureaplasma urealyticum	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Ureaplasma parvum	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Treponema pallidum	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Gardnerella vaginalis	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Mobiluncus mulieris	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Bacteroides fragilis	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Peptostreptococcus anaerobius	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Candida albicans	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Lactobacillus acidophilus	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
HSV1	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
HSV2	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Neisseria menigitidis	0 / 5	0 / 5	5 / 5	0 / 5	0 / 5	No cross-reactivity
Neisseria lactamica	0 / 5	0 / 5	5 / 5	5 / 5 (Tm 5 / 5 < 64.5°C)	0 / 5	No cross-reactivity

NOTE

Neisseria gonorrhoeae is discriminated from the rest of the other *Neisseria species* through the analysis of the melting Temperature (Tm). If the Tm value is higher than or equal to 64.5°C the sample is *Neisseria gonorrhoeae* detected. If the Tm value is lower than 64.5°C the sample is Neisseria DNA determined, species not determined, the sample must be tested with other methods of analysis.

11.5 Potentially interfering organisms: Inhibition

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 (ATCC, Vircell and DSMZ) spiked with *C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis* reference materials (ZeptoMetrix and DSMZ).

The results are reported in the following table.

Table 16

Organism	Positive / Replicates					Outcome
	TV	CT	IC	NG	MG	
Mycoplasma hominis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Ureaplasma urealyticum	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Ureaplasma parvum	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Treponema pallidum	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Gardnerella vaginalis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Mobiluncus mulieris	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Bacteroides fragilis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Peptostreptococcus anaerobius	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Candida albicans	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Lactobacillus acidophilus	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
HSV1	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
HSV2	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Neisseria menigitidis	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	No interference
Neisseria lactamica	5 / 5	5 / 5	5 / 5	5 / 5 (Tm 5 / 5 < 64.5°C)	5 / 5	Interference NG detected

All potentially interfering organisms tested showed no inhibition of the target amplification using the STI PLUS ELITe MGB Kit with the exception of *N. lactamica*.

NOTE

In the case of co-infection of *N. Gonorrhoeae* and *N. lactamica*, in the presence of a high titer of *N. lactamica* (100,000 copies/reaction), we can detect the presence of *N. Gonorrhoeae* by Tm when the latter is found at concentrations greater than 3,000 copies/reaction.

11.6 Potentially interfering substances: Cross-reactivity

The cross-reactivity 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 concentration.

The results are reported in the following table.

Table 17

Sample	Pos / Rep					Outcome
	TV	CT	IC	NG	MG	
Reference	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Acid Urine	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Alkaline Urine	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Mucin	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Semen	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Whole Blood	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Acyclovir	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Azithromycin	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Clotrimazole	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Fosfomycin	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Nonoxinol-9	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity
Vaseline Oil	0 / 6	0 / 6	6 / 6	0 / 6	0 / 6	No cross-reactivity

The test showed that all the tested substances do not cross-react with the targets using the STI PLUS ELITe MGB Kit.

11.7 Potentially interfering substances: Inhibition

The potential inhibition of 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 concentration in samples of first void urine collected without preservatives spiked with the targets.

The results are reported in the following table.

Table 18

Sample	Pos / Rep					Outcome
	TV	CT	IC	NG	MG	
Reference	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Acid Urine	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Alkaline Urine	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Mucin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Semen	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Whole Blood	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Acyclovir	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Azithromycin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Clotrimazole	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference

Table 18 (continued)

Sample	Pos / Rep					Outcome
	TV	CT	IC	NG	MG	
Fosfomycin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Nonoxinol-9	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Vaseline Oil	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference

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

11.8 Repeatability

The Repeatability of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of first void urine samples negative or spiked with certified reference material of *C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis* (Zeptometrix and Qnostics).

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

Table 19 ELITe BeGenius Intra-Session Repeatability Day 1

Target	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	9	32.78	0.85	2.58	100%
3 X LOD *C. trachomatis*	9	38.07	1.08	2.84	100%
3 X LOD *N. gonorrhoeae*	9	31.47	0.27	0.86	100%
3 X LOD *M. genitalium*	9	33.30	0.52	1.58	100%
IC	36	26.88	0.75	2.80	-

Table 20 ELITe InGenius Intra-Session Repeatability Day 1

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	9	32.24	0.98	3.05	100%
3 X LOD *C. trachomatis*	9	37.49	0.66	1.76	100%
3 X LOD *N. gonorrhoeae*	9	31.46	0.58	1.86	100%
3 X LOD *M. genitalium*	9	33.12	0.70	2.11	100%
IC	36	25.59	0.22	0.85	-

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

Table 21 ELITe BeGenius Inter-Session Repeatability Day 1/Day 3

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	18	32.37	0.98	3.04	100%
3 X LOD *C. trachomatis*	18	37.12	1.34	3.61	100%
3 X LOD *N. gonorrhoeae*	18	30.86	0.69	2.22	100%

Table 21 ELITe BeGenius Inter-Session Repeatability Day 1/Day 3 (continued)

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *M. genitalium*	18	33.06	0.59	1.78	100%
IC	71	26.94	0.74	2.75	-

Table 22 ELITe InGenius Inter-Session Repeatability Day 1/Day 3

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	18	31.80	1.04	3.26	100%
3 X LOD *C. trachomatis*	18	36.36	1.43	3.92	100%
3 X LOD *N. gonorrhoeae*	18	30.68	0.91	2.96	100%
3 X LOD *M. genitalium*	18	32.59	1.01	3.09	100%
IC	72	25.66	0.24	0.93	-

In the Repeatability test, the STI PLUS 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%.

11.9 Reproducibility

The Reproducibility of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of first void urine samples negative or spiked with certified reference material of *C. trachomatis, N. gonorrhoeae, M. genitalium* and *T. vaginalis* (Zeptometrix and Qnostics).

An example of Inter-Batch Reproducibility (on two lots) is shown in the tables below.

Table 23 ELITe BeGenius Inter-Batch Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	18	33.18	1.05	3.16	100%
3 X LOD *C. trachomatis*	18	37.72	1.23	3.26	100%
3 X LOD *N. gonorrhoeae*	18	31.81	0.49	1.55	100%
3 X LOD *M. genitalium*	18	33.56	0.50	1.49	100%
IC	72	26.95	0.75	2.78	-

Table 24 ELITe InGenius Inter-Batch Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	18	32.34	0.85	2.63	100%
3 X LOD *C. trachomatis*	18	37.07	1.06	2.86	100%
3 X LOD *N. gonorrhoeae*	18	31.48	0.45	1.44	100%
3 X LOD *M. genitalium*	18	33.46	0.68	2.03	100%
IC	72	25.68	0.27	1.04	-

An example of Inter-Instrument Reproducibility(on two instruments)is shown in the tables below.

Table 25 ELITe BeGenius Inter-Instrument Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	18	32.66	1.17	3.57	100%
3 X LOD *C. trachomatis*	18	36.93	1.64	4.43	100%
3 X LOD *N. gonorrhoeae*	18	31.67	0.36	1.15	100%
3 X LOD *M. genitalium*	18	32.84	0.81	2.46	100%
IC	72	26.76	0.63	2.34	-

Table 26 ELITe InGenius Inter-Instrument Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3 X LOD *T. vaginalis*	18	32.45	1.13	3.48	100%
3 X LOD *C. trachomatis*	18	36.44	1.70	4.67	100%
3 X LOD *N. gonorrhoeae*	18	31.42	0.47	1.49	100%
3 X LOD *M. genitalium*	18	32.82	1.17	3.58	100%
IC	72	25.71	0.35	1.35	-

In the Reproducibility test, the STI PLUS 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%.

11.10 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 analysis of clinical samples of first void urine collected without preservatives, concentrated first void urine and cervical vaginal swabs, certified negative for each target.

As ELITe InGenius has equivalent analytical performances to ELITe BeGenius, 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 27

Negative first void urine samples	N	Positive	Negative	% Diagnostic Specificity
C. trachomatis	62	0	62	100%
N. gonorrhoeae	63	0	63	100%
M. genitalium	63	0	63	100%
T. vaginalis	63	0	63	100%

Table 28

Negative concentrated first void urine samples	N	Positive	Negative	% Diagnostic Specificity
C. trachomatis	61	0	61	100%
N. gonorrhoeae	63	0	63	100%

Table 28 (continued)

Negative concentrated first void urine samples	N	Positive	Negative	% Diagnostic Specificity
M. genitalium	62	0	62	100%
T. vaginalis	63	0	63	100%

Table 29

Negative cervical-vaginal swab samples	N	Positive	Negative	% Diagnostic Specificity
C. trachomatis	69	0	69	100%
N. gonorrhoeae	69	0	69	100%
M. genitalium	69	0	69	100%
T. vaginalis	69	0	69	100%

The IC Ct cut-off value is set at 31 for all matrices.

11.11 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 of first void urine collected without preservatives, concentrated first void urine and cervical vaginal swabs, certified positive for each target or spiked with reference materials.

As ELITe InGenius has equivalent analytical performances to ELITe BeGenius, 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 30

Positive/spiked first void urine	N	Positive	Negative	% Diagnostic Sensitivity
Positive for *C. trachomatis*	58	56	2	96.6%
Positive for *N. gonorrhoeae*	59	58	1	98.3%
Positive for *M. genitalium*	11	7	4	93%
Spiked for *M. genitalium*	46	46	0	
Positive for *T. vaginalis*	12	11	1	98%
Spiked for *T. vaginalis*	38	38	0	

Table 31

Positive/spiked concentrated first void urine	N	Positive	Negative	% Diagnostic Sensitivity
Positive for *C. trachomatis*	58	58	0	100%
Positive for *N. gonorrhoeae*	59	58	1	98.3%

Table 31 (continued)

Positive/spiked concentrated first void urine	N	Positive	Negative	% Diagnostic Sensitivity
Positive for *M. genitalium*	11	8	3	94.8%
Spiked for *M. genitalium*	47	47	0	
Positive for *T. vaginalis*	12	11	1	98%
Spiked for *T. vaginalis*	38	38	0	

Table 32

Positive/spiked Cervical-vaginal swab	N	Positive	Negative	% Diagnostic Sensitivity
Positive for *C. trachomatis*	52	52	0	100%
Positive for *N. gonorrhoeae*	19	19	0	100%
Spiked for *N. gonorrhoeae*	36	36	0	
Positive for *M. genitalium*	39	31	8	87.3%
Spiked for *M. genitalium*	24	24	0	
Positive for *T. vaginalis*	17	17	0	100%
Spiked for *T. vaginalis*	33	33	0	

For all the four targets in all the matrices, the above results are from single-infected and multi-infected samples. In case of single infection, the sensitivity was at least 96%.

11.12 Method agreement

The Diagnostic performances of the assay, as agreement with reference method, was evaluated with the Cohen's kappa value.

The comparison of the results is summarized in the following tables.

Table 33 First void urine

		C. trachomatis				
		Reference method			**AUC**	**Cohen's kappa**
		Pos	**Neg**	**Tot**		
STI PLUS ELITe MGB Kit	**Pos**	56	0	56	**98.3%**	**0.967**
	Neg	2	62	64		
	Tot	58	62	120		

		N. gonorrhoeae				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	58	0	58	99.2%	0.984
	Neg	1	63	64		
	Tot	59	63	122		

		M. genitalium				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	53	0	53	96.7%	0.933
	Neg	4	63	67		
	Tot	57	63	120		

		T. vaginalis				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	49	0	49	99.1%	0.982
	Neg	1	63	64		
	Tot	50	63	113		

Table 34 Concentrated first void urine

		C. trachomatis				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	58	0	58	100%	1.000
	Neg	0	61	61		
	Tot	58	61	119		

		N. gonorrhoeae				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	58	0	58	99.2%	0.984
	Neg	1	63	64		
	Tot	59	63	122		

		M. genitalium				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	55	0	55	97.5%	0.950
	Neg	3	62	65		
	Tot	58	62	120		

		T. vaginalis				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	49	0	49	99.1%	0.982
	Neg	1	63	64		
	Tot	50	63	113		

Table 35 Cervical-vaginal swabs

		C. trachomatis				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	52	0	52	100%	1.000
	Neg	0	69	69		
	Tot	52	69	121		

		N. gonorrhoeae				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	55	0	55	100%	1.000
	Neg	0	69	69		
	Tot	55	69	124		

		M. genitalium				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	55	0	55	93.9%	0.878
	Neg	8	69	77		
	Tot	63	69	132		

		T. vaginalis				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
STI PLUS ELITe MGB Kit	Pos	50	0	50	100%	1.000
	Neg	0	69	69		
	Tot	50	69	119		

The STI PLUS ELITe MGB Kit generated an Area Under Curve (AUC) and a Cohen's kappa value corresponding to a perfect agreement with the results obtained with the reference method, for all the four targets and for all the 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 "STI PLUS ELITe MGB Kit", FTP 400ING.

12 REFERENCES

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13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: first void urine collected without preservatives and cervical-vaginal swab.

Do not use this product with samples containing mucin at high concentration: mucin inhibits the amplification reaction of nucleic acids and causes invalid results.

Currently there are no data available concerning product performance with other clinical samples: sperm, rectal swabs, oropharyngeal swabs.

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 contaminations from positive clinical samples, positive controls and PCR products. Cross-contaminations cause false positive results. The product format is designed to limit cross-contaminations. However, cross-contaminations 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 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 means 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.

In case of co-infections, the sensitivity for one target can be affected by the amplification of a second target (see 11 PERFORMANCE CHARACTERISTICS page 18).

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 36

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 37

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.

Table 37 (continued)

Invalid Negative Control reaction	
Possible Causes	**Solutions**
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 38

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 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. Prepare 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 39

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 40

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 41

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. At the moment of the current revision of the IFU, no serious incident or recall with impact on product performance and safety of the device has occurred.

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, 7381818, 7541454, 7671218, 7718374, 7723038, 7759126, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1687609, 1781675, 1789587, 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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Appendix A STI PLUS 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 at www.elitechgroup.com.

INTENDED USE

The product **STI PLUS ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids Real-Time PCR assay **for the detection and identification of the DNA of *Chlamydia trachomatis***, ***Neisseria gonorrhoeae***, ***Mycoplasma genitalium*** and ***Trichomonas vaginalis*** extracted from clinical specimens.

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 swabs.

The product is intended for use as an aid in the diagnosis of urogenital tract infections in patients suspected of having *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium* or *Trichomonas vaginalis* infections.

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

Amplified sequence

Sequence	Gene	Fluorophore	Channel
C. trachomatis	dnaB-like and ompA	AP525	CT
N. gonorrhoeae	pivNG	AP593	NG
M. genitalium	23S rRNA	AP639	MG
T. vaginalis	L23861	FAM	TV
Internal Control	Human Beta Globin	AP559	IC

Validated matrix

first void urine collected without preservatives
cervical-vaginal swabs

Kit content and related products

STI PLUS PLUS ELITe MGB Kit Kit (RTS400ING)		STI PLUS PLUS - ELITe Positive Control (CTR400ING)	
 X 8		 X 3	
STI PLUS PLUS PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube		STI PLUS PLUS Positive Control 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 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 SP200 Consumable Set: INT032CS. › ELITe InGenius PCR Cassette: INT035PCR. › ELITe InGenius Waste Box: F2102-000.	› 300 µL Filter Tips Axigen: TF-350-L-R-S. › 1000 µL Filter Tips Tecan: 30180118. **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 › Total elution volume	200 µL 100 µL	› Eluate PCR input volume › PCR Mix volume › Frequency of controls	20 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Target	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity	Method Agreement	
					AUC	Cohen's kappa
First Void Urine	CT	21 organism/mL	96.6% (56/58)	100%	**98.3%**	**0.967**
	NG	59 organism/mL	98.3% (58/59)	100%	**99.2%**	**0.984**
	MG	244 organism/mL	93% (53/57)	100%	**96.7%**	**0.933**
	TV	17 organism/mL	98% (49/50)	100%	**99.1%**	**0.982**
Concentrated First Void Urine	CT	21 organism/mL	100% (58/58)	100%	**100%**	**1.000**
	NG	59 organism/mL	98.3% (58/59)	100%	**99.2%**	**0.984**
	MG	244 organism/mL	94.8% (55/58)	100%	**97.5%**	**0.950**
	TV	17 organism/mL	98% (49/50)	100%	**99.1%**	**0.982**

Matrix	Target	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity	Method Agreement	
					AUC	Cohen's kappa
Cervical-vaginal swab	CT	21 organism/mL	100% (52/52)	100%	**100%**	**1.000**
	NG	59 organism/mL	100% (55/55)	100%	**100%**	**1.000**
	MG	244 organism/mL	87.3 (55/63)	100%	**93.9%**	**0.878**
	TV	17 organism/mL	100% (50/50)	100%	**100%**	**1.000**

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.

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 swab	eSwab® (COPAN)	≤ 2 days	≤ 2 days	≤ 1 month	≤ 1 month

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.

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

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** 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: STI PLUS ELITe_U_200_100 or STI PLUS ELITe_CS_200_100	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix in the Inventory Block
7. Load PCR Cassettes, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**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: STI PLUS ELITe_PC or STI PLUS ELITe_NC or STI PLUS ELITe_U_200_100 or STI PLUS ELITe_CS_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, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	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

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** 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: STI PLUS ELITe_Be_U_200_100 or STI PLUS 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 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)

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: STI PLUS ELITe_Be_PC or STI PLUS ELITe_Be_NC or STI PLUS ELITe_Be_U_200_100 or STI PLUS ELITe_Be_CS_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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