

Instructions for use

UroGen ELITe MGB® Kit

reagents for DNA Real-Time PCR



REF RTS404ING

UDI 08033891487362

CE **IVD**
0123

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yyyy)
00	new product development	13/05/2024

TABLE OF CONTENT

1 INTENDED USE

2 ASSAY PRINCIPLE

3 PRODUCT DESCRIPTION

4 MATERIALS PROVIDED IN THE PRODUCT

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT.....

6 OTHER PRODUCTS REQUIRED.....

7 WARNINGS AND PRECAUTIONS

8 SPECIMENS AND CONTROLS

9 ELITe InGenius PROCEDURE.....

10 ELITe BeGenius PROCEDURE

11 PERFORMANCE CHARACTERISTICS.....

12 REFERENCES.....

13 PROCEDURE LIMITATIONS

14 TROUBLESHOOTING

15 SYMBOLS

16 NOTICE TO THE USERS.....

17 NOTICE TO PURCHASER: LIMITED LICENSE

Appendix A QUICK START GUIDE.....

4

4

4

5

5

5

6

7

9

14

17

30

31

32

34

34

34

36

1 INTENDED USE

The product **UroGen ELITE MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the genomic DNA of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum*, 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 swab.

The product is intended for use as an aid in the diagnosis of urogenital tract infections in patients suspected of having *Mycoplasma hominis*, *Ureaplasma urealyticum* or *Ureaplasma parvum* 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 hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum* DNA isolated from specimens and amplified using the assay reagent **UroGen PCR Mix**, that contains primers and probes with ELITE MGB Kit technology.

The ELITE MGB Kit 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 Kit 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 **UroGen ELITE MGB Kit** provides the assay reagent **UroGen PCR Mix**, an optimized and stabilized PCR Mixture that contains the specific primers and probes for:

- *Mycoplasma hominis* **gap** gene, detected in Channel **MH**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye,
- *Ureaplasma urealyticum*, **ureC** gene, detected in Channel **UU**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor® 639 (AP639) dye,
- *Ureaplasma parvum*, **ureC** gene, detected in Channel **UP**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 593 (AP593) 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 525 (AP525) dye.

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

The Internal Control monitors for the cellularity of sample, extraction and PCR efficiency.

The assay test can be performed in two different ways:

- testing the first void urine, using as exogenous template for Internal Control the **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 **UroGen 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 **UroGen 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
UroGen PCR Mix ref. RTS404ING	Mixture of reagents for Real-Time PCR in tube with WHITE 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 (~3,000 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).
- 15 mL Generic screw cap tube (e.g., Sarstedt ref. 62.554.502)
- 50 mL Generic screw cap tube (e.g., Sarstedt ref. 62.547.254)
- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

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

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

Table 2

Instruments and softwares	Products and reagents
ELiTe InGenius (ELiTechGroup S. p. A., EG SpA, ref. INT030) ELiTe InGenius Software version 1.3.0.19 (or later) UroGen ELiTe _PC , Assay Protocol with parameters for Positive Control analysis UroGen ELiTe _NC , Assay Protocol with parameters for Negative Control analysis UroGen ELiTe_U_200_100 , Assay Protocol with parameters for Urine specimen analysis UroGen 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. CTCPE) for Urine specimens only UroGen - ELiTe Positive Control (EG SpA, ref. CTR404ING) eSWAB® (COPAN Italia S. p. A., ref. 480CE), or an equivalent device, for cervical-vaginal swab specimens only
ELiTe BeGenius (EG SpA, ref. INT040) ELiTe BeGenius Software version 2.2.1 (or later) UroGen ELiTe_Be_PC , Assay Protocol with parameters for Positive Control analysis UroGen ELiTe_Be_NC , Assay Protocol with parameters for Negative Control analysis UroGen ELiTe_Be_U_200_100 , Assay Protocol with parameters for Urine specimen analysis UroGen 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)
UroGen 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	≤ 24 hours	≤ 48 hours	≤ 1 months	≤ 1 month
Cervical-vaginal swabs	eSwab® (COPAN)	≤ 48 hours	≤ 48 hours	≤ 1 months	≤ 1 month

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

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 Kit Kits and the **ELITE InGenius** or **ELITE BeGenius** with the indicated matrices.

Table 5

Assay Protocols for UroGen ELITE MGB Kit Kit				
Specimen	Instrument	Assay Protocol Name	Report	Characteristics
First void Urine	ELITE InGenius	UroGen 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	UroGen ELITE_Be_U_200_100	Positive / Negative	
Cervical- vaginal swabs	ELITE InGenius	UroGen ELITE_CS_200_100	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITE BeGenius	UroGen 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 “7 WARNINGS AND PRECAUTIONS page 6” 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 **UroGen - ELITE Positive Control** (not provided with this kit) with the **UroGen ELITE_PC** or **UroGen ELITE_Be_PC** Assay Protocols.
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **UroGen ELITE_NC** or **UroGen 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 **UroGen 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	A) Validation of Positive Control and Negative Control results
		B) Validation of sample results
		C) 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 (**UroGen Positive Control**, **UroGen Negative Control**) are approved and valid (Status) for the **UroGen PCR Mix** lot to be used. If no valid PCR Controls are available for the **UroGen 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 “[8 SPECIMENS AND CONTROLS page 7](#)”).

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 **UroGen 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 **UroGen 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 8 SPECIMENS AND CONTROLS page 7).	Select the Assay Protocol in the "Assay" column (see 8 SPECIMENS AND CONTROLS page 7).	Select the Assay Protocol in the "Assay" column (see 8 SPECIMENS AND CONTROLS page 7). 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.

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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.
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 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 **UroGen 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 **UroGen 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 reactions with the **UroGen ELiTe_PC** and **UroGen 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 **MH**, **UU**, **UP**) and the Internal Control (channel **IC**) with the **UroGen ELiTe_U_200_100** and **UroGen ELiTe_CS_200_100** Assay Protocols 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
UroGen Positive Control	APPROVED
2) Negative Control	Status
UroGen 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
MH:DNA Detected	<i>Mycoplasma hominis</i> DNA was detected in the sample.
UU:DNA Detected	<i>Ureaplasma urealyticum</i> DNA was detected in the sample.
UP:DNA Detected	<i>Ureaplasma parvum</i> DNA was detected in the sample.
MH:DNA Not Detected or below the LoD	<i>Mycoplasma hominis</i> DNA was not detected in the sample. The sample is negative for the <i>Mycoplasma hominis</i> DNA, or its concentration is below the assay Limit of Detection.
UU:DNA Not Detected or below the LoD	<i>Ureaplasma urealyticum</i> DNA was not detected in the sample. The sample is negative for the <i>Ureaplasma urealyticum</i> DNA, or its concentration is below the assay Limit of Detection.
UP:DNA Not Detected or below the LoD	<i>Ureaplasma parvum</i> DNA was not detected in the sample. The sample is negative for the <i>Ureaplasma parvum</i> 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 32](#)").

Samples reported as "Xxx: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 17](#)").

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 **UroGen 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	A) Validation of Positive Control and Negative Control results
		B) Validation of sample results
		C) 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 (**UroGen Positive Control**, **UroGen Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **UroGen 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 “[8 SPECIMENS AND CONTROLS page 7](#)”).

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 **UroGen ELITE MGB Kit** can be used on the **ELITE BeGenius** to perform:

- Sample run (Extract + PCR),
- Eluted sample run (PCR Only),
- 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 **UroGen 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 2 mL 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.
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 " 8 SPECIMENS AND CONTROLS page 7 ").	Select the Assay Protocol in the "Assay" column (see " 8 SPECIMENS AND CONTROLS page 7 ").	Select the Assay Protocol in the "Assay" column (see " 8 SPECIMENS AND CONTROLS page 7 ").
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

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
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 Cooler Unit 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 **UroGen 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 **UroGen 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 pool of first void urine spiked with reference materials of *Mycoplasma hominis* (ZeptoMetrix, US), *Ureaplasma parvum* (ATCC, US) and *Ureaplasma urealyticum* (NCTC, UK). 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 urine samples and ELiTe BeGenius			
Pathogen	LoD (organisms / mL)	95% confidence interval limits	
		Lower limit	Upper limit
<i>M. hominis</i>	223	143	477
<i>U. parvum</i>	79	54	158
<i>U. urealyticum</i>	152	101	310

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 *M. hominis*, *U. urealyticum* and *U. parvum* certified reference material at the claimed concentration.

The results obtained confirmed the claimed concentration for all the targets of UroGen ELiTe MGB Kit 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 *M. hominis*, *U. urealyticum* and *U. parvum*, 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 *M. hominis*, *U. urealyticum* and *U. parvum* reference materials (ATCC, NCTC, Zeptomatrix and Vircell).

The results are reported in the following table.

Table 13

Genomic DNA Reference material			
Sample	Strain	Pos. / Rep.	Outcome
<i>M. hominis</i>	ZK-CU2	3 / 3	MH detected
	LBD-4	3 / 3	MH detected
<i>U. parvum</i>	7	3 / 3	UP detected
<i>U. urealyticum</i>	960	3 / 3	UU detected

Table 14

Bacterial culture Reference material			
Sample	Strain	Pos. / Rep.	Outcome
<i>M. hominis</i>	Z317	3 / 3	MH detected
	LBD-4	3 / 3	MH detected
<i>U. parvum</i>	27	3 / 3	UP detected
<i>U. urealyticum</i>	354	3 / 3	UU detected
	960	3 / 3	UU detected
	960 (CX8)	3 / 3	UU detected

All samples were correctly detected by the UroGen ELITE MGB Kit Kit.

11.3 Interference among targets

The potential interference among targets of the assay was evaluated by a test of co-amplification of *M. hominis*, *U. parvum* and *U. urealyticum*.

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

Table 15

Interference among targets			
Target in test (low copies)	Interfering target at ~10 ⁵ copies / reaction		
	<i>M. hominis</i>	<i>U. parvum</i>	<i>U. urealyticum</i>
<i>M. hominis</i>	-	500 c. / rxn	500 c. / xn
<i>U. parvum</i>	1,500 c. / rxn	-	1,500 c. / rxn
<i>U. urealyticum</i>	500 c. / rxn	500 c. / rxn	-

The UroGen ELITE MGB Kit Kit shows a minimal interference among targets. Despite this interference, one target can be detected even when it has a concentration about 100 times less than the other ones in the same sample.

11.4 Bacterial Load Evaluation

The bacterial load evaluation using this assay was implemented through the calculation of defined Ct values intervals for each target corresponding to its approximative logarithmic concentration in the samples.

The Ct intervals for the evaluation of the bacterial load for each target using the UroGen ELITE MGB Kit Kit are reported in the following table.

Table 16

MH Ct Interval	UP Ct Interval	UU Ct Interval	Estimated Quantity Log organisms / mL
18.32 - 21.69	19.64 - 22.96	17.50 - 20.88	About 7
21.70 - 25.07	22.97 - 26.30	20.89 - 24.27	About 6
25.08 - 28.46	26.31 - 29.64	24.28 - 27.65	About 5
28.47 - 31.84	29.65 - 32.97	27.66 - 31.04	About 4
31.85 - 35.22	32.98 - 36.31	31.05 - 34.43	About 3
35.23 - 38.58	36.32 - 39.62	34.44 - 37.79	About 2

11.5 Potentially 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 showed no significant homology with other unintended organisms (viruses, bacteria, protozoa and fungi). Therefore, no 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 results are reported in the following table.

Table 17

Sample	Positive / Replicates				Outcome
	MH	IC	UU	UP	
<i>Chlamydia trachomatis</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Neisseria gonorrhoeae</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Mycoplasma genitalium</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Trichomonas vaginalis</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Treponema pallidum</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Gardnerella vaginalis</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Mobiluncus mulieri</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Bacteroides fragilis</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Peptostreptococcus anaerobius</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Lactobacillus acidophilus</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Escherichia coli</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Staphylococcus aureus</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
<i>Candida albicans</i>	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
HSV1	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
HSV2	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
HPV16	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity

All potentially interfering organisms tested showed no cross-reactivity for the targets using the UroGen ELITE MGB Kit.

11.6 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 *M. hominis*, *U. urealyticum* and *U. parvum* reference materials (Vircell).

The results are reported in the following table.

Table 18

Sample	Positives / Replicates				Outcome
	MH	IC	UU	UP	
<i>Chlamydia trachomatis</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Neisseria gonorrhoeae</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Mycoplasma genitalium</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Trichomonas vaginalis</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Treponema pallidum</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference

Table 18 (continued)

Sample	Positives / Replicates				Outcome
	MH	IC	UU	UP	
<i>Gardnerella vaginalis</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Mobiluncus mulieri</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Bacteroides fragilis</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Peptostreptococcus anaerobius</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Lactobacillus acidophilus</i>	3 / 3	3 / 3	3 / 3	6 / 6	No interference
<i>Escherichia coli</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Staphylococcus aureus</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
<i>Candida albicans</i>	3 / 3	3 / 3	3 / 3	3 / 3	No interference
HSV1	3 / 3	3 / 3	3 / 3	3 / 3	No interference
HSV2	3 / 3	3 / 3	3 / 3	3 / 3	No interference
HPV16	3 / 3	3 / 3	3 / 3	3 / 3	No interference

All potentially interfering organisms tested showed no inhibition of the target amplification using the UroGen ELITe MGB Kit.

11.7 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 19

Sample	Pos / Rep				Out come
	MH	IC	UU	UP	
Mucin	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Whole Blood	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Semen	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Alkaline Urine	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Acid Urine	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Acyclovir	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Fosfomycin	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Azithromycin	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Clotrimazole	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Vaseline Oil	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity
Nonoxinol-9	0 / 3	3 / 3	0 / 3	0 / 3	No cross-reactivity

The test showed that all the tested substances do not cross-react with the targets using the UroGen ELITE MGB Kit Kit.

11.8 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 20

Sample	Pos / Rep				Outcome
	MH	IC	UU	UP	
Mucin	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Whole Blood	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Semen	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Alkaline Urine	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Acid Urine	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Acyclovir	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Fosfomycin	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Azithromycin	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Clotrimazole	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Vaseline Oil	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Nonoxinol-9	3 / 3	3 / 3	3 / 3	3 / 3	No interference

The test showed that the tested substances do not inhibit the targets detection using the UroGen ELITE MGB Kit Kit.

11.9 Cross-contamination

The possible Cross-contamination during analysis was evaluated for the assay by testing 60 replicates of a negative first void urine specimen alternated to 60 replicates of a positive first void urine specimen in 5 sessions.

The results are reported in the following table.

Table 21

Samples	N	Positive	Negative	%Agreement
Positive	60	60	0	100%
Negative	60	0	60	100%

In this test with the UroGen ELITE MGB Kit Kit the cross-contamination was neither detected within sessions nor between sessions.

11.10 Whole system failure

The Whole system failure rate for the assay was evaluated by testing a panel of first void urine samples spiked with certified reference material of *M. hominis* (Zeptomatrix) at concentration of 3x LoD.

The results are reported in the following table.

Table 22

Samples	N	Positive	Negative	Whole system failure rate
First void urine spiked at 3x LoD	50	50	0	0%

In this test with the UroGen ELiTe MGB Kit Kit none of the *M. hominis* positive samples gave false negative results. In this test the whole system failure rate was equal to 0%.

11.11 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 *M. hominis*, *U. urealyticum* and *U. parvum* (Zeptomatrix, NCTC and ATCC).

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

Table 23

ELiTe BeGenius Intra-Session Repeatability (Day 1)						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	MH	8	-	-	-	100%
3x LoD		8	36.36	0.58	1.60	100%
10x LoD		8	34.57	0.40	1.16	100%
Neg	UP	8	-	-	-	100%
3x LoD		8	36.65	0.70	1.91	100%
10x LoD		8	34.58	0.50	1.45	100%
Neg	UU	8	-	-	-	100%
3x LoD		8	34.83	0.44	1.27	100%
10x LoD		8	32.75	0.33	1.00	100%

Table 24

ELiTe InGenius Intra-Session Repeatability (Day 1)						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	MH	8	-	-	-	100%
3x LoD		8	35.35	0.42	1.19	100%
10x LoD		8	33.14	0.12	0.36	100%
Neg	UP	8	-	-	-	100%
3x LoD		8	36.05	0.17	0.48	100%
10x LoD		8	33.99	0.24	0.70	100%

Table 24 (continued)

ELiTe InGenius Intra-Session Repeatability (Day 1)						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	UU	8	-	-	-	100%
3x LoD		8	34.14	0.61	1.79	100%
10x LoD		8	31.86	0.30	0.94	100%

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

Table 25

ELiTe BeGenius Inter-Session Repeatability (Day 1 + Day 2)						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	MH	16	-	-	-	100%
3x LoD		16	36.29	0.46	1.26	100%
10x LoD		16	34.45	0.39	1.14	100%
Neg	UP	16	-	-	-	100%
3x LoD		16	36.57	0.59	1.62	100%
10x LoD		16	34.71	0.47	1.35	100%
Neg	UU	16	-	-	-	100%
3x LoD		16	35.05	0.57	1.64	100%
10x LoD		16	32.72	0.29	0.90	100%

Table 26

ELiTe InGenius Inter-Session Repeatability (Day 1 + Day 2)						
Neg	MH	16	-	-	-	100%
3x LoD		16	35.26	0.42	1.19	100%
10x LoD		16	33.18	0.16	0.48	100%
Neg	UP	16	-	-	-	100%
3x LoD		16	36.10	0.36	1.00	100%
10x LoD		16	34.05	0.28	0.81	100%
Neg	UU	16	-	-	-	100%
3x LoD		16	34.28	0.59	1.74	100%
10x LoD		16	32.02	0.31	0.98	100%

In the Repeatability test, the UroGen ELiTe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 1.91%.

11.12 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 *M. hominis*, *U. urealyticum* and *U. parvum* (Zeptomatrix, NCTC and ATCC).

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

Table 27

ELiTe BeGenius Inter-Batch Reproducibility						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	MH	48	-	-	-	100%
3x LoD		48	36.17	0.42	1.17	100%
10x LoD		48	34.26	0.41	1.19	100%
Neg	UP	48	-	-	-	100%
3x LoD		48	36.44	0.50	1.37	100%
10x LoD		48	34.60	0.40	1.15	100%
Neg	UU	48	-	-	-	100%
3x LoD		48	35.04	0.54	1.53	100%
10x LoD		48	32.62	0.40	1.24	100%

Table 28

ELiTe InGenius Inter-Batch Reproducibility						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	MH	48	-	-	-	100%
3x LoD		48	35.05	0.51	1.46	100%
10x LoD		48	33.02	0.25	0.77	100%
Neg	UP	48	-	-	-	100%
3x LoD		48	35.85	0.52	1.45	100%
10x LoD		48	33.91	0.28	0.84	100%
Neg	UU	48	-	-	-	100%
3x LoD		48	34.28	0.56	1.63	100%
10x LoD		48	31.99	0.28	0.89	100%

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

Table 29

ELiTe BeGenius Inter-Instrument Reproducibility						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	MH	24	-	-	-	100%
3x LoD		24	36.04	0.63	1.74	100%
10x LoD		24	33.71	0.43	1.27	100%
Neg	UP	24	-	-	-	100%
3x LoD		24	36.34	0.61	1.68	100%
10x LoD		24	34.30	0.33	0.96	100%
Neg	UU	24	-	-	-	100%
3x LoD		24	35.73	0.67	1.89	100%
10x LoD		24	32.92	0.38	1.14	100%

Table 30

ELiTe InGenius Inter-Instrument Reproducibility						
Sample	Target	N	Mean Ct	SD	%CV	%Agreement
Neg	MH	24	-	-	-	100%
3x LoD		24	35.18	0.40	1.15	100%
10x LoD		24	33.04	0.36	1.09	100%
Neg	UP	24	-	-	-	100%
3x LoD		24	36.24	0.40	1.12	100%
10x LoD		24	34.04	0.31	0.91	100%
Neg	UU	24	-	-	-	100%
3x LoD		24	34.81	0.97	2.78	100%
10x LoD		24	32.12	0.35	1.08	100%

In the Reproducibility test, the UroGen ELiTe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 2.78%.

11.13 Diagnostic Specificity: Confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with ELiTe BeGenius by analysing clinical samples of first void urine collected without preservatives, concentrated first void urine and cervical vaginal swabs collected in eSWAB kit, 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 BeGenius is also applicable to ELiTe InGenius.

The results are summed up in the following table.

Table 31

Negative first void urine	N	Positive	Negative	% Diagnostic Specificity
<i>M. hominis</i>	174	0	174	100%
<i>U. parvum</i>	155	2	153	98.7%
<i>U. urealyticum</i>	152	1	151	99.3%

Table 32

Negative Concentrated first void urine	N	Positive	Negative	% Diagnostic Specificity
<i>M. hominis</i>	176	4	172	97.7%
<i>U. parvum</i>	151	0	151	100%
<i>U. urealyticum</i>	152	2	150	98.7%

Table 33

Negative Cervical-vaginal swab	N	Positive	Negative	% Diagnostic Specificity
<i>M. hominis</i>	178	1	177	99.4%
<i>U. parvum</i>	149	0	149	100%
<i>U. urealyticum</i>	171	2	169	98.8%

The IC Ct cut-off value is set at 30 for all matrices.

11.14 Diagnostic Sensitivity: Confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with ELiTe BeGenius 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 BeGenius is also applicable to ELiTe InGenius.

The results are summed up in the following table.

Table 34

Positive/spiked first void urine	N	Positive	Negative	% Diagnostic Sensitivity
Positive for <i>M. hominis</i>	31	31	0	100%
Spiked for <i>M. hominis</i>	20	20	0	
Positive for <i>U. parvum</i>	55	52	3	94.5%
Positive for <i>U. urealyticum</i>	53	52	1	98.1%

Table 35

Positive/spiked concentrated first void urine	N	Positive	Negative	% Diagnostic Sensitivity
Positive for <i>M. hominis</i>	31	31	0	100%
Spiked for <i>M. hominis</i>	20	20	0	
Positive for <i>U. parvum</i>	54	52	2	96.3%
Positive for <i>U. urealyticum</i>	53	52	1	98.1%

Table 36

Positive Cervical-vaginal swab	N	Positive	Negative	% Diagnostic Sensitivity
Positive for <i>M. hominis</i>	52	51	1	98.1%
Positive for <i>U. parvum</i>	78	73	5	93.6%
Positive for <i>U. urealyticum</i>	51	48	3	94.1%

For all the three targets in all the matrices, the above results are from single-infected and multi-infected samples. In case of single infection, all the samples were correctly detected as positives and the sensitivity was equal to 100%.

11.15 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 37

		First void urine				
		<i>M. hominis</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit Kit	Pos	51	0	51	100%	1.000
	Neg	0	174	174		
	Tot	51	174	225		
		<i>U. parvum</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit Kit	Pos	52	2	54	97.6%	0.938
	Neg	3	153	156		
	Tot	55	155	210		

Table 37 (continued)

		<i>U. urealyticum</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit Kit	Pos	52	1	53	99.0%	0.975
	Neg	1	151	152		
	Tot	53	152	205		

Table 38

		Concentrated first void urine				
		<i>M. hominis</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit	Pos	51	4	55	98.2%	0.951
	Neg	0	172	172		
	Tot	51	176	227		
		<i>U. parvum</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit	Pos	52	0	52	99.0%	0.975
	Neg	2	151	153		
	Tot	54	151	205		
		<i>U. urealyticum</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit	Pos	52	2	54	98.5%	0.962
	Neg	1	150	151		
	Tot	53	152	205		

Table 39

		Cervical-vaginal swabs				
		<i>M. hominis</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit	Pos	51	1	52	99.1%	0.975
	Neg	1	177	178		
	Tot	52	178	230		
		<i>U. parvum</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit	Pos	73	0	73	97.8%	0.950
	Neg	5	149	154		
	Tot	78	149	227		
		<i>U. urealyticum</i>				
		Reference method			AUC	Cohen's kappa
		Pos	Neg	Tot		
UroGen ELiTe MGB Kit	Pos	48	2	50	97.7%	0.936
	Neg	3	169	172		
	Tot	51	171	222		

The UroGen 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 three 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 "UroGen ELITE MGB Kit", FTP 404ING.

12 REFERENCES

- A. Baczynska et al (2004) BMC Microbiology 4: 35, doi: 10.1186/1471-2180-4-35.
- J. A. Robertson et al. (2002) Int. J. Syst. Evol. Microbiol. 52: 587 - 597, doi: 10.1099/ijls.0.01965-0.
- S. A. Cunningham et al. (2013) Int. J. Bacteriol. 2013: 168742
- J. Maryne et al. (2019) J. Clin. Method 165: doi: 10.1016/j.mimet.2019.105700.
- K. Linnet et al. (2004) Clin. Chem. 50: 732 - 740.
- E. A. Lukhtanov et al. (2007) Nucleic Acids Res. 35: e30

13 PROCEDURE LIMITATIONS

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

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

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 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 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.

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 17](#)).

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 40

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 41

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 42

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.

Table 42 (continued)

Invalid Sample reaction	
Possible Causes	Solutions
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 43

Anomalous dissociation curve	
Possible causes	Solutions
Absence of a defined peak. Defined peak but T _m 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 44

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 45

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



Catalogue Number.



Upper limit of temperature.



Batch code.



Use by (last day of month).



in vitro diagnostic medical device.



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.



Unique Device Identification



Contains sufficient for "N" tests.



Caution, consult instructions for use.



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.

MGB®, Eclipse Dark Quencher®, AquaPhluor®, ELiTe MGB®, the ELiTe MGB® logo, ELiTe InGenius® and ELiTe BeGenius® are registered trademarks of ELiTechGroup within the European Union.
eSwab® is registered trademark of COPAN Italia S.p.A.

Appendix A UroGen ELiTe MGB Kit® 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 **UroGen ELiTe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids Real-Time PCR assay for the detection and identification of the genomic DNA of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum*, 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 swab.

The product is intended for use as an aid in the diagnosis of urogenital tract infections in patients suspected of having *Mycoplasma hominis*, *Ureaplasma urealyticum* or *Ureaplasma parvum* infection.

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



Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	<i>Mycoplasma hominis</i> , gap gene	FAM	MH
Target 2	<i>Ureaplasma urealyticum</i> , ureC gene	AP639	UU
Target 3	<i>Ureaplasma parvum</i> , ureC gene	AP593	UP
Internal Control	beta globin gene	AP525	IC

Validated matrix

› First void Urine (“as is” and concentrated)	
› Cervical-vaginal swabs	

Kit content and related products

UroGen ELiTe MGB Kit Kit (RTS404ING)	UroGen - ELiTe Positive Control (CTR404ING)
 X 8	 X 3
UroGen PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube	UroGen Positive Control 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles

UroGen ELiTe MGB Kit Kit (RTS404ING)		UroGen - ELiTe Positive Control (CTR404ING)	
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

<ul style="list-style-type: none"> › 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. 	<ul style="list-style-type: none"> › CPE - Internal Control: CTCPE (for first void Urine specimens only) › 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	200 µL	› Eluate PCR input volume	20 µL
› CPE volume	10 µL	› UroGen PCR Mix volume	20 µL
› Total elution volume	100 µL	› Frequency of controls	15 days

ELiTe InGenius and ELiTe BeGenius Performances

Matrix	Target	Limit of Detection	Sensitivity	Specificity	Method Agreement	
					AUC	Cohen's kappa
First void Urine	<i>Mycoplasma hominis</i>	223 organism / mL	100% (51/51)	100% (174/174)	100%	1.000
	<i>Ureaplasma urealyticum</i>	152 organism / mL	98.1% (52/53)	99.3% (151/152)	99.0%	0.975
	<i>Ureaplasma parvum</i>	79 organism / mL	94.5% (52/55)	98.7% (153/155)	97.6%	0.938
Concentrated First void Urine	<i>Mycoplasma hominis</i>	223 organism / mL	100% (51/51)	97.7% (172/176)	98.2%	0.951
	<i>Ureaplasma urealyticum</i>	152 organism / mL	98.1% (52/53)	98.7% (150/152)	98.5%	0.962
	<i>Ureaplasma parvum</i>	79 organism / mL	96.3% (52/54)	100% (151/151)	99.0%	0.975
Cervical-vaginal swabs	<i>Mycoplasma hominis</i>	223 organism / mL	98.1% (51/52)	99.4% (177/178)	99.1%	0.975
	<i>Ureaplasma urealyticum</i>	152 organism / mL	94.1% (48/51)	98.8% (169/171)	97.7%	0.936
	<i>Ureaplasma parvum</i>	79 organism / mL	93.6% (73/78)	100% (149/149)	97.8%	0.950

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	Transport/Storage conditions			
	+16 / +26 °C (room temperature)	+2 / +8 °C	- 20 ± 10 °C	- 70 ± 15 °C
First void Urine (collected without preservatives)	≤ 24 hours	≤ 48 hours	≤ 1 month	≤ 1 month
Cervical-vaginal swabs (eSwab® COPAN)	≤ 48 hours	≤ 48 hours	≤ 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

1. Switch on ELiTe InGenius. Log in with username and password. Select the mode " CLOSED ".	2. Verify controls: UroGen Positive Control and UroGen Negative Control in the "Controls" menu. Note: Both must have been run, approved and not expired.	3. Thaw the UroGen PCR Mix and the CTR CPE (if required) 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: UroGen ELiTe_U_200_100 or UroGen 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 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: UroGen ELiTe_U_200_100 or UroGen ELiTe_Cs_200_100 or UroGen ELiTe_PC or UroGen 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, 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: UroGen Positive Control and UroGen Negative Control in the "Controls" menu. Note: Both must have been run, approved and not expired.	3. Thaw the UroGen PCR Mix and the CTR CPE (if required) 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: UroGen ELiTe_Be_U_200_100 or UroGen 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)

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: UroGen ELITE_Be_U_200_100 or UroGen ELITE_Be_CS_200_100 or UroGen ELITE_Be_PC or UroGen 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	

ELITechGroup S.p.A.
C.so Svizzera, 185, 10149 Torino ITALY
Tel. +39-011 976 191
Fax +39-011 936 76 11
E. mail: emd.support@elitechgroup.com
WEB site: www.elitechgroup.com

