Instructions for use

Pneumocystis ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS150ING

UDI 08033891486723

CE 0123

IVD

CHANGE HISTORY

Revision	Notice of change	Date (dd/mm/yyyy)
04–R	Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements. Upgrade of the analytical and diagnostic performances in PERFORMANCE CHARACTERISTICS paragraph Update of the Intended use.	09/03/2026
03	Replacement of 2mL tube 953-217 and white cap 953-223 with 2mL tube 953-065 related to PCR Mix component tubes. Update of the Intended use. Update of the packaging of the PCR Mix tube (paragraph “Materials provided in the product”. Update of the paragraph “Other product required”. Update of the paragraph “Notice to the users”. Update of the paragraph “Notice to purchaser: limited license”. Update of the paragraph “Symbols” with the symbol “Consult instructions for use” New graphics and content setting of the IFU.	09/09/2025
02	Extended use of the product in association with «ELITe BeGenius®» instrument (REF INT040) and Sputum matrix. Update of PERFORMANCE CHARACTERISTICS: — update of ULoQ value for sputum matrix — update of Linear measuring range for sputum performed in matrix instead of PBS. New graphics and content setting of the IFU.	25/07/2024
01	Extended use of the product on «ELITe BeGenius®» instrument (REF INT040) and BAL matrix. Updated ULoQ/LLoQ value calculated on BAL matrix. Description of IC cut off value.	15/05/2023
00	New product development	09/11/2019

NOTE

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

PRODUCT REF.	Lot Number	Expiry date
RTS150PLD	U01224–002	31/12/2026
RTS150PLD	U0425–100	28/02/2027
RTS150PLD	U1025–037	31/05/2027
RTS150PLD	U0226–034	28/02/2028

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

TABLE OF CONTENT

1 INTENDED USE

The product **Pneumocystis ELITe MGB Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a quantitative nucleic acids Real-Time PCR assay for the **detection and the quantification of the genomic DNA** of *Pneumocystis jirovecii* **(PJ)** 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 broncho-alveolar lavage (BAL) / bronchial aspirate (BA) and Sputum

The product is intended for use as an aid in diagnosis and monitoring of Pneumocystis *jirovecii* infections in patients suspected of having or undergoing monitoring of *Pneumocystis jirovecii* infections.

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

2 ASSAY PRINCIPLE

The assay is a quantitative Real-Time PCR detecting *Pneumocystis jirovecii* **(PJ)** DNA, isolated from specimens and amplified using the assay reagent **PJ PCR Mix** that contains primers and probes with ELITe MGB® technology.

The ELITe MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycles (Ct) and the melting temperatures (Tm). The PJ DNA quantity is calculated based on a stored calibration curve.

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

3 PRODUCT DESCRIPTION

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

- The **mitochondrial Large Subunit of rRNA gene (mtLSU)** of PJ, detected in Channel **PJ**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye,
- Internal Control, artificial sequence **IC2,** detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 525 (AP525) dye.

The **PJ PCR Mix** also contains buffer, magnesium chloride, triphosphate nucleotides and hot start DNA Polymerase.

The **Pneumocystis ELITe MGB Kit** contains sufficient reagents for 96 tests in association with **ELITe InGenius** and **ELITe BeGenius (12 tests each tube)**, with 20 µL used per reaction.

The **Pneumocystis ELITe MGB Kit** can be also used in association with other equivalent instruments.

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
PJ PCR Mix ref. RTS150ING	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 (volume range: 0.5-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, ref. 72.694.005).
- 0.5 mL sterile screw capped tubes (Sarstedt, ref. 72.730.005)
- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA, the extraction and inhibition internal control, the amplification positive and negative controls, the DNA standards 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) **PJ ELITe _STD**, Assay Protocol with parameters for Calibrators analysis **PJ ELITe _PC**, Assay Protocol with parameters for Positive Control analysis **PJ ELITe _NC**, Assay Protocol with parameters for Negative Control analysis **PJ ELITe_BAL_200_100**, Assay Protocol with parameters for BAL/BA specimen analysis **PJ ELITe_SP_200_100**, Assay Protocol with parameters for sputum specimen analysis	**Pneumocystis - ELITe Positive Control** (EG SpA, ref. CTR150PLD). **Pneumocystis ELITe Standard** (EG SpA, ref. STD150PLD) **ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **CPE - Internal Control** (EG SpA, ref. CTRCPE). **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use)
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.3.0. (or later) **PJ ELITe _Be_STD**, Assay Protocol with parameters for Calibrators analysis **PJ ELITe _Be_PC**, Assay Protocol with parameters for Positive Control analysis **PJ ELITe _Be_NC**, Assay Protocol with parameters for Negative Control analysis **PJ ELITe_Be_BAL_200_100**, Assay Protocol with parameters for BAL/BA specimen analysis **PJ ELITe_Be_SP_200_100**, Assay Protocol with parameters for sputum 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.

Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

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 to prevent dispersion into the environment and to avoid contamination of the instrument's working area.

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

7.3 Warnings and precautions specific for the components

Table 3

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

* with intermediate freezing

8 SPECIMENS AND CONTROLS

8.1 Specimens

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

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ± 10 °C**	**-70 ± 15 °C**
BAL/BA	in sterile physiological solution or sterile PBS*	≤ 3 days	≤1 week	≤ 30 days	≤ 30 days
SP	-	≤ 3 days	≤1 week	≤ 30 days	≤ 30 days

BAL, bronchoalveolar lavage; BA, broncho aspirate;SP, Sputum; PBS, Phosphate Buffered Saline solution

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.

If BAL/BA samples are particularly viscous, they can be liquefied by dithiothreitol based reagents (e.g. Sputasol, Oxoid, Thermo Fisher Scientific) as per laboratory guidelines.

Samples of Sputum must be liquefied by dithiothreitol based reagents (e. g. Sputasol, Oxoid, Thermo Fisher Scientific) as per laboratory guidelines.

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 Pneumocystis ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
Bronchoal-veolar Lavage / bronchial aspirate (BAL/BA)	**ELITe InGenius**	**PJ ELITe_BAL_200_100**	copies/mL	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	**PJ ELITe_Be_ BAL_200_100**		Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Sputum (SP)	**ELITe InGenius**	**PJ ELITe_SP_200_100**	copies/mL	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	**PJ ELITe_Be_SP_200_100**		

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 5 section.

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

Refer to “Potentially Interfering Substances” in the 11 PERFORMANCE CHARACTERISTICS page 19 section to check data concerning interfering substances.

Quantities of human genomic DNA higher than 1 µg extracted from the sample could inhibit the real-time amplification

8.2 PCR calibrators and controls

The Calibration curve must be generated and approved for each lot of PCR reagent.

- For the calibration curve, use the four levels of the product **Pneumocystis ELITe Standard** (not provided with this kit) with the **PJ ELITe_STD** or **PJ ELITe_Be_STD** Assay Protocols.

NOTE

The concentration of Q – PCR Standards are expressed in copies / reaction (10^5 copies / rxn, 10^4 copies / rxn, 10^3 copies / rxn, 10^2 copies /rxn). Refer to “Standard Curve Uncertainty in the 11 PERFORMANCE CHARACTERISTICS page 19 section.

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

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

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the calibration curve and PCR control validation for each lot of PCR reagent.

Calibration curves expire after **60 days**, at which time it is necessary to re-run the calibration.

PCR control results expire after **15 days**, at which time it is necessary to re-run the Positive and Negative Controls.

The Calibrators and 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** instruments.

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 **Pneumocystis 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) Calibration run (PCR Only)
		D) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Calibration curve
		2) Validation of Positive Control and Negative Control results
		3) Validation of sample results
		4) Sample result reporting

9.1 STEP 1 – Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and log in "**CLOSED**" mode,
- in the "Calibration" menu on the Home page, verify the Calibrators (**Q - PCR Standard**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- in the "Controls" menu on the Home page, verify that 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,
- select the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and use 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 **Pneumocystis ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run, (PCR Only),

C. Calibration run (PCR Only),

D. 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**. 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 four types of run follow the steps below while referring to the GUI:

	A. Sample run (Extract + PCR)	**B. Eluted sample run (PCR Only)**
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. For this assay, 200 µL of sample must be transferred in an Extraction tube previously labelled. **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 reactions.	**Thaw** the **Elution tube** 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.
2	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen.
3	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.
4	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.

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
5	**Select** the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
6	Ensure the "Protocol" displayed is: "Extract + PCR".	Select "PCR Only" in the "Protocol" column.
7	Select the sample loading position as "Extraction Tube" in the "Sample Position" column. Ensure the "**Dilution factor**" is "**1**".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Ensure the "**Dilution factor**" is "**1**".
8	Click "Next" to continue.	Click "Next" to continue.
9	**Load CPE** and the **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** the **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.
10	Click "Next" to continue.	Click "Next" to continue.
11	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace "Tip Rack(s)" if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace "Tip Rack(s)" if necessary.
12	Click "Next" to continue.	Click "Next" to continue.
13	**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.
14	Click "Next" to continue.	Click "Next" to continue.
15	Close the instrument door.	Close the instrument door.
16	Press "Start".	Press "Start".

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) at room temperature for 30 minutes. 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. **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.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL.
4	For the Q-PCR Standard, assign the "Track", **select** the **Assay Protocol** (see "Specimen and Controls") in the "Assay" column and enter the reagent lot number and expiry date.	**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.
5	Ensure "PCR Only" is selected in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
6	Ensure the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
7	**Load** the **PCR Mix** on the "Inventory Block" referring to the Load List and enter the PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter the PCR Mix lot number, expiry date and number of reactions for each tube.
8	Click "Next" to continue.	Click "Next" to continue.
9	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
10	Click “Next” to continue.	Click “Next” to continue.
11	**Load** the PCR Cassette and the Q-PCR Standard tubes.	**Load** PCR Cassette, Positive Control and Negative Control.
12	Click “Next” to continue.	Click “Next” to continue.
13	Close the instrument door.	Close the instrument door.
14	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 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 **Q - PCR Standard** can be removed from the instrument, capped, and stored at -20 °C or below. Avoid spilling the Q - PCR Standard.

NOTE

The **Q - PCR Standard** can be used for 4 separate sessions of 2 hours each.

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 **Pneumocystis ELITe MGB Kit** through the following procedure:

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

9.3.1 Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the target of the Calibrator reactions with the **ELITe STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

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

The Calibration curve expires **after 60 days**.

NOTE

If the Calibration curve does not meet the acceptance criteria, the “Failed” message is shown on the “Calibration” screen. In this case, the results cannot be approved and the Calibrator amplification reactions must be repeated. In addition, if samples were included in the run, these are not quantified and must also be repeated to generate quantitative results.

9.3.2 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the target of the Positive Control and Negative Control reactions with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and 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 **ELITe InGenius software** processes the Positive Control and Negative Control results and generates Control Charts. The approval of the Positive Control is based on the evaluation of the obtained logarithmic quantity that should be within the expected logarithmic quantity range (PC Chart). This ensures the system performance is within the acceptance criteria. The second chart (L-J chart) is dedicated exclusively to monitoring the Positive Control trend over time. 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.

9.3.3 C. Validation of Sample results

The **ELITe InGenius** software interprets the PCR results for the target (Channel **PJ**) and the Internal Control (Channel **IC**) with the **PJ ELITe_BAL_200_100** and **PJ ELITe_SP_200_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Results Display" screen.

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

1) Calibration Curve	**Status**
PJ Q-PCR Standard	APPROVED
2) Positive Control	**Status**
PJ Positive Control	APPROVED
3) Negative Control	**Status**
PJ 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.

Result of sample run	**Interpretation**
PJ:DNA Detected, quantity equal to XXXcopies / mL	**PJ DNA was detected** in the sample within the assay measurement range, its concentration is shown.
PJ:DNA Detected, quantity below LLoQcopies/mL	**PJ DNA was detected** in the sample, its concentration is below the assay -Lower Limit of Quantification
PJ:DNA Detected, quantity beyond ULoQcopies/mL	**PJ DNA was detected** in the sample, its concentration is above the assay Upper Limit of Quantification
PJ:DNA Not detected or below LoDcopies/mL	**PJ DNA was not detected** in the sample. The sample is negative for the target 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 26)

Samples reported as “PJ:DNA Not detected or below LoDcopies/mL” are suitable for analysis but PJ DNA was not detected. In this case the sample may be either negative for PJ DNA or the PJ DNA is present at a concentration below the Limit of Detection of the assay (see 11 “Performance Characteristics” page 19).

PJ DNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as “PJ:DNA Detected, quantity below LLoQcopies/mL” (see 11 “Performance Characteristics” page 19).

PJ DNA positive samples within the Linear Measuring Range are detected and are reported as “PJ:DNA Detected, quantity equal to XXXcopies/mL”. (see 11 “Performance Characteristics” page 19)

PJ DNA positive samples that are above the Upper Limit of Quantification are reported as “PJ:DNA Detected, quantity beyond ULoQcopies/mL” and they are not suitable for quantification. If needed the sample may be diluted before extraction or PCR and retested to yield results within the Linear Measuring Range of the assay (see 11 “Performance Characteristics” page 19).

NOTE

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

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

9.3.4 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 **Pneumocystis ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 7

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

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login “**CLOSED**” mode,
- in the “Calibrations” menu on the Home page, verify the Calibrators (**Q - PCR Standard**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- 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,

- select the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and use 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 **Pneumocystis ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Calibration run (PCR Only),

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

All the required parameters are included in the Assay Protocol 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**. 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 four types of run follow the steps below while referring to the GUI:

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. For this assay, 200 µL of sample must be transferred in a 2 mL Sarstedt Tube previously labelled. **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.	If needed, **thaw the Elution tube** 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.
2	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.
3	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.
4	Select the “Run mode”: “Extract + PCR”.	Select the “Run mode”: “PCR Only”.
5	Load the samples into the “Sample Rack”. (Note: when secondary tubes “2 mL Tubes” are loaded, use the blue adaptors for the “Sample Rack”).	Load the samples into the “Elution Rack”.
6	**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).
7	Click “Next” to continue.	Click “Next” to continue.

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

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) for 30 minutes at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw the 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. **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.
2	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen
3	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) from 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.

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
4	Select the “Run mode: PCR Only”.	Select the “Run mode”: “PCR Only”.
5	**Load the Q-PCR** Standard tubes into the “Elution Rack”.	**Load** the **Positive Control and Negative Control tubes** into the “Elution Rack”.
6	**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).	**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).
7	Click “Next” to continue.	Click “Next” to continue.
8	Select the **Assay Protocol** in the “Assay” column (see “Specimens and Controls”).	Select the **Assay Protocol** in the “Assay” column (see “Specimens and Controls”).
9	Click “Next” to continue.	Click “Next” to continue.
10	**Load** the **PCR Mix** into “Reagent/Elution Rack”.	**Load** the **PCR Mix** into “Reagent/Elution Rack”.
11	**Insert** the “**Reagent/Elution Rack**” into the “Cooler Unit” in “Lane 2” (L2) If needed, for each PCR Mix 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 needed, for each PCR Mix enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
12	Click “Next” to continue.	Click “Next” to continue.
13	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Rack(s) if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Rack(s) if necessary.
14	Click “Next” to continue.	Click “Next” to continue.
15	**Load** the “**PCR Rack**” with “**PCR Cassette**” in the Inventory Area.	**Load** the “**PCR Rack**” with “**PCR Cassette**” in the Inventory Area.
16	Click “Next” to continue.	Click “Next” to continue.
17	Close the instrument door.	Close the instrument door.
18	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 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 **Q - PCR Standard** can be removed from the instrument, capped and stored at -20 °C or below. Avoid spilling the Q - PCR Standard.

NOTE
The **Q- PCR Standard** can be used for 4 separate sessions of 2 hours each.

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 **Pneumocystis ELITe MGB Kit** through the following procedure:

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

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

11 PERFORMANCE CHARACTERISTICS

11.1 Analytical sensitivity: Limit of Detection (LoD)

The analytical sensitivity of the Pneumocystis ELITe MGB Kit, as Limit of Detection (LoD) was defined in association with BAL/BA samples and **ELITe InGenius** system.

The LoD was calculated by testing a panel of negative BAL/BA samples spiked with *Pneumocystis jirovecii* (PJ) certified reference material at known titre (Qnostics). The LoD was obtained by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive result.

The final results are reported in the following table.

Table 8 Limit of Detection in BAL/BA with ELITe InGenius

LoD	95% confidence interval	
	lower bound	upper bound
97 copies / mL	60 copies / mL	275 copies / mL

The calculated LoD value was verified for each matrix by testing on **Elite InGenius** and on **ELITe BeGenius** a pool of BAL/BA and SP spiked by Pneumocystis reference material at the claimed concentration.

The results obtained confirmed the claimed concentration for the target of Pneumocystis ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius for each matrix.

11.2 Linear measuring range and Limits of Quantification

The linear measuring range of the assay was determined in association with each matrix on **ELITe InGenius** and **ELITe BeGenius** using a panel of PJ DNA - negative matrix, spiked with dilutions of PJ target DNA (quantified plasmid DNA containing the amplicon of the mtLSU).

The results, for each matrix, are reported in the following paragraphs.

Sputum

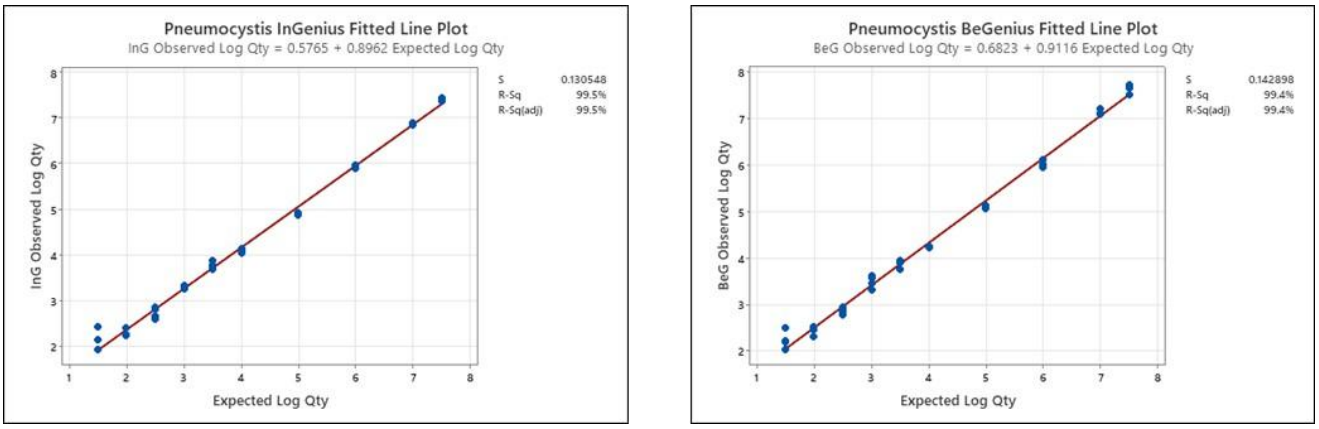


Fig. 1

BAL/BA

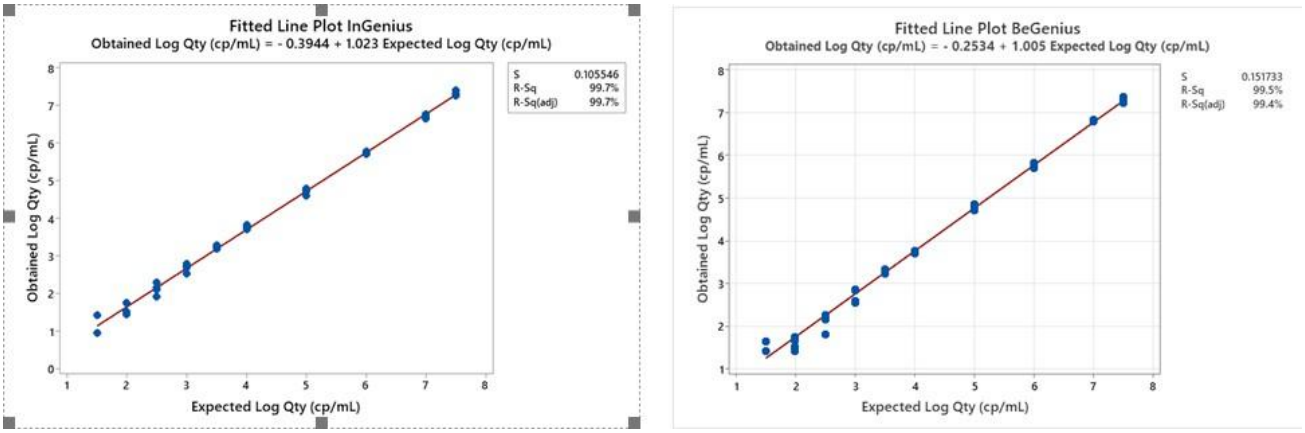


Fig. 2

The final results are summarized in the following table.

Table 9 Linear measuring range for Sputum and BAL/BA on ELITe InGenius and ELITe BeGenius

Lower Limit	Upper limit
97 copies / mL	31,622,777 copies / mL

11.3 Standard Curve Uncertainty

The Uncertainty value of the Standard curve was calculated by combining the random errors (SD) of all level quantifications and multiplying for the Coverage factor k = 2 (Expanded Combined Uncertainty) and is equal to 0.3078 Log copies / reaction.

Table 10

Standard curve levels	Theoretical	Measured	SD	Expanded Combined Uncertainty
	Log c/rxn	Log c/rxn		
PJ - PCR Standard 10^5	5.0000	4.9934	0.0841	0.3078
PJ - PCR Standard 10^4	4.0000	4.0233	0.0619	
PJ - PCR Standard 10^3	3.0000	2.9733	0.0892	
PJ - PCR Standard 10^2	2.0000	2.0100	0.0694	

11.4 Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

The inclusivity of the assay, as efficiency of detection for different strains of *Pnemocystis jirovecii* was evaluated by *in silico* analysis of the sequences available in nucleotide databases. The analysis showed mtLSU sequence conservation and absence of significant mutations. So, an efficient detection of the different strains or isolates is expected.

11.5 Potential interfering organisms: Cross-reactivity

The Potential cross-reactivity of unintended organisms that may be found in clinical specimens was evaluated by *in silico* analysis. The analysis showed no significant homology with other unintended organisms (viruses, prokaryotes, invertebrates, fungi, phages and human). Therefore, no cross-reactivity should be expected.

11.6 Potential interfering organisms: Inhibition

The potential inhibition of unintended organisms that may be found in clinical specimens was evaluated by *in silico* analysis. The analysis showed no significant homology with other unintended organisms (viruses, prokaryotes, invertebrates, fungi, phages and human). Therefore, no inhibition should be expected.

11.7 Potential 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 PJ positive sputum samples.

The results are reported in the following table.

Table 11 Sputum

Sample	PJ Pos. / Rep	Outcome
Ambroxol hydrochloride	5/5	No inhibition
Sulfamethoxazole	5/5	No inhibition
Trimetoprim	5/5	No inhibition

Table 11 Sputum (continued)

Ampicillin	5/5	No inhibition
Azithromicin,	5/5	No inhibition
Benzocaine	5/5	No inhibition
Beclometasone	5/5	No inhibition
Prednison	5/5	No inhibition
Phenylephrine	5/5	No inhibition
Bilastine,	5/5	No inhibition
Oseltamivir	5/5	No inhibition
Nicotine	5/5	No inhibition
Sodium chloride	5/5	No inhibition
mucin	5/5	No inhibition
whole blood	5/5	No inhibition
albumin	5/5	No inhibition
BAL	5/5	No inhibition

The tested substances do not interfere with the PJ or Internal Control amplification using the Pneumocystis ELITe MGB Kit on sputum samples.

11.8 Repeatability

The Repeatability of the assay was evaluated on **ELITe InGenius** and **ELITe BeGenius** instruments by analysis of a panel of BAL/BA samples, including one negative sample and two samples spiked with PJ DNA.

Results are shown in the tables below.

Table 12 Intra - Session Repeatability on ELITe InGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	8	NA	NA	NA	100%
3X LoD	8	37.08	0.42	1.13	100%
10X LoD	8	35.04	0.48	1.37	100%

Table 13 Inter - Session Repeatability on ELITe InGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	16	NA	NA	NA	100%
3X LoD	16	37.23	0.72	1.93	100%
10X LoD	16	35.28	0.46	1.30	100%

Table 14 Intra - Session Repeatability on ELITe BeGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	8	NA	NA	NA	100%
3X LoD	8	37.45	0.32	0.86	100%
10X LoD	8	35.37	0.44	1.25	100%

Table 15 Inter - Session Repeatability on ELITe BeGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	16	NA	NA	NA	100%
3X LoD	16	37.98	1.05	2.76	100%
10X LoD	16	35.79	0.63	1.76	100%

In the Repeatability test, the Pneumocystis ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

11.9 Reproducibility

The Reproducibility of the assay was evaluated on **ELITe InGenius** and **ELITe BeGenius** instruments by analysis of a panel of BAL/BA samples, including one negative sample and two samples spiked with PJ DNA.

A summary of Inter-Instrument Reproducibility is shown in the tables.

Table 16 Inter – Instrument Reproducibility on ELITe InGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	8	NA	NA	NA	100%
3X LoD	8	36.63	0.63	1.72	100%
10X LoD	8	34.42	0.48	1.39	100%

Table 17 Inter – Instrument Reproducibility on ELITe BeGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	8	NA	NA	NA	100%
3X LoD	8	37.26	0.41	1.11	100%
10X LoD	8	34.92	0.40	1.16	100%

Table 18 Inter – Batch Reproducibility on ELITe InGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	8	NA	NA	NA	100%
3X LoD	8	36.99	0.48	1.29	100%
10X LoD	8	35.03	0.44	1.25	100%

Table 19 Inter – Batch Reproducibility on ELITe BeGenius

Sample	N	PJ			
		Mean Ct	SD	% CV	% Agreement
Negative	8	NA	NA	NA	100%
3X LoD	8	37.59	0.38	1.02	100%
10X LoD	8	35.70	0.59	1.65	100%

In the Reproducibility test, the Pneumocystis ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %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 analyzing BAL/BA and Sputum samples that were negative for PJ DNA (tested with a validated real time amplification product). As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic specificity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius**.

The results are summarized in the following table.

Samples	N	Positive	Negative	%Diagnostic Specificity
PJ Negative BAL/BA	72	2	70	97.2
PJ Negative Sputum	65	1	64	98.5

The Internal Control Ct (IC Ct) cut-off value is set at 34 with **ELITe InGenius** and at 35 with **ELITe BeGenius** for BAL/BA samples.

The Internal Control Ct (IC Ct) cut-off value is set at 37 for sputum samples when tested with **ELITe InGenius** and **ELITe BeGenius**.

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 analyzing clinical BAL/BA and Sputum samples positive for PJ DNA (tested with a validated real time PCR kit) or spiked with PJ reference material. As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summarized in the following table

Samples	N	Positive	Negative	%Diagnostic Sensitivity
PJ positive BAL/BA	74	74	0	**100**
PJ positive Sputum	54	53	1	**98.4**
PJ spiked Sputum	7	7	0	
Total Sputum	**61**	**60**	**1**	

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 "PJ ELITe MGB Kit", FTP150ING.

12 REFERENCES

C. Valero et al. (2016) *Front. Microbiol*. 7:1413

M. Maillet et al. (2014) *Eur J Clin Microbiol Infect Dis*.33(3):331-6

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

C. N. Kotton et al. (2025) Transplantation 109: 1066-1110

13 PROCEDURE LIMITATIONS

Use this product only with the following clinical sample: BAL/BA, Sputum.

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

Do not use this product with samples containing too much mucin: samples with high viscosity inhibit the amplification reaction of nucleic acids and can cause invalid results.

There are no data available concerning product performance with DNA extracted from the following clinical samples: respiratory 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 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 Performance Characteristics). In this case the result could be a false negative.

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

Possible polymorphisms, insertions or deletions within the region of the DNA targeted by the product primers and probes may impair detection and quantification 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 20

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Q-PCR Standards and Positive Control. Check the volumes of PCR Mix, Q-PCR Standards 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 three 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.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 4 independent sessions (2 hours each in the Extraction Area or in the Cooler Unit). 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 new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 21

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 PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the Negative Control.	Do not use the Negative Control for more than one session. Use a new aliquot of molecular biology grade water.
Contamination of the extraction area, Racks or Inventory Area Cool Block or in the Cooler Unit	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 22

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. Use a new aliquot of PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. In case a new extraction is needed, perform or repeat the liquefaction step of the primary sample as reported in par. 8.1, “Specimens” and repeat the extraction in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 23

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 Standards or 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 24

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the 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 or - repeat the extraction of the sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 25

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

15 SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE 0123	Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.
UDI	Unique Device Identification
	Contains sufficient for "N" tests.
	Consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

16 NOTICE TO THE USERS

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

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

17 NOTICE TO PURCHASER: LIMITED LICENSE

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

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

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

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

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

CAUTION

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

INTENDED USE

The product **Pneumocystis ELITe MGB Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a quantitative nucleic acids Real-Time PCR assay for the **detection and the quantification of the genomic DNA** of *Pneumocystis jirovecii* **(PJ)** 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 broncho-alveolar lavage (BAL) / bronchial aspirate (BA) and Sputum

The product is intended for use as an aid in diagnosis and monitoring of Pneumocystis *jirovecii* infections in patients suspected of having or undergoing monitoring of *Pneumocystis jirovecii* infections.

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

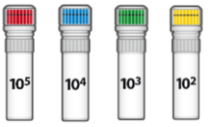
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	mtLSU	FAM	**PJ**
Internal Control	artificial sequence (IC2)	AP525	**IC**

Validated matrices

- BAL/BA
- Sputum samples

Kit content and related products

Pneumocystis ELITe MGB Kit	Pneumocystis ELITe Standard	Pneumocystis - ELITe Positive Control
 X 8	 X 2	 X 3
Ready-to-use PCR Mix 8 tubes of 280 µL 96 reactions per kit 7 freeze-thaw cycles per tube	Ready-to-use 4 levels: 10^5, 10^4, 10^3, 10^2 2 set of 4 tubes of 200 µL 4 freeze-thaw cycles per tube	Ready-to-use PC 3 tubes of 160 µL 12 reactions per kit 4 freeze-thaw cycles per tube

Maximum shelf-life: **24 months**

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.	• CPE - Internal Control: CTRCPE • **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use)

ELITe InGenius and ELITe BeGenius protocol

Table 26

› Sample volume	200 µL	› PJ volume	20 µL
› CPE volume	10 µL	› Frequency of controls	15 days
› Total elution volume	100 µL	› Frequency of calibration	60 days
› Eluate PCR input volume	20 µL	Unit of quantitative result	Copies/mL

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Linearity Range	Diagnostic Sensitivity	Diagnostic Specificity
BAL/BA	**97 cp / mL**	**97 – 31,622,777**	**100%** 74/74*	**97.2%** 70/72*
Sputum	**97 cp / mL**	**97 – 31,622,777**	**98.4%** 60/61*	**98.5%** 64/65*

**confirmed samples/ tested samples*

Sample preparation

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

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 ° C**	**-20 ± 10 °C**	**-70 ± 15 °C**
Bronchoalveolar Lavage / bronchial aspirate (BAL/BA)	in sterile physiological solution or sterile PBS*	≤ 3 days	≤1 week	≤ 30 days	≤ 30 days
Sputum (SP)	-	≤ 3 days	≤1 week	≤ 30 days	> 30 days

BAL, bronchoalveolar lavage; BA, broncho aspirate;SP, Sputum; PBS, Phosphate Buffered Saline solution.

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

Before analysis

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

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

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

NOTE

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

Procedure 2: PCR Only (e.g., eluates, standards, 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: PJ ELITe_PC and PJ ELITe_ NC)	**5.** Select the method "PCR Only" and the sample position "Elution Tube"	**6.** Load the PCR Mix in the Inventory Block
7. Load: PCR Cassette rack and the Elution tube rack with the extracted nucleic acid	**8.** Close the door. Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

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

Before analysis

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

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

1. Select “Perform Run” on the touch screen 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 (PJ ELITe_Be_BAL_200_100 or PJ ELITe_Be_SP_200_100 or **Note:** If a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Basket” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

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

Procedure 2: PCR Only (e.g., eluates, standards, 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.** For Controls: for each “Position” enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions). For eluates: for each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).
4. Select the “Assay protocol” of interest (PJ ELITe_Be_PC and PJ ELITe_Be_NC or PJ ELITe_Be_STD)	**5.** Load the complete reaction mixture 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