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EMPOWERING IVD

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NOTICE of CHANGE dated 28/07/2023

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«Aspergillus spp. ELITe MGB Kit»
Ref. RTS110PLD

This new revision of the Instruction for Use (IFU) contains the following changes:

- *Update for the use of the product for BAL and Plasma matrices in association with «ELITe BeGenius®» instrument (REF INT040).*

- *Description of IC cut off value already adopted in the Assay protocol of the product (section "Diagnostic specificity")*

- *Confirmed ULoQ/LLoQ value calculated on BAL and Plasma matrices*

Composition and use of the product remain unchanged.

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIESE FASSUNG DER GEBRAUCHSANLEITUNG IST KOMPATIBEL MIT DER VORHERIGEN VERSION DES TESTKITS

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ASPERGILLUS spp. ELITe MGB® Kit

reagent for DNA Real Time PCR

REF RTS110PLD CE -20°C

TABLE OF CONTENTS

INTENDED USE

The **ASPERGILLUS spp. ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as qualitative and quantitative nucleic acids amplification assay for the detection and quantification of the DNA of the Aspergillus genus (Aspergillus spp.) in DNA samples 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 bronchoalveolar lavage (BAL), bronchial aspirate (BA) and plasma collected in EDTA.

The assay can detect and dose the DNA of the following species of the *Aspergillus* genus: *Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Aspergillus flavus, Aspergillus versicolor, Aspergillus glaucus.*

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

ASSAY PRINCIPLES

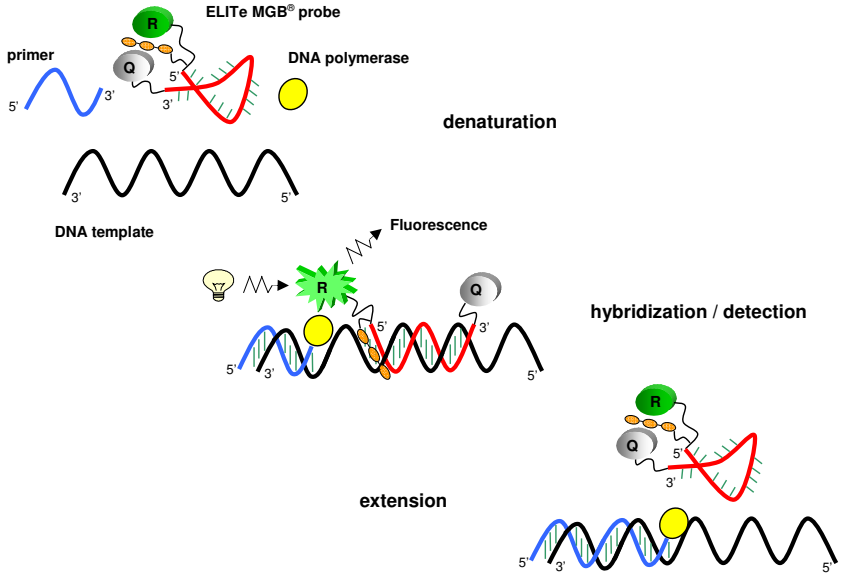
The assay consists of a Real-Time PCR reaction in a microplate with a programmable thermostat provided with a fluorescence detection optical system (real time amplification thermal cycler).

In each well, two amplification reactions are performed starting from DNA extracted from the samples being tested: a specific reaction for the region of the **rDNA 18S** gene of *Aspergillus spp.* (present in multiple copies in the genome of the fungus) and a specific reaction for a region of the human **beta Globin** gene (Internal Control of inhibition). The *Aspergillus spp.* specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the *Aspergillus spp.* amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with AP525 fluorophore (analogous to VIC), is activated when hybridizes with the specific product of the Internal Control amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data allows detecting the presence and the titre of *Aspergillus spp.* DNA in the starting sample.

At the end of the amplification session, dissociation curve (melting curve) analysis can be carried out in order to determine the dissociation temperature (melting temperature) and to confirm the presence of the correct target or to identify the presence of mutations.

The assay is validated with the systems described in this user manual.

The ELITe MGB technology is depicted in the illustration below. 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 (Tm) calculation.



PRODUCT DESCRIPTION

The **ASPERGILLUS spp. ELITe MGB Kit** provides the **ASP. Q - PCR Mix,** an optimized and stabilized PCR mixture **aliquoted into four ready-to-use tubes (TRANSPARENT cap)**. Each tube contains **540 µL** and is sufficient for **24 tests** on **ELITe InGenius** and **ELITe BeGenius** if processing at least 2 samples per session and 25 tests in association with other systems.

The **ASP. Q-PCR Mix** contains the specific primers and probe for:
Primers and probe for (ASP) are specific for *Aspergillus spp* **rDNA 18S** gene region. The probe ASP is stabilized by MGB®, quenched by Eclipse Dark Quencher®, and labelled by FAM fluorophore.
Primers and probe for Internal Control (**IC**) are specific for the human **beta globin** gene sequence. The probe **IC** is stabilized by MGB®, quenched by Eclipse Dark Quencher®, and labelled with AquaPhluor® AP525 dye.
The **ASP. Q-PCR Mix** also contains buffer, magnesium chloride, nucleotides triphosphate, AP593 fluorophore (analogous to ROX or to Cy5) as passive reference for fluorescence normalisation, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product, and the hot start DNA Polymerase.

The **ASP. Q-PCR Mix** contains sufficient reagents for **96 tests** on the **ELITe InGenius** and **ELITe BeGenius**, with 20 µL used per reaction.

The **ASP. Q-PCR Mix** contains sufficient reagents for **100 tests** in association with other systems, including standards and controls, with 20 µL used per reaction.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
ASP. Q - PCR Mix	Mixture of reagents for real-time PCR tube with **TRANSPARENT cap**	**4 x 540 µL**	-

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 (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.
- Programmable thermostat with optical fluorescence detection system 7300 Real Time PCR System or System o 7500 Fast Dx Real-Time PCR Instrument calibrated following manufacturer's instructions.
- Sarstedt screw cap microtube 2,0 mL (Sarstedt ref. 72.694.005).
- Generic screw cap tube 15 mL (e.g., Sarstedt ref. 62.554.502).
- Generic screw cap tube 50 mL (e.g., Sarstedt ref. 62.547.254).

OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA, the extraction and inhibition internal control template, the amplification positive control 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.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

Instrument and software	Product and reagents
ELITe InGenius (ELITechGroup S.p.A., ref. INT030) **ELITe InGenius Software** version 1.3.0 (or later) **ASP ELITe_STD** parameters for the Standard analysis **ASP ELITe_PC**, parameters for Positive Control analysis **ASP ELITe_NC**, parameters for Negative Control analysis **ASP ELITe_BAL_1000_100**, parameters for BAL specimen analysis **ASP ELITe_PL_1000_100**, parameters for Plasma collected in EDTA specimen analysis	**ELITe InGenius® SP 1000** (ELITechGroup S.p.A., ref. INT033SP1000) **ELITe InGenius® SP 200 Consumable Set** (ELITechGroup S.p.A., ref. INT032CS) **ELITe InGenius® PCR Cassette** (EG SpA, ref. INT035PCR), **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) **ELITe InGenius® Waste Box** (ELITechGroup S.p.A., ref. F2102-000) **CPE - Internal Control** (ELITechGroup S.p.A., ref. CTRCPE) **ASPERGILLUS spp. - ELITe Positive Control** (ELITechGroup S.p.A., ref. CTR110PLD) **ASPERGILLUS spp. - ELITe Standard** (ELITechGroup S.p.A., ref. STD110PLD)
ELITe BeGenius (ELITechGroup S.p.A., ref. INT040) **ELITe BeGenius Software** version 2.1.0 (or later) **ASP ELITe_Be_STD** parameters for the Standard analysis **ASP ELITe_Be_PC**, parameters for Positive Control analysis **ASP ELITe_Be_NC**, parameters for Negative Control analysis **ASP ELITe_Be_BAL_1000_100**, parameters for BAL specimen analysis **ASP ELITe_Be_PL_1000_100**, parameters for Plasma collected in EDTA specimen analysis	**ELITe InGenius SP 1000** (ELITechGroup S.p.A., ref. INT033SP1000) **ELITe InGenius SP 200 Consumable Set** (ELITechGroup S.p.A, ref. INT032CS) **ELITe InGenius® PCR Cassette** (EG SpA, ref. INT035PCR), **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) **ELITe InGenius® Waste Box** (ELITechGroup S.p.A, ref. F2102-000) **CPE - Internal Control** (ELITechGroup S.p.A., ref. CTRCPE) **ASPERGILLUS spp. - ELITe Positive Control** (ELITechGroup S.p.A., ref. CTR110PLD) **ASPERGILLUS spp. - ELITe Standard** (ELITechGroup S.p.A., ref. STD110PLD)
7300 Real-Time PCR System (ThermoFisher Scientific, ref. 4351101)	**«MicroAmp™ Optical 96-Well Reaction Plate»** (Life Technologies, ref. N8010560) **CPE - Internal Control** (ELITechGroup S.p.A., ref. CTRCPE) **ASPERGILLUS spp. - ELITe Positive Control** (ELITechGroup S.p.A., ref. CTR110PLD) **ASPERGILLUS spp. - ELITe Standard** (ELITechGroup S.p.A., ref. STD110PLD)
7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific, ref. 4406985)	**«MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL»** (Life Technologies, ref. 4346906) **CPE - Internal Control** (ELITechGroup S.p.A., ref. CTRCPE) **ASPERGILLUS spp. - ELITe Positive Control** (ELITechGroup S.p.A., ref. CTR110PLD) **ASPERGILLUS spp. - ELITe Standard** (ELITechGroup S.p.A., ref. STD110PLD)

WARNINGS AND PRECAUTIONS

This product is designed for *in-vitro* use only.

General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with the biological samples. Avoid splashing or spraying. 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. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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 neutralised before disposal.

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 in the product before running the assay.
While running the assay, follow the product instructions provided.
Do not use the product after the indicated expiry date.
Only use the reagents provided in the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

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.

When amplification session is manually setup, it is necessary to have available separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never introduce an amplification product in the area designated for extraction / preparation of amplification reactions.

When amplification session is manually setup, it is necessary to have available laboratory coats, gloves and tools which are exclusively used for the extraction / preparation of the amplification reactions and for the amplification / detection of amplification 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.

When amplification session is automatically setup, it is necessary to have available 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.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

The PCR Cassettes must be handled carefully and never opened in order to avoid PCR product diffusion into the environment and sample and reagent contamination.

Warnings and precautions specific for the components

The **ASP. Q-PCR Mix** must be stored at temperature of -20 °C or below and protected from light.
The **ASP Q - PCR Mix** must be used within one month from the first opening.
The **ASP. Q-PCR Mix** can be frozen and thawed up to **five times**: further freeze / thaw cycles may cause a loss of product performances.

The **ASP Q - PCR Mix** can be kept on board on the **ELITe InGenius** or on the **ELITe BeGenius** up to **five separate sessions of three hours each** (Extract + PCR mode, with intermediate freeze / thaw cycles) or for **three consecutive sessions of three hours each** (Extract + PCR mode).

SPECIMENS AND CONTROLS

Specimens

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			
		RT (~+21°C)	+2° / +8°C	-20°C	-70°C
Bronchoalveolar Lavage and Broncho Aspirate	Collected without preservatives	N. A.	≤ 7days	≤ 1 months	≤ 1 year
Plasma	EDTA	N.A.	≤ 3 days	≤ 1 months	≤ 1 year

N.A., not applicable

Samples of Broncho-alveolar lavage (BAL), intended for DNA extraction, must be collected in sterile physiological solution or sterile PBS according to laboratory guidelines,
If BAL samples are particularly mucous, they can be liquefied by dithiothreitol based reagents (e.g. Sputasol, Oxoid, Thermo Fisher Scientific) as per laboratory guidelines.
It is recommended to divide the samples into aliquots before freezing to prevent repeated freez / thaw cycles. When using frozen samples, thaw the samples just before the extraction in order to avoid possible nucleic acid degradation.

To perform nucleic acid extraction from samples on the **ELITe InGenius** and **ELITe BeGenius**, the following protocols must be used. For all protocols, 1000 µL of sample is processed, and nucleic acid is eluted in 100 µL.

Specimen	Instrument	Assay Protocol	Sample transfer
Bronchoalveolar Lavage and Broncho Aspirate	**ELITe InGenius**	**ASP ELITe_BAL_1000_100**	Required, in Extraction tube
	ELITe BeGenius	**ASP ELITe_Be_BAL_1000_100**	Required, in 2 mL Tube
Plasma	**ELITe InGenius**	**ASP ELITe_PL_1000_100**	Required, in Extraction tube
	ELITe BeGenius	**ASP ELITe_Be_PL_1000_100**	Required, in 2 mL Tube

Note: Pipetting samples from the swab primary tube to the **Extraction tube** or to the **2 mL Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the "Warnings and Precautions" section.

Purified nucleic acids can be stored at +2 / +8 °C for 16 hours or at ~-20 °C for 1 month.
Primary tube cannot be used

Interfering substances

Available data concerning inhibition caused by drugs and other substances are reported in Potentially Interfering Substances in the Performance Characteristics section.

PCR calibrators and amplification controls

Calibration curve must be generated and approved for each lot of PCR reagent.
- For the calibration curve, use the four concentration levels of the **ASPERGILLUS spp. ELITe Standard**, with the **ASP ELITe_STD** or **ASP ELITe_Be_STD** Assay Protocols.

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

- For the Positive Control, use the **ASPERGILLUS spp. - ELITe Positive Control** with the **ASP ELITe_PC** or **ASP ELITe_Be_PC** Assay Protocols,
- For the Negative Control, use molecular biology grade water (not provided), with **ASP ELITe_NC** or **ASP ELITe_Be_NC** Assay Protocols.

Note: 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** and **ELITe BeGenius**.

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.

ELITe InGenius

PROCEDURE

Using the **ASPERGILLUS spp. ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:
- Verification of the system readiness,
- Setup of the session,
- Review and approval of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Calibration" menu on the Home page, verify that the Calibrators (**ASP Q-PCR Standard**) are approved and valid (Status) for the **ASP Q - PCR Mix** lot to be used. If no valid Calibrators are available for the **ASP Q - PCR Mix** lot, perform calibration as described in the following sections,
- in the "Controls" menu on the Home page, verify that the amplification Controls (Controls, **ASP Positive Control, ASP Negative Control**) are approved and valid (Status) for the **ASP Q - PCR Mix** lot to be used. If no valid amplification Controls are available for the **ASP Q - PCR Mix** lot, run the amplification 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 ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB Kits and **ELITe InGenius** with the indicated matrices.

The Assay Protocols available for sample testing with the product **ASPERGILLUS spp. ELITe MGB Kit** are described in the table below.

Assay protocol for ASPERGILLUS spp. ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
ASP ELITe_BAL_1000_100	BAL / BA	Positive / copies/mL / Negative	Extraction Input Volume: 1000 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

Assay protocol for ASPERGILLUS spp. ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
ASP ELITe_PL_1000_100	Plasma	Positive / copies/mL / Negative	Extraction Input Volume: 1000 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

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

Setup of the session

The **ASPERGILLUS spp. ELITe MGB Kit** can be used on **ELITe InGenius** to perform:
A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Calibration run (PCR only),
D. Amplification run for Positive Control and Negative Control (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 loading the session information. Refer to the instrument manual for more details.

The main steps for the setup of the four types of run are described here below.

A. Integrated run (Extract + PCR)

To setup an integrated run with sample extraction and amplification, follow the steps below while referring to the GUI:

1. Identify the samples and handle according to laboratory guidelines and the "Specimens and Controls" section.
2. If needed, thaw samples at room temperature (~+21 °C) and handle according to laboratory guidelines and to the "Specimens and Controls" section. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block (+2 / +8 °C).

Note: For this assay, 1000 µL of sample must be transferred in an **Extraction tube** previously labeled. Exceeding volume will be left in the **Extraction Tube** by the **ELITe InGenius**.

3. Thaw the needed **ASP. Q-PCR Mix** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 24 reactions in in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

Note: Protect **ASP. Q-PCR Mix** from light while thawing because this reagent is photosensitive.

4. Thaw the needed **CTR CPE** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 12 extractions in optimized conditions (2 or more extraction per session). Mix gently then spin down the contents for 5 seconds.
5. Select "Perform Run" from the "Home" screen.
6. Ensure that the "Extraction Input Volume" is 1000 µL and the Extracted Elute Volume is 100 µL.
7. For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.
8. Select the Assay Protocol to be used in the "Assay" column (e.g. ASP ELITe_BAL_1000_100).
9. Ensure that the "Protocol" displayed is: "Extract + PCR".
10. Select the sample loading position "Extraction Tube" in the "Sample Position" column. Click "Next" to continue.
11. Load **CTR CPE** and **ASP. Q-PCR Mix** on the designated "Inventory Block" referring to the "Load List" and enter the reagent lot number, expiry date and number of reactions for each tube. Click "Next" to continue.

12. Verify the tips in the "Tip Racks" in the "Inventory Area" and replace **Tip Racks** if necessary. Click "Next" to continue.
13. Load the **PCR Cassettes**, the **ELITe InGenius SP 1000** extraction cartridges, and all the required consumables and the samples to be extracted, following the GUI instruction. Click "Next" to continue.
14. Close the instrument door.
15. Press "Start" to start the run.

When the session is finished, the **ELITe InGenius** allows users to view, approve, and store the results and to 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 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

Note: The **ASP. Q-PCR Mix** can be used for 5 separate sessions of 3 hours each or can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

B. Amplification run (PCR only)

To set up the amplification run starting from extracted DNA, follow the steps below while referring to the GUI:

1. If needed, thaw the **Elution tubes** containing the extracted nucleic acids at room temperature (~+21 °C). Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block (+2 / +8 °C).
2. Thaw the needed **ASP. Q-PCR Mix** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 24 reactions in in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

Note: Protect **ASP. Q-PCR Mix** from light while thawing because this reagent is photosensitive.

3. Select "Perform Run" from the "Home" screen.
4. Ensure that the "Extraction Input Volume" is 1000 µL and the Extracted Elute Volume is 100 µL, even if extraction is not being performed.
5. For each sample, assign the Track and enter the SID by typing or by scanning the sample barcode.
6. Select the Assay Protocol to be used in the "Assay" column (e.g. ASP ELITe_BAL_1000_100).
7. Select "PCR Only" in the "Protocol" column.
8. Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Click "Next" to continue.
9. Load **ASP. Q-PCR Mix** on the "Inventory Block" referring to the "Load List" and enter the reagent lot number, expiry date and number of reactions for each tube. Click "Next" to continue.
10. Verify the tips in the "Tip Racks" in the "Inventory Area" and replace **Tip Racks** if necessary. Click "Next" to continue.
11. Load the **PCR Cassettes** and **Elution tubes** with extracted nucleic acids samples following the GUI instruction. Click "Next" to continue.
12. Close the instrument door.
13. Press "Start" to start the run.

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results and to 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 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

Note: The **ASP. Q-PCR Mix** can be used for 5 separate sessions of 3 hours each or can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7

hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run (PCR only)

To setup Calibration run, follow the steps below while referring to the GUI:

1. Thaw the needed **ASP. Q-PCR Mix** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

Note: Protect the **ASP. Q-PCR Mix** from light while thawing because this reagent is photosensitive.

2. Thaw the needed **ASP Q-PCR Standard** tubes (Cal1: ASP Q-PCR Standards 10^2, Cal2: ASP Q-PCR Standards 10^3, Cal3: ASP Q-PCR Standards 10^4, Cal4: ASP Q-PCR Standards 10^5) at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 4 reactions. **Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block (+2 / +8 °C)** .
3. Select "Perform Run" from the "Home" screen.
4. Ensure that the "Extraction Input Volume" is 1000 µL and the "Extracted Elute Volume" is 100 µL, even if extraction is not being performed.
5. For the **ASP Q-PCR Standard**, assign the Track, select the Assay Protocol "ASP ELITe_STD in the "Assay" column and enter the reagent lot number and expiry date.
6. Ensure that "PCR Only" is selected in the "Protocol" column.
7. Ensure that the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".
8. Load **ASP. Q-PCR Mix** on the "Inventory Block" referring to the Load List and enter the reagent lot number, expiry date and number of reactions for each tube. Click "Next" to continue.
9. Verify the tips in the "Tip Racks" in the "Inventory Area" and replace **Tip Racks** if necessary. Click "Next" to continue.
10. Load the **PCR Cassettes** and the **ASP Q-PCR Standard** tubes following the GUI instruction. Click "Next" to continue.
11. Close the instrument door.
12. Press "Start" to start the run.

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

Note: At the end of the run the remaining **ASP Q-PCR Standard** can be removed from the instrument, capped and stored at ~ -20 °C.

Note: The **ASP Q-PCR Standard** can be used for 4 separate sessions of 3 hours each.

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

Note: The **ASP. Q-PCR Mix** can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

D. Amplification run for Positive Control and Negative Control (PCR only)

To setup the amplification run for Positive Control and Negative Control, follow the steps below while referring to the GUI:

1. Thaw the needed **ASP. Q-PCR Mix** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

Note: Protect the **ASP. Q-PCR Mix** from light while thawing because this reagent is photosensitive.

2. If needed, thaw **ASP Positive Control** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for preparing 4 reactions. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block (+2 / +8 °C).
3. Prepare the ASP **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**.
4. Select "Perform Run" from the "Home" screen.
5. Ensure that the "Extraction Input Volume" is 1000 µL and the "Extracted Elute Volume" is 100 µL, even if extraction is not being performed.
6. For the **ASP Positive Control**, assign the "Track", select the Assay Protocol "ASP ELITe_PC" in the "Assay" column and enter the lot number and expiry date.
7. For the **Negative Control**, assign the "Track", select the Assay Protocol "ASP ELITe_NC" in the "Assay" column and enter the molecular biology the lot number and expiry date.Click "Next" to continue.
8. Ensure that "PCR Only" is selected in the "Protocol" column.
9. Ensure that the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".
10. Load **ASP. Q-PCR Mix** on the "Inventory Block" referring to the "Load List" and enter the reagent lot number, expiry date and number of reactions for each tube. Click "Next" to continue..
11. Verify the tips in the "Tip Racks" in the "Inventory Area" and replace **Tip Racks** if necessary. Click "Next" to continue.
12. Load the **PCR Cassettes**, the **ASP Positive Control tube** and the **Negative Control** tubes following the GUI instruction. Click "Next" to continue.
13. Close the instrument door.
14. Press "Start" to start the run.

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

Note: At the end of the run the remaining **ASP Positive Control** can be removed from the instrument, capped and stored at ~-20 °C. The remaining **Negative Control** must be discarded.

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

Note: At the end of the run the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

Note: The **ASP. Q-PCR Mix** can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

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 are shown. From this screen results can be approved, amd 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 Server" (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 product **ASPERGILLUS spp. ELITe MGB Kit** through the following procedure:

A. Validation of Calibration curve,
B. Validation of Positive Control and Negative Control results,
C. Validation of sample results,
D. Sample result reporting.

A. Validation of amplification Calibration curve

The **ELITe InGenius software** interprets the PCR results for the ASP probe (channel "ASP") of the Calibrators reactions with the **ASP 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 expire **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 result cannot be approved, and the 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.

B. Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the ASP probe (channel "ASP") of the Positive Control and Negative Control reactions with the **ASP ELITe_PC** and **ASP 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 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. Four approved Positive Control and Negative Control results are used to set up the initial Control Chart. For subsequent controls, the results are analyzed by the software to ensure the system performances are within the acceptance criteria, shown in the Control Chart plots. 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, it 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.

C. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the ASP probe (channel "ASP") and the Internal Control probe (Channel "**IC**") with the **ASP ELITe_BAL_1000_100** and **ASP ELITe_PL_1000_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Result Display" screen.

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

1) Calibration Curve	**Status**
ASP Q-PCR Standards	APPROVED
2) Positive Control	**Status**
ASP Positive Control	APPROVED
3) Negative Control	**Status**
ASP 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
ASP:DNA detected Not significant, quantity below LLoQ copies / mL	**ASP DNA was detected but it is not significant** taking into account the basal level of this target.
ASP: DNA detected Significant, quantity equal to XXX copies / mL	**ASP DNA was detected and significant,** quantity within the measurement range of the assay, quantity as shown.
ASP: DNA Detected Significant, quantity beyond ULoQ copies / mL	**ASP DNA was detected and significant**, quantity beyond the upper limit of quantification of the assay.
ASP:DNA not detected or below the LoD copies / mL	**ASP DNA was not detected** in the sample. The sample is negative for the target DNA or its concentration is below the Limit of Detection of the assay.
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 are not suitable for result interpretation. In this case, the Internal Control DNA was not efficiently detected, which could be due to problems in the CR or extraction step (degradation or loss of RNA 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, see “Troubleshooting”) 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..

Samples reported as “ASP DNA Not Detected or below LoD" are suitable for analysis but *Aspergillus spp.* DNA it was not detect. In this case, the sample may be either negative for ASP DNA or ASP DNA is present at a concentration below the Limit of Detection of the assay (see “Performance characteristics”).

Samples reported as “DNA Detected, Not significant, qty below LLoQ copies / mL" are suitable for analysis, the *Aspergillus spp.* DNA has been detected but is present in amounts below the threshold established for this assay taking into account the basal level of this target (see “Performance characteristics”). In this case the result of the assay is negative because the presence of *Aspergillus spp.* DNA in the sample is not significant.

Samples reported as “DNA Detected, Significant, qty below LLoQ copies / mL" are suitable for analysis, the *Aspergillus spp.* DNA has been detected.

Samples reported as “DNA Detected Significant, quantity beyond ULoQ copies / mL” are not suitable for quantification. The concentration of ASP DNA detected in the sample is above the level at which it can be accurately quantified. The sample may be diluted before extraction or PCR and retested to yield results within the linear range of the assay.

If the result of the amplification reaction of a sample is **Quantity ≥ 120** copies / mL for BAL/BA or **Quantity ≥ 50** copies/mL for plasma, it means that DNA of *Aspergillus spp.* has been detected and its quantity is above the threshold established for this assay taking into account the basal level of this target (see “Performance characteristics”). In this case, the result of the assay is positive and the presence of DNA of *Aspergillus spp.* in the sample is significant.

Note: If the result of the amplification reaction of a sample is **120 ≤ Quantity ≤ 210** copies / mL for BAL/BA or **50 ≤ Quantity ≤ 90** copies / mL for plasma it means that the *Aspergillus spp.* DNA has been detected in quantities close to the threshold established for this assay taking into account the basal level of this target (see “Performance characteristics”). In this case the result should be considered as “undeterminate”. The test should be repeated from the extraction of a new sample in order to confirm its positivity.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

Note: The results obtained with this assay must be interpreted along with all other clinical data and other laboratory test for the patient.

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

D. Sample result reporting

The sample results are stored in the database and can be viewed or 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.

ELITe BeGenius

PROCEDURE

Using the **ASP ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

- Verification of the system readiness
- Session setup
- Review and approval of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the **ELITe BeGenius** and login in “**CLOSED**” mode,
- - in the “Calibrations” menu on the Home page, verify that the Calibrators (**ASP Q-PCR Standard**) are approved and valid (Status) for the **ASP Q-PCR Mix** lot to be used. If no valid Calibrators are available for the **ASP Q-PCR Mix** lot, perform calibration as described in the following sections,
- - in the “Controls” menu on the Home page, verify that the amplification Controls (**ASP Positive Control, ASP Negative Control**) are approved and valid (Status) for the **ASP Q-PCR Mix** lot to be used. If no valid amplification Controls are available for the **ASP Q-PCR Mix** lot, run the amplification Controls as described in the following sections,
- - choose the type of run, follow the instructions on the Graphical User Interface (GUI) for the session setup and use the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB kits and the **ELITe BeGenius** instrument and the indicated matrices.

The Assay protocols available for sample testing with the product **ASPERGILLUS spp. ELITe MGB Kit** are described in the table below.

Assay protocols for ASPERGILLUS spp. ELITe MGB Kit			
Name	**Matrix**	**Report unitage**	**Characteristics**
ASP ELITe_Be_BAL_1000_100	BAL / BA	Positive / copies/mL / Negative	Extraction Input Volume: 1000 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
ASP ELITe_Be_PL_1000_100	Plasma	Positive / copies/mL / Negative	Extraction Input Volume: 1000 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

If the assay protocol of interest is not in the system, contact your local ELITechGroup Customer Service.

Setup of the session

The **ASPERGILLUS spp. ELITe MGB Kit** can be used on **ELITe BeGenius** to perform:

A. Integrated run, (EXTR + PCR),
B. Amplification run (PCR only),
C. Calibration run (PCR only),
D. Amplification run for Positive and Negative Control run (PCR only).

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

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

The main steps for the setup of the four types of runs are described here below.

A. Integrated run (Extract + PCR)

To set up an integrated run with sample extraction and amplification, follow the steps below while referring to the GUI:

1. Identify the samples and handle according to laboratory guidelines and the "Specimens and Controls" section.
2. If needed, thaw samples at room temperature (~+21 °C) and handle according to laboratory guidelines and "Specimens and Controls" section. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block (+2 / +8 °C).

Note: For the test, 1000 µL of sample must be transferred in a **2 mL Sarstedt Tube** (not provided) previously labeled. Exceeding volume will be left in the **2 mL Sarstedt Tube** by the **ELITe BeGenius**.

3. Thaw the needed **ASP Q-PCR Mix** tubes at room temperature (~ +21 °C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently, then spin down the content for 5 seconds.

Note: Protect the **ASP Q - PCR Mix** from light while thawing because this reagent is photosensitive.

4. Thaw the needed **CTR CPE** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 12 extractions in optimized conditions (2 or more extraction per session). Mix gently then spin down the contents for 5 seconds..
5. Select "Perform Run" from the "Home" screen.
6. Remove all the Racks from the "Cooler Unit" and place them on the preparation table.
7. Select the run mode: "Extract + PCR".
8. Load the samples into the "**Sample Rack**".

Note: When secondary tubes "**2 mL Tube**" are loaded, use the blue adaptors for the "**Sample Rack**".

9. Insert the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5) by following the GUI instruction. Click "Next" to continue.
10. If needed, insert the "Sample ID" (SID) for each "Position" used.

Note: If secondary tubes are loaded, flag "**2 mL Tube**". If secondary tubes are not barcoded, type manually the "Sample ID".

11. Check the "Extraction Input Volume" (1000 µL) and the "Extraction Elution Volume" (100 µL).

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF **RTS110PLD**

12. Select the Assay Protocol to be used in the "Assay" column (i.e. **ASP ELITe_Be_BAL_1000_100**). Click "Next" to continue.
13. Load the **"Elution tubes"** into the **"Elution Rack"**.

Note: Elution tubes can be labelled with barcode to improve traceability.

14. Insert the **"Elution Rack"** into the "Cooler Unit" starting from "Lane 3" (L3) by following the GUI instruction. Click "Next" to continue.
15. Load **ASP Q-PCR Mix** and **CTR CPE** into the **"Reagent/Elution Rack"**.
16. Insert the **"Reagent/Elution Rack"** into the "Cooler Unit" in "Lane 2" (L2) by following the GUI instruction. Click "Next" to continue.
17. If needed, for each **PCR Mix** and / or **CTR CPE** enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
18. Verify the tips in the **"Tip Racks"** in the "Inventory Area" and replace Tip Rack(s) if necessary. Click "Next" to continue.
19. Load the **"PCR Basket"** with **"PCR Cassette"** in the Inventory Area by following the GUI instruction. Click "Next" to continue.
20. Load the **"Extraction Basket"** with the **ELITe InGenius SP1000** extraction cartridges and the required extraction consumables by following the GUI instruction. Click "Next" to continue.
21. Close the instrument door.
22. Press "Start" to start the run.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, and store the results and to 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 °C. Avoid the spilling of the Extracted Sample.

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

Note: The **ASP Q-PCR Mix** can be used for 5 independent work sessions of 3 hours each or can be kept on board in the Cooler Unit up to 3 consecutive work sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

B. Amplification run (PCR only)

To set up the amplification run, with eluted samples, carry out the steps below while referring to the GUI:

1. If needed, thaw eluted samples at room temperature (~+21 °C). Mix gently then spin down the contents for 5 seconds.
2. Thaw the needed **ASP Q - PCR Mix** tubes at room temperature (~+21 °C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

Note: Protect the **ASP Q - PCR Mix** from light while thawing because this reagent is photosensitive.

3. Select "Perform Run" from the "Home screen".
4. Remove the "**Racks**" from "Lane 1, 2 and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table.
5. Select the "Run mode: PCR Only"
6. Load the samples into the "**Elution Rack**".
7. Insert the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3) by following the GUI instruction.
8. If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).

9. Select the Assay Protocol to be used in the "Assay" column (e.g., **ASP ELITe_Be_BAL_1000_100**). Click "Next" to continue.
10. Load **ASP Q-PCR Mix** into "Reagent/Elution Rack".
11. Insert the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) by following the GUI instruction. Click "Next" to continue.
12. 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).
13. Verify the tips in the "**Tip Racks**" in the "Inventory Area" and replace Tip Rack(s) if necessary. Click "Next" to continue.
14. Load the **"PCR Basket"** with **"PCR Cassette"** in the Inventory Area by following the GUI instruction. Click "Next" to continue.
15. Close the instrument door.
16. Press "Start" to start the run.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, store the results and to 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 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid the spilling of the reaction products.

Note: The **ASP Q-PCR Mix** can be used for 5 independent work sessions of 3 hours each or can be kept on board in the Cooler Unit up to 3 consecutive work sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run (PCR only)

To set up the Calibration with the Q-PCR Standards, follow the steps below while referring to the GUI:

1. Thaw the needed **ASP Q-PCR Mix** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently, then spin down the contents for 5 seconds.

Note: Protect the **ASP Q-PCR Mix** from light while thawing because this reagent is photosensitive.

2. Thaw the needed **ASP Q - PCR Standards** (Cal1: ASP Q-PCR Standards 10^2, Cal2: ASP Q-PCR Standards 10^3, Cal3: ASP Q-PCR Standards 10^4, Cal4: ASP Q-PCR Standards 10^5) at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home" screen.
4. 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: PCR Only".
6. Load the **ASP Q-PCR Standard** into the "**Elution Rack**".
7. Insert the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3) by following the GUI instruction. Click "Next" to continue.
8. 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).
9. Check the "Extraction Input Volume" (1000 µL) and the "Extraction Elution Volume" is (100 µL), even if extraction is not being performed.
10. Select the Assay Protocol to be used in the "Assay" column "ASP ELITe_Be_STD". Click "Next" to continue.
11. Load the **ASP Q-PCR Mix** into the "**Reagent/Elution Rack**".

12. Insert the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) by following the GUI instruction. Click "Next" to continue.
13. Verify the tips in the "**Tip Racks**" in the "Inventory Area" and replace Tip Rack(s) if necessary. Click "Next" to continue.
14. Load the "**PCR Basket**" with "**PCR Cassette**" in the Inventory Area by following the GUI instruction. Click "Next" to continue.
15. Close the instrument door.
16. Press "Start" to start the run.

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

Note: At the end of the run the remaining Q-PCR Standards can be removed from the instrument, capped and stored at ~-20 °C. Avoid spilling the Q-PCR Standards.

Note: At the end of the run the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid the spilling of the reaction products.

Note: Note: The **ASP Q-PCR Mix** can be used for 5 independent work sessions of 3 hours each or can be kept on board in the "Cooler Unit" for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

D. Amplification run for Positive Control and Negative Control (PCR only)

To set up the amplification run Positive Control and Negative Control, follow the steps below while referring to the GUI:

1. Thaw the needed **ASP Q-PCR Mix** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently, then spin down the contents for 5 seconds.

Note: Protect the **ASP Q - PCR Mix** from light while thawing because this reagent is photosensitive

2. Thaw the **ASP - ELITe Positive Control** tubes at room temperature (~+21°C) for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently, then spin down the contents for 5 seconds.
3. Prepare the ASP 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**.
4. Select "Perform Run" from the "Home" screen.
5. Remove the "**Racks**" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
6. Select the "Run mode: PCR Only".
7. Load the Positive Control and Negative Control tubes into the "**Elution Rack**".
8. Insert the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3) by following the GUI instruction. Click "Next" to continue.
9. 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).
10. Select the Assay Protocol to be used "ASP ELITe_Be_PC" and "ASP ELITe_Be_NC" in the "Assay" column. Click "Next" to continue.
11. Load the **ASP Q-PCR Mix** into the "**Reagent/Elution Rack**".
12. Insert the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) by following the GUI instruction. Click "Next" to continue.
13. Verify the tips in the "**Tip Racks**" in the "Inventory Area" and replace Tip Rack(s) if necessary. Click "Next" to continue.
14. Load the "**PCR Basket**" with "**PCR Cassette**" in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.
15. Close the instrument door.

16. Press “Start” to start the run.

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

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

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

Note: The **ASP Q-PCR Mix** can be used for 7 independent work sessions of 3 hours each or can be kept on board in the “Cooler Unit” for up to 2 sessions of 3 hours each and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

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 using the **ASP ELITe MGB Kit** through the following procedures:

A. Validation of Calibration curve result,
B. Validation of Positive Control and Negative Control results,
C. Validation of sample results,
D. Sample result reporting.

Note: Please, refer to the same **ELITe InGenius** chapters for the details.

PERFORMANCE CHARACTERISTICS
ELITe InGenius and ELITe BeGenius

Basal level and threshold value of target DNA

The basal level for target DNA was established using water for molecular biology as the negative control in association with **ELITe InGenius**. The negative control was amplified in 152 replicates in three different instruments with three different lots of products ELITechGroup S.p.A. The basal level for target DNA was calculated as the mean of the results obtained plus four times the standard deviation (4.09 copies / reaction).

The results are shown in the following table.

Samples	N	Mean	Deviazione Standard	Mean + 4 DS
Water	152	1.22 copies / reaction	0.72 copies / reaction	4.09 copies /reaction

In this assay the basal level established in association with **ELITe InGenius** for target DNA is 4.09 copies of the 18S rDNA gene in the amplification reaction. The basal level value was verified in association with **ELITe BeGenius** and it was confirmed at 4.09 copies / reaction.

The threshold value of the target DNA in matrix was determined by analyzing the results of 61 samples of BAL / BA and 59 samples of plasma presumably negative for *Aspergillus spp.*. The samples were extracted and amplified with products ELITechGroup S.p.A. The threshold value was calculated by adding 3 standard deviation to the mean copies / mL obtained (119.14 copies/mL approximated at 120 copies/mL for BAL / BA and 52.16 copies / mL approximated at 50 copies / mL for plasma).

Samples	N	Mean	Std. deviation	Mean + 3 SD	Mean + 6 SD
BAL / BA negative	61	28.12 copies / mL	30.34 copies / mL	119.14 copies / mL	210.16 copies / mL
Plasma negative	59	13.46 copies / mL	12.90 copies / mL	52.16 copies / mL	90.86 copies / mL

In this assay the threshold value established for target DNA is 120 copies / mL of DNA of the 18S rDNA gene of *Aspergillus spp.* for BAL / BA samples and 50 copies / mL for plasma samples.

The threshold value of target DNA of ASPERGILLUS spp. ELITe MGB Kit with **BAL** and **Plasma EDTA** matrices in association to **ELITe InGenius** and **ELITe BeGenius** was verified by testing 30 different samples of BAL and Plasma collected in EDTA samples tested negative for Aspergillus spp. DNA (ASP). Each extracted sampled was performed in two replicates in amplification with two different lots. The samples were performed in 3 different days, on 3 different instruments and were processed on **ELITe InGenius** and on **ELITe BeGenius** in “Extract + PCR” mode.

The results are shown in the following table:

Matrix	Thresholds of target DNA in the amplification reaction ELITe InGenius and ELITe BeGenius	
BAL / BA	**Significant presence**	**Quantity ≥ 120** copies / mL (Mean + 3SD)
	Non-significant presence	**Quantity < 120** copies/mL (Mean + 3SD)
Plasma	**Significant presence**	**Quantity ≥ 50** copies / mL (Mean + 3SD)
	Non-significant presence	**Quantity < 50** copies/mL (Mean + 3SD)

Note: If the result of the amplification reaction of a sample is **120 ≤ Quantity ≤ 210** copies / mL (Mean + 6SD) for BAL / BA or **50 ≤ Quantity ≤ 90** copies / mL (Mean + 6SD) for plasma it means that the *Aspergillus spp.* DNA has been detected in quantities close to the threshold (Mean + 3SD). In this case the result should be considered as “undetermined”. The test should be repeated from the extraction of a new sample in order to confirm its positivity.

Linear measuring range

The analytical sensitivity of this assay, as linear measuring range, allows the quantification from about 1,000,000 to about 10 copies per reaction.

The linearity of this assay was determined using a panel of dilutions (1 Log dilution steps) of a plasmid DNA containing the amplification product, whose initial concentration was measured by spectrophotometer. The dilutions from 10^6 copies per reaction to 10^1 copies per reaction were tested in 4 replicates carrying out carrying out the amplification by the ELITechGroup S.p.A. (EG SpA) products. The analysis of the results by linear regression demonstrated that the assay shows a linear response for all the dilutions (square correlation coefficient greater than 0.99).

The lower limit of the linear measuring range was set at the threshold value before calculated.

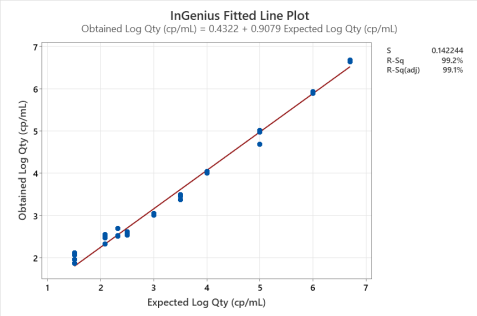
The upper limit of the linear measuring range was set at the highest concentration that gives quantitative results sufficiently accurate and precise.

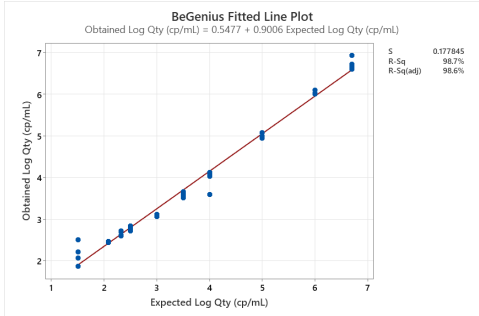
For BAL:

The linear measuring range of ASPERGILLUS spp. ELITe MGB Kit used in association with **BAL** and **ELITe InGenius** and **ELITe BeGenius** was verified and was tested using a panel prepared by diluting Aspergillus reference material (Zeptometrix for Aspergillus fumigatus, code 0801716) in ASP DNA - negative or not significant positive matrix. The panel consisted of ten dilution points from 5 x10^6 to 3.2 x 10^1 copies /mL. Each sample of the panel was tested in 4 replicates carrying out the whole analysis procedure by **ELITe InGenius** and **ELITe BeGenius** in “Extract + PCR” mode and EG SpA products.

The analysis of the obtained data, performed by linear regression, demonstrated that the assay in association with BAL samples shows a linear response for all the dilution levels with a Square Correlation Coefficient (R2) equal to 0.992 for **ELITe InGenius** and 0.987 for **ELITe BeGenius**.

The results are reported in the following figure.





The Lower Limit of Quantification (LLoQ) was set at threshold value of the target DNA and gives quantitative results precise (Standard Deviation equal to 0.1401 Log copies / mL for ELITe InGenius and 0.0947 Log copies / mL for ELITe BeGenius) and accurate (Bias equal to 0.2026 Log copies / mL for ELITe InGenius and 0.2179 for ELITe BeGenius): 120 copies/mL.

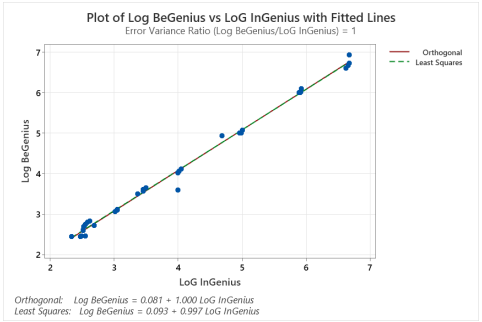
The Upper Limit of Quantification (ULoQ) on was set at the highest concentration that gives quantitative results precise (Standard Deviation equal to 0.0223 Log copies / mL for ELITe InGenius and 0.1398 Log copies / mL for ELITe BeGenius) and accurate (Bias equal to 0.0395 Log copies / mL for ELITe InGenius and 0.0290 Log copies / mL for for ELITe BeGenius): 5,000,000 copies / mL.

The final results are summarized in the following table.

Linear measuring range for BAL / BA samples and ELITe InGenius and ELITeBeGenius		
Unit of measure	lower limit	upper limit
copies / mL	**120**	**5,000,000**

The results obtained by **ELITe InGenius** and **ELITe BeGenius** were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



The Orthogonal Regression analysis (Figure 5) generated an intercept equal to 0.081 (95% 0.0030, 0.1922) and a slope equal to 1.000 (95% CI: 0.9734, 1.026). The Linear regression analysis generated a R2 of 0.994.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

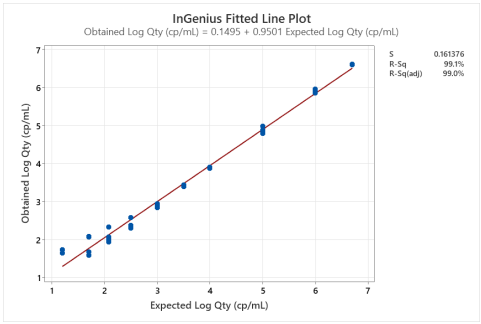
REF RTS110PLD

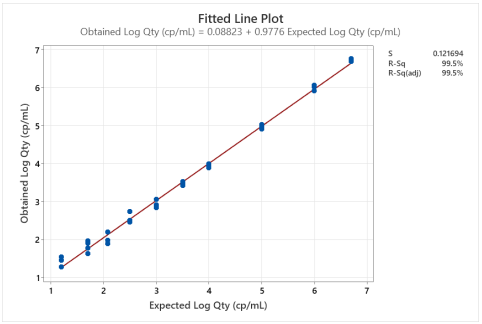
For Plasma collected in EDTA:

The linear measuring range of ASPERGILLUS spp. ELITe MGB Kit used in association with **Plasma** and **ELITe InGenius** and **ELITe BeGenius** was verified and was tested using a panel prepared by plasmid DNA dilution in ASP DNA - negative or not significant positive matrix. The panel consisted of ten dilution points from 5 x10^6 to 1.6 x 10^1 copies /mL. Each sample of the panel was tested in 4 replicates carrying out the whole analysis procedure by **ELITe InGenius** and **ELITe BeGenius** in “Extract + PCR” mode and EG SpA products.

The analysis of the obtained data, performed by linear regression, demonstrated that the assay in association with Plasma samples shows a linear response for all the dilution levels with a Square Correlation Coefficient (R2) equal to 0.991 for **ELITe InGenius** and 0.995 for **ELITe BeGenius**.

The results are reported in the following figure.





The Lower Limit of Quantification (LLoQ) was set at threshold value of the target DNA and gives quantitative results precise (Standard Deviation equal to 0.1471 Log copies / mL for ELITe InGenius and 0.1523 Log copies / mL for ELITe BeGenius) and accurate (Bias equal to 0.1480 Log copies / mL for ELITe InGenius and 0.2210 Log copies / mL for ELITe BeGenius): 50 copies/mL.

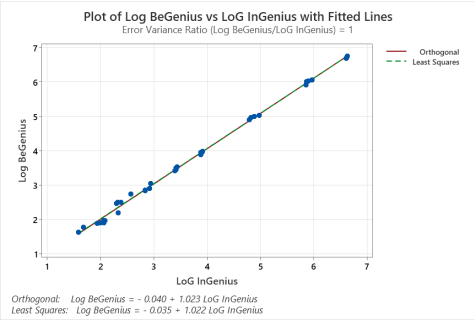
The Upper Limit of Quantification (ULoQ) was set at the highest concentration that gives quantitative results precise (Standard Deviation equal to 0.0102 Log copies / mL for ELITe InGenius and 0.0362 Log copies / mL for ELITe BeGenius) and accurate (Bias equal to 0.0842 Log copies / mL for ELITe InGenius and 0.0011 Log copies / mL for ELITe BeGenius): 5,000,000 copies / mL.

The final results are summarized in the following table.

Linear measuring range for plasma samples and ELITe InGenius and ELITe BeGenius		
Unit of measure	lower limit	upper limit
copies / mL	**50**	**5,000,000**

The results obtained by **ELITe InGenius** and **ELITe BeGenius** were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



The Orthogonal Regression analysis (Figure 10) generated an intercept equal to 0.040 (95% CI -0.1046; -0.0255) and a slope equal to 1.023 (95% CI: 1.0074; 1.0391). The Linear regression analysis generated a R2 of 0.998.

Repeatability

The Repeatability of results obtained by the product ASPERGILLUS spp. ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of BAL samples. The panel included one negative sample and two samples spiked by Aspergillus reference material (Zeptometrix for Aspergillus fumigatus, code 0801716): a sample at medium titer (about 360 copies / mL) and a sample at high titer (about 1200 copies / mL).

The Intra – Session Repeatability on **ELITe InGenius** and on **ELITe BeGenius** were obtained through the analysis of panel samples in eight replicates, with the same lot of product, with the same instrument, by the same operator, on the same day. Samples were processed in randomized positions.

The Inter – Session Repeatability on **ELITe InGenius** and on **ELITe BeGenius** were obtained through the analysis of panel samples in eight replicates, with the same lot of product, with the same instrument, by the same operator, on two different days. Samples were processed in randomized positions.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability ELITe InGenius

Sample	ASP				Internal Control			
	Pos.§ / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 8	N.A.	N.A:	N.A.	24 / 24	21.21	0.23	1.07
Medium titre	8 / 8	33.88	0.14	0.40				
High titre	8 / 8	32.14	0.15	0.48				

Inter – Session Repeatability ELITe InGenius

Sample	ASP				Internal Control			
	Pos.§ / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 16	N.A.	N.A.	N.A.	48 / 48	21.25	0.23	1.09
Medium titre	16 / 16	33.79	0.21	0.61				
High titre	16 / 16	32.18	0.14	0.43				

Intra – Session Repeatability ELITe BeGenius

Sample	ASP				Internal Control			
	Pos.§ / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 8	N.A.	N.A.	N.A.	24 / 24	24.64	0.35	1.41
Medium titre	8 / 8	34.77	0.26	0.75				
High titre	8 / 8	32.92	0.22	0.66				

Inter – Session Repeatability ELITe BeGenius

Sample	ASP				Internal Control			
	Pos.§ / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 16	N.A.	N.A.	N.A.	48 / 48	24.47	0.38	1.54
Medium titre	16 / 16	34.87	0.31	0.90				
High titre	16 / 16	32.97	0.18	0.56				

** Negative as Negative or Not Significant Positive sample*
§Positive as significant positive for ASP sample

In the Repeatability test on **ELITe InGenius** and **on ELITe BeGenius**, the assay detected the ASP target as expected and showed Ct values with %CV below 5% for ASP and for Internal Control.

Reproducibility

The Reproducibility of results obtained by the product ASPERGILLUS spp. ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** was tested by analysing a panel of BAL. The panel included one negative sample and two samples spiked by Aspergillus reference material (Zeptometrix for Aspergillus fumigatus, code 0801716): a sample at medium titer (about 360 copies / mL) and a sample at high titer (about 1200 copies / mL).

The Reproducibility was obtained through the analysis of panel samples in four replicates. Two different lots of product were used in two different days on two different instruments by two different operators. Samples were processed on **ELITe InGenius** and on **ELITE BeGenius** in “Extract + PCR” mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe InGenius

Sample	ASP				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 8	N.A.	N.A.	N.A.	24 / 24	24.90	0.17	0.70
Medium titre	8 / 8	33.57	0.39	1.17				
High titre	8 / 8	31.89	0.30	0.93				

Inter – Batch Reproducibility ELITe InGenius

Sample	ASP				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 8	N.A.	N.A.	N.A.	24 / 24	25.12	0.32	1.29
Medium titre	8 / 8	33.83	0.20	0.59				
High titre	8 / 8	32.06	0.12	0.38				

Inter – Instrument Reproducibility ELITe BeGenius

Sample	ASP				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 8	N.A.	N.A.	N.A.	24 / 24	27.16	0.46	1.70
Medium titre	8 / 8	33.32	0.42	1.25				
High titre	8 / 8	31.50	0.21	0.67				

Inter – Batch Reproducibility ELITe BeGenius								
Sample	ASP				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative*	0 / 8	N.A.	N.A.	N.A.	24 / 24	28.02	0.70	2.51
Medium titre	8 / 8	33.67	0.23	0.67				
High titre	8 / 8	31.88	0.34	1.06				

** Negative as Negative or Not Significant Positive sample*
§Positive as significant positive for ASP sample

In the Reproducibility test on **ELITe InGenius** and on **ELITe BeGenius**, the assay detected the ASP target as expected and showed Ct values with %CV below 5% for ASP and for Internal Control.

Reproducibility with certified reference material

The analytical sensitivity of the assay was evaluated using as reference material the proficiency panel QCMD 2017 Aspergillus DNA EQA Panel (Qnostics Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** system and ELITechGroup S.p.A. products.

The results obtained were reported in the following table:

Tests with certified reference materials and ELITe InGenius						
Reference material				ASPERGILLUS spp. ELITe MGB Kit		
Sample	Sample description	Detection Frequency	Sample Status	Target Ct	mean copies/mL	Outcome
ASPDNA17S-01	*A. fumigatus*	Detected	Core	30.25	4,576	Positive
ASPDNA17S-02	*A. fumigatus*	Detected	Educational	33.09	645	Positive
ASPDNA17S-03	*A. fumigatus*	Detected	Core	32.79	803	Positive
ASPDNA17S-04	Negative	Negative	Core	42.20	1	Negative
ASPDNA17S-05	*A. niger*	Detected	Educational	31.42	2,027	Positive
ASPDNA17S-06	*A. fumigatus*	Frequently Detected	Core	29.81	6,154	Positive
ASPDNA17S-07	Negative	Negative	Core	40.53	5	Negative
ASPDNA17S-08	*A. niger*	Detected	Educational	28.23	18,395	Positve

All samples were correctly detected by the product in association with the ELITe InGenius instrument.

Further tests were carried out using as reference material the panel Aspergillus Evaluation Panel 01 (Qnostics Ltd, UK). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** system and ELITechGroup S.p.A. products.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

The results obtained were reported in the following table:

Tests with certified reference materials and ELITe InGenius				
Reference material		ASPERGILLUS spp. ELITe MGB Kit		
Sample	Sample description	Target Ct	mean copies/mL	Outcome
ASPEP01-S01	*A. fumigatus*	31.34	2,152	Positive
ASPEP01-S02	*A. fumigatus*	34.91	186	Positive
ASPEP01-S03	*A. terreus*	30.28	4,470	Positive
ASPEP01-S04	*A. terreus*	33.61	460	Positive
ASPEP01-S05	Negative	39.11	13	Negative

All samples were correctly detected by the product in association with the ELITe InGenius instrument.

Diagnostic specificity: confirmation of negative samples

BAL

The Diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with **ELITe InGenius** by analyzing 44 BAL / BA samples that were negative for *Aspergillus spp.* DNA.

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 samples were tested by the assay in association with **ELITe InGenius** in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	Positive	negative	invalid
BAL / BA negative samples	44	1	43	0

43 out of 44 BAL / BA samples resulted valid and negative for *Aspergillus spp.* DNA; one sample resulted positive at significant level with a titre of 144 copies / mL (within the "undetermined interval").

In these tests, the assay Diagnostic specificity for BAL / BA was equal to 98%.

Plasma collected in EDTA

The Diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with **ELITe InGenius** by 50 Plasma samples collected in EDTA that were negative for *Aspergillus spp.* DNA.

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 samples were tested by the assay in association with **ELITe InGenius** in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	Positive	negative	invalid
Plasma negative samples	50	1	49	0

49 out of 50 plasma samples resulted valid and negative for *Aspergillus spp.* DNA; one sample resulted positive at significant level with a titre of 83 copies/mL that is in the "undetermined interval".

In these tests, the assay Diagnostic specificity for plasma was equal to 98%.

Note: If the "undetermined result rule" is applied, the Diagnostic specificity of the product was equal to 100% in association with both matrices. In fact, the positive BAL / BA sample was retested from extraction and resulted negative (38 copies / mL) and it was considered concordant. The plasma primary sample was no longer available, so it was not possible to retest it starting from the extraction and it was excluded from the analysis.

The Internal Control Ct (IC Ct) cut-off value is set at 35 for BAL and Plasma collected in EDTA samples.

Diagnostic sensitivity: confirmation of positive samples

BAL

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing 19 clinical BAL / BA samples positive for *Aspergillus spp*. DNA and 20 BAL/BA samples spiked with Aspergillus 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 samples were tested by the assay in association with ELITe InGenius system in "Extract + PCR" mode.

The results, after invalid and discrepant resolution, are summarized in the following table.

Samples	N	positive	negative	invalid
ASP positive BAL / BA	19	19	0	0
ASP spiked BAL / BA	20	20	0	0
Total BAL / BA samples	**39**	**39**	**0**	**0**

All BAL/BA samples resulted positive.

In these tests, the assay diagnostic sensitivity for BAL/BA was equal to 100%.

Plasma collected in EDTA

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing 2 clinical Plasma samples collected in EDTA positive for *Aspergillus spp.* DNA and 30 plasma samples spiked with *Aspergillus* 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 samples were tested by the assay in association with ELITe InGenius system in "Extract + PCR" mode.

The results, after invalid and discrepant resolution, are summarized in the following table.

Samples	N	positive	negative	invalid
ASP positive Plasma EDTA	2	2	0	0
ASP spiked Plasma EDTA	30	30	0	0
Total Plasma samples EDTA	**32**	**32**	**0**	**0**

All BAL/BA and plasma samples resulted positive.

In these tests, the assay diagnostic sensitivity for plasma was equal to 100%.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics are recorded in the Section 7 of the Product Technical File "ASPERGILLUS spp. ELITe MGB® Kit ", FTP110PLD.

ABI 7500 Fast Dx Real-Time PCR Instrument
ABI 7300 Real-Time System

SAMPLES AND CONTROLS

Samples

This product must be used with **DNA extracted** from the following clinical samples: bronchoalveolar lavage (BAL) and bronchial aspirate (BA).

Bronchoalveolar lavage and bronchial aspirate

The bronchoalveolar lavage and bronchial aspirate samples for nucleic acids extraction must be collected in sterile physiological solution or sterile PBS according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing.

Note: when you carry out the DNA extraction from bronchoalveolar lavage or bronchial aspirate (cellular samples) using **EXTRAblood prelysis** and **EXTRAblood** kit, please, follow the instructions in the manual of instructions for the Pretreatment of clinical samples, start from a sample of 1 mL (a maximum of **1.000.000 Cells**) add **10 µL** of **CPE** for internal control at the beginning of the extraction, recover the DNA with 100 µL of elution buffer.

Interfering substances

The DNA extracted from the sample must not contain mucoproteins, haemoglobin, ethanol or 2-propanol in order to prevent the problem of inhibition and the possibility of frequent invalid results.

High quantity of human genomic DNA in the DNA extracted from the sample may inhibit the amplification reaction.

There are no data available concerning inhibition caused by antifungal drugs, antiviral drugs, chemotherapeutic drugs or immunosuppressants.

Amplification controls

It is absolutely mandatory to validate each amplification session with a negative control reaction and a positive control reaction.

For the negative control, use sterile bidistilled water (not provided with this product) added to the reaction in place of the DNA extracted from the sample.

For the positive control, use the **ASPERGILLUS spp. ELITe Standard** product.

Quality controls

It is recommended to validate the whole analysis procedure of each extraction and amplification session by processing a negative tested sample and a positive tested sample or a calibrated reference material.

PROCEDURE

Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

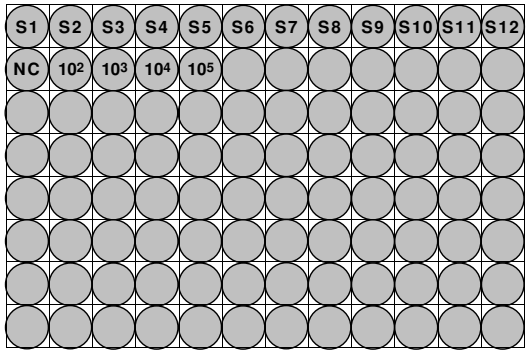
If you use a tool **7300 Real-Time PCR System**:

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session;
- set (Detector Manager) the "detector" for the ASP. probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "ASP.";
- set (Detector Manager) the "detector" for the Internal Control probe with the "reporter" = "VIC" (AP525 is analogous to VIC) and the "quencher" = "none" (non fluorescent) and call it "IC";

- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "ROX" (AP593 is analogous to ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

Note: In order to determine the DNA titre in the starting sample, set up a series of reactions with the **Q - PCR Standards** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard curve.**

See below, by way of example, how you can organise the quantitative analysis of 12 samples.



Legend: S1 - **S12**: Samples to be analysed; **NC**: Negative Control of amplification;
10^2: 10^2 standard copies; **10^3**: 10^3 standard copies; **10^4**: 10^4 standard copies; **10^5**: 10^5 standard copies.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:
- add to amplification stage the step (Add Step) of **extension at 72 °C**;

Note: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60 °C.

- modify timing as indicated in the table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;
- optional: add dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**.

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (fluorescence acquisition)	30 sec.
	72 °C	20 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	30 sec.
	80 °C	15 sec.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF **RTS110PLD**

If you use a tool **7500 Fast Dx Real-Time PCR Instrument**:

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session and set"Run mode: Fast 7500";
- set (Detector Manager) the "detector" for the ASP. probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “ASP.”;
- set (Detector Manager) the "detector" for the Internal Control probe with the "reporter" = "VIC" (AP525 is analogous to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”;
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference “= Cy5" (AP593 is analogous to Cy5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

The mode of organization of a qualitative analysis of 12 samples is illustrated by way of example in the previous section on the procedure for the **7300 Real Time PCR System** instrument.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:
- add to amplification stage the step (Add Step) of **extension at 72 °C**;

Note: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60 °C.

- modify timing as indicated in the table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;
- optional: add dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**.

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (fluorescence acquisition)	30 sec.
	72 °C	20 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	1 min.
	80 °C	15 sec.
	60 °C	15 sec

Amplification set-up
(To be performed in the extraction / preparation of the amplification reaction area)

Before starting the session, it is necessary to:
- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **ASP. Q-PCR Mix** tubes required for the session, remembering that each tube is sufficient for preparing **25 reactions**. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **ASP. Q - PCR Standard** tubes. Mix them gently, spin down the content for 5 seconds and keep them on ice;
- take the **Amplification Microplate** that will be used during the session, being careful to handle it with powder-free gloves and not to damage the wells.

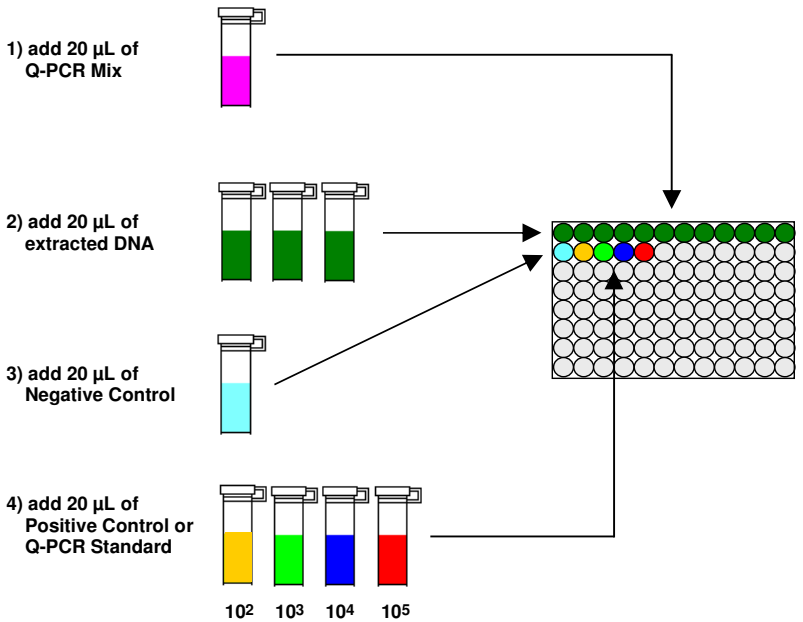
1. Accurately pipet **20 µL** of reaction mixture **ASP. Q-PCR Mix** on the bottom of the **Amplification Microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

Note: If not all the reaction mixture is used, store the remaining volume in the dark at -20 °C for no longer than one month. Freeze and thaw the reaction mixture from a maximum of **3 TIMES**.

2. Accurately pipet, by placing into the reaction mixture, **20 µL** of **extracted DNA** from the first sample in the corresponding well of **Amplification Microplate**, as previously established in the **Work Sheet**. Mix well the sample by pipetting the **extracted DNA** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other samples of **extracted DNA**.
3. Accurately pipet, by placing into the reaction mixture, **20 µL** of **Sterile bidistilled water** (not provided with this product) in the well of **Amplification Microplate** of the negative control of amplification, as previously established in the **Work Sheet**. Mix well the negative control by pipetting the **Sterile bidistilled water** three times into the reaction mixture. Avoid creating bubbles.
4. Accurately pipet, by placing into the reaction mixture, **20 µL** of **ASP. Q - PCR Standard 10^2** in the corresponding well of **Amplification Microplate**, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **ASP. Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the **ASP. Q - PCR Standards 10^3, 10^4, 10^5**.
5. Accurately seal the **Amplification Microplate** with the **Amplification Sealing Sheet**.
6. Transfer the **Amplification Microplate** into the real time thermal cycler in the amplification / detection of amplification products area and start the thermal cycle for the amplification saving the session setting with an univocal and recognizable file name (e.g. "year-month-day-ASP-EGSpA").

Note: At the end of the thermal cycle the Amplification Microplate with the reaction products must be removed from the instrument and eliminated without producing environmental contaminations. In order to avoid the spilling of the reaction products, the **Amplification Sealing Sheet must not to be removed from the Amplification Microplate**.

The following figure shows synthetically the preparation of the amplification reaction.



ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

Analysis of the results

The recorded values of the fluorescence emitted by the specific *Aspergillus spp.* probe (FAM detector "ASP") and by the specific Internal Control probe (VIC detector "IC") in the amplification reactions must be analysed by the instrument software.

Before starting the analysis, referring to the instrument documentation, it is necessary to:
- set manually (Results > Amplification plot > delta Rn vs Cycle) the calculation range for the **Baseline** (**fluorescence background level**) from cycle 6 to cycle 15;

NOTE: In the case of a positive sample with a high titre of *Aspergillus spp.* DNA, the FAM fluorescence of the *Aspergillus spp.* specific probe may begin to increase before the cycle 15. In this case the calculation range for the **Baseline** must be adapted from cycle 6 to the cycle in which the FAM fluorescence of the sample begins to increase, as detected by the instrument software (Results > Component).

If you use a tool **7300 Real-Time PCR System:**

- set manually the **Threshold** for the FAM detector "ASP" to **0.1**;
- set manually the **Threshold** for the VIC detector "IC" to **0.05**.

If you use a tool **7500 Fast Dx Real-Time PCR Instrument:**

- set manually the **Threshold** for the FAM detector "ASP" to **0.2**;
- set manually the **Threshold** for the VIC detector "IC" to **0.1**.

The values of fluorescence emitted by the specific probes in the amplification reaction and the **Threshold** value of fluorescence allow determining the **Threshold cycle (Ct)**, the cycle in which the fluorescence reached the **Threshold** value.

In the amplification reactions of the four **ASP. Q - PCR standards**, the **Ct** values of *Aspergillus spp.* are used to calculate the **Standard Curve** (Results > Standard Curve) for the amplification session and to validate the amplification and the detection as described in the following table:

Q - PCR Standard 10^5 reaction detector FAM "ASP"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

Standard Curve detector FAM "ASP"	Acceptability range	Amplification / Detection
Correlation coefficient (R2)	0.990 ≤ R2 ≤ 1.000	CORRECT

If the result of the **Q - PCR Standard 10^5** amplification reaction is **Ct > 25** or **Ct Undetermined** or if the **Correlation coefficient (R2)** value does not fall within the limits, this means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mixture or of the standards, degradation of the reaction mixture or of the standards, incorrect setting of the position of the standards, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

In the **Negative control** amplification reaction, the **Ct** value of *Aspergillus spp.* and the **Standard Curve** of the amplification session are used to calculate the **Quantity** of target DNA present in the reaction and for to validate the session amplification and detection as described in the following table:

Negative control reaction detector FAM "ASP"	*Aspergillus spp.* DNA in the reaction	Amplification / Detection
Ct Undetermined or Quantity < 8	NOT DETECTED or FEWER THAN 8 COPIES	CORRECT

Note: In this assay the baseline established for the target DNA in the amplification reaction of the **negative control** is 8 copies of 18S rDNA (see paragraph on Performance Characteristics on page 14).

If the result of the **Negative control** amplification reaction is **≥ 8 copies**, it means that DNA of 18S rDNA has been detected in the amplification reaction above the baseline. In this case, problems have occurred during the amplification phase (contamination) which may cause incorrect results. The session is invalid and must be repeated from the amplification phase.

In the amplification reaction of each **sample**, the **Ct** value of *Aspergillus spp.* and the **Standard Curve** of the amplification session, are used to to calculate the **Quantity** of target DNA present in the amplification reactions of samples. The **Ct** value of Internal Control is used to validate extraction, amplification and detection.

Note: Verify with the instrument software (Results > Amplification plot > delta Rn vs Cycle) that the **Ct** was determined by a fast and regular increase of the fluorescence values and not by peaks or an increase of the background (irregular or high background).

This product is able to quantity from 1,000,000 to 10 copies of DNA of 18S rDNA gene of *Aspergillus spp.* in the amplification reaction.

In this assay the threshold value established for target DNA in the amplification reaction of the sample is **1300** copies of 18S rDNA of *Aspergillus spp.* Significance limits have been defined around the threshold limit for the presence of target DNA (see paragraph on Performance Characteristics on page 14).

The results as a **Ct** and as a **Quantity** of amplification reactions for each sample (Results> Report) are used as described in the following table:

Sample result		Sample suitability	Assay result	*Aspergillus spp.* DNA
detector FAM "ASP"	**detector VIC "IC"**			
Ct Undetermined	**Ct > 35 or Undetermined**	**not suitable**	**invalid**	
	Ct ≤ 35	**suitable**	**valid, negative**	**NOT DETECTED**
Quantity < 1170	**Ct > 35 or Undetermined**	**not suitable**	**invalid**	**-**
	Ct ≤ 35	**suitable**	**valid, negative**	**PRESENT but NOT SIGNIFICANT**
1170 ≤ Quantity ≤ 1430	**Ct > 35 or Undetermined**	**not suitable**	**invalid**	**-**
	Ct ≤ 35	**suitable**	**valid, undetermined**	**PRESENT but UNDETERMINED**
Quantity > 1430	**Ct > 35 or Undetermined**	**suitable***	**valid, positive**	**PRESENT and SIGNIFICANT**
	Ct ≤ 35	**suitable**	**valid, positive**	**PRESENT and SIGNIFICANT**

If the result of the amplification reaction of a sample is **Ct Undetermined** or **Quantity ≤ 1430** copies for *Aspergillus spp.* and **Ct > 35** or **Ct Undetermined** for the Internal Control, it means that it was impossible to detect efficiently the DNA for the Internal Control. In this case problems occurred during the amplification step (inefficient or absent amplification) or during the extraction step (degradation of the DNA sample, the sample with insufficient numbers of cells, loss of DNA during the extraction or presence of inhibitors) which may lead to incorrect results and false negatives. The sample is not suitable, the assay is invalid and it needs to be repeated starting from the extraction of a new sample.

If the result of the amplification reaction of a sample is **Ct Undetermined** for *Aspergillus spp.* and **Ct ≤ 35** for the Internal Control, it means that the *Aspergillus spp.* DNA was not detected or is present in amounts below the threshold of significance established for this assay (see the paragraph about Performance Characteristics, page 14). In this case the result is negative and is not detected the presence of DNA of Aspergillus spp. in the sample.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF **RTS110PLD**

If the result of the amplification reaction of a sample is **Quantity < 1170** copies for *Aspergillus spp.* and **Ct ≤ 35** for the Internal Control, it means that the *Aspergillus spp.* DNA has been detected but is present in amounts below the threshold of significance established for this assay (see the paragraph about Performance Characteristics, page 14). In this case the result is negative and the presence of *Aspergillus spp.* DNA in the sample is not significant.

If the result of the amplification reaction of a sample is **1170 ≤ Quantity ≤ 1430** copies for *Aspergillus spp.* and **Ct ≤ 35** for the Internal Control, it means that the *Aspergillus spp.* DNA has been detected in quantities close to the limit of significance set for this assay but insufficient to overcome (see the paragraph about Performance Characteristics, page 14). In this case the result is indeterminate. The sample is suitable, but the test must be repeated from the extraction of a new sample.

If the result of the amplification reaction of a sample is **Quantity > 1430** copies for *Aspergillus spp.* and **Ct ≤ 35** for the Internal Control, it means that DNA of *Aspergillus spp.* has been detected and it is available in quantities above the significance limit established for this assay (see the paragraph about Performance Characteristics, page 14). In this case, the test is positive and the presence of DNA of *Aspergillus spp.* in the sample is significant.

The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes about the patient.

Note: When in the amplification reaction of a sample the *Aspergillus spp.* DNA has been detected a Quantity > 1430 copies, the Internal Control may result as Ct > 35 or Ct Undetermined. In fact, the low efficiency amplification reaction for the Internal Control may be displaced by competition with the high efficiency amplification reaction for *Aspergillus spp.* DNA. In this case the sample is nevertheless suitable and the positive result of the assay is valid.

PERFORMANCE CHARACTERISTICS

Basal level and threshold value of target DNA

In this assay the basal level established for target DNA is 8 copies of DNA of the 18S rDNA gene in the amplification reaction.

The basal level for target DNA was established using ultrapure water for molecular biology as the negative control. The water was used in 176 repeats in two different amplification sessions with products ELITechGroup S.p.A. The baseline level for target DNA was calculated as the mean of the results obtained plus three times the standard deviation (8.13 copies of DNA approximate to 8 copies).

The results are shown in the following table.

Samples	No.	Mean	Std. deviation	Basal level
Water	176	1.16 copies of DNA	2.32 copies of DNA	8.13 copies of DNA

In this assay the threshold value established for target DNA is 1300 copies of DNA of the 18S rDNA gene of *Aspergillus spp.* in the amplification reaction.

The threshold value of the target DNA was determined by analyzing the results of 45 samples of BAL and BA certificates negative in culture, extracts and amplified with products ELITechGroup S.p.A. Three samples were considered "outliers" and were excluded from the analysis. The threshold value was calculated by multiplying by ten the value of the average number of copies of target DNA of the 42 negative samples remaining plus three standard deviations (1329.7 DNA copies approximated to 1300 copies). Around the threshold value of the target DNA were determined limits of significance of the presence of target DNA in the amplification reaction.

The results are shown in the following table:

Samples	N	Mean	Std. deviation	Average + 3 SD
BAL and BA negative	42	29,70 copies of DNA	34,42 copies of DNA	132,97 copies of DNA

Limits for target DNA in the amplification reaction	
Significant presence	**Quantity > 1430** copies of DNA (1300 + 10%)
Undetermined result	**1170 ≤ Quantity ≤ 1430** copies of DNA (1300 ± 10%)
Insignificant presence	**Quantity <1170** copies of DNA (1300 - 10%)

Linear measuring range

The analytical sensitivity of this assay allows the quantification from 1,000,000 to 10 molecules of target DNA in the 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of this assay, as linear measuring range, was determined using a panel of dilutions (1 $\log_{10}$ between one dilution and the next) of a plasmid DNA containing the amplification product whose initial concentration was measured by a spectrophotometer. The dilutions from 10^7 molecules per reaction to 10^1 molecules per reaction were tested in 9 replicates carrying out the amplification by ELITechGroup S.p.A. products.

The analysis of the obtained data, performed by linear regression, demonstrated that the assay displays a linear response for all the dilutions (linear correlation coefficient greater than 0.99).

The upper limit of the linear measuring range was set at 10^6 molecules per reaction, corresponding to the genome Equivalents per reaction, within one logarithm from the highest concentration Q - PCR Standard amplification standard (10^5 molecules / 20 µL).

The lower limit of the linear measuring range was set at 10 molecules per reaction, corresponding to the genome Equivalents per reaction, within one logarithm from the lowest concentration Q - PCR Standard amplification standard (10^2 molecules / 20 µL).

The final results are summed up in the following table.

Linear measuring range	
Upper limit	**1,000,000** DNA copies / reaction
Lower limit	**10** DNA copies / reaction

Precision and Accuracy

The precision of the assay, as the variability of results obtained with several replicates of a sample tested within the same amplification session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 21.9% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The accuracy of the assay, as the difference between the mean of results obtained with several replicates of a sample within the same amplification session and the theoretical concentration value of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 11.0% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The precision and the accuracy were determined using data obtained for the study of the linear measuring range.

Efficiency of detection and quantification of different species

The efficiency of detection and quantification of different species of the genus *Aspergillus* was evaluated by comparison of sequences with nucleotide databases.

An examination of selected regions for hybridization of oligonucleotide primer and fluorescent probe on the alignment of the sequences available in the database of 18S rDNA region of *Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Aspergillus flavus, Aspergillus versicolor, Aspergillus glaucus* showed their conservation and the absence of mutations.

The detection efficiency of the assay on the species of the genus *Aspergillus*, was assessed using clinical samples positive for *Aspergillus fumigatus, Aspergillus niger and Aspergillus terreus* (tested with a culture method). These samples were tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products. The three species of Aspergillus have yielded positive results as expected.

Diagnostic sensitivity: positive samples

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested using some clinical samples positive for *Aspergillus spp.*

The diagnostic sensitivity was evaluated using as reference material 11 bronchoalveolar lavage (BAL) or bronchial aspirate (BA) samples all positive for *Aspergillus spp.*(tested with a culture method). These samples were tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF **RTS110PLD**

The results are summed up in the following table:

Samples	No.	positive	negative
BAL positive for *Aspergillus fumigatus*	6	6	0
BAL positive for *Aspergillus niger*	2	2	0
BAL positive for *Aspergillus terreus*	1	1	0
BA positivo for *Aspergillus fumigatus*	1	1	0
BA positivo for *Aspergillus fumigatus* e *Aspergillus terreus*	1	1	0

This assay allowed us to identify as positive all samples tested.

Markers potentially interfering

The cross-reactivity of the assay with other potentially interfering markers, was evaluated by comparison of sequences with nucleotide databases.

The analysis of the alignment of the sequences of the primers and of the fluorescent probe with the sequences available in databases for different organisms other than species of interest of the genus Aspergillus has shown:
- Their specificity (no significant homology) compared to the following species of the genus Candida: Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei;
- Their non-specificity and the presence of significant homology compared to other species of fungi including several species of the genus Aspergillus and Penicillium like that so that can give rise to false positive results.

The cross-reactivity was checked using samples of culture of *Candida albicans, Candida glabrata* and *Candida krusei* (tested with culture method). These samples were tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table:

Samples	No.	positive	negative
Culture of *Candida albicans*	2	0	2
Culture of *Candida glabrata*	1	0	1
Culture of *Candida krusei*	1	0	1
BAL negative for *Aspergillus spp.* and positive for *Candida albicans*	1	0	1
BAL negativ e for *Aspergillus spp.* and positive for *Candida spp.*	2	0	2

All samples of *Candida* have been found negative as expected.

Diagnostic specificity: negative samples

The Diagnostic specificity of the assay, as confirmation of negative clinical samples, was tested using some clinical samples negative for *Aspergillus spp* and was equal to 97.8%.

The Diagnostic specificity was evaluated using as reference material 45 bronchoalveolar lavage (BAL) or bronchial aspirate (BA) samples, all negative for *Aspergillus spp.* (tested with culture method). These samples were tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
BAL negative for *Aspergillus spp.*	39	1	38
BA negative for *Aspergillus spp.*	6	0	6

One sample tested negative in culture was identified as positive by the assay. This result can be explained in two ways: the sample was positive for DNA of Aspergillus spp. but the fungus was not viable and has not grown in culture or in the test sample was positive for the DNA of another fungus (Penicillium, for example) but the fungus was not viable and / or has not grown in culture in the test.

N. B.: The complete data and results of the tests carried out to evaluate the product performance characteristics are recorded in the Section 7 of the Product Technical File "ASPERGILLUS spp. ELITe MGB® Kit ", FTP RTS110PLD.

REFERENCES

Loeffler. J et al. (2001) *J Clin Microbiology* 39: 1626 - 1629
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30
K. Linnet et al. (2004) Clin. Chem. 50: 732 - 740.

PROCEDURE LIMITATIONS

Use this product only with the following clinical sample: bronchoalveolar lavage (BAL), bronchial aspirate (BA) and plasma collected in EDTA.

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

Do not use DNA extract that is contaminated with mucoproteins, haemoglobin, ethanol or 2-propanol with this product: these substances inhibit the amplification reaction of nucleic acids and may causes invalid results.

No data are available regarding the performance of this product with DNA extracted from following clinical specimens: whole blood collected in EDTA, the cerebrospinal fluid.

Do not use with this product extracted DNA containing high quantity of human genomic DNA that may inhibit the amplification reaction of nucleic acids.

There are no data available concerning inhibition caused by antifungal drugs, antiviral drugs, chemotherapeutic drugs or immunosuppressants.

The results obtained with this product depend on an adequate 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 products for nucleic acids extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the *Aspergillus spp.* positive clinical samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations; however, the cross-contaminations can be avoided only by good laboratory practices and following carefully these instructions for use manual.

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 work clothes 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 must be handled by qualified personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid incorrect results.

It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results.

This product requires the use of special clothing and instruments for extraction/preparation of amplification reactions and for amplification/detection of amplification products to avoid false positive results.

To avoid incorrect results, this product must be handled by professional personnel, qualified.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A negative result obtained with this product means that the target DNA is not detected in the DNA extracted from the sample; but it cannot be excluded that the target DNA has a lower titre 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.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

Possible polymorphisms within the region of the target DNA covered 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 taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, false positive and false negative results obtained 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.

TROUBLESHOOTING

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Take care when dispensing reactions into the microplate wells and comply with the work sheet. Check the volumes of reaction mixture dispensed. Check the volumes of positive control or standard dispensed.
Instrument setting error on ELITe InGenius and ELITe BeGenius	Check the position of complete reaction mixture, Q-PCR Standards and Positive Control. Check the volumes of complete reaction mixture, Q-PCR Standards and Positive Control.
PCR Mix degradation.	Not use the PCR Mix for more than five sessions 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.	Not use the standard and positive control for more than four sessions in association with ELITe InGenius and ELITe BeGenius. Use a new aliquot of standard or positive control.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, negative control, positive control or standards into the microplate wells and comply with the work sheet.
Instrument setting error on ELITe InGenius and ELITE BeGenius	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Error while setting the instrument.	Check the position settings of the samples, negative control, positive control or standards on the instrument.
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of the Negative Control.	Use a new aliquot of molecular grade water.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction / preparation of amplification reactions area of Racks or of Inventory Block or Cooler Unit on ELITe InGenius and ELITe BeGenius	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Take care, by pipetting three times, when mixing samples, negative controls and positive controls or standards into the reaction mixture. Avoid creating bubbles.
Baseline setting error.	Set the baseline calculation range within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g. from cycle 6 to cycle 15. Use the automatic baseline calculation by setting the "Auto Baseline" option.

Invalid Sample reaction	
Possible causes	**Solutions**
Incorrect dispensing into the microplate wells.	Take care when dispensing reactions into the microplate wells and comply with the work sheet. Check the volumes of reaction mixture dispensed. Check the volumes of sample.
Incorrect setting error on ELITe InGenius and ELITe BeGenius	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control and sample.
Internal Control degradation.	Use new aliquots of Internal Control.
Inhibition due to sample interfering substances.	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 primary sample in a “Extract + PCR” session.
PCR Mix degradation.	Not use the PCR Mix for more than five sessions (3 hours each in the Inventory Area). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

Error in Ct calculation on ELITe InGenius and ELITe BeGenius	
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 with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of sample in an “Extract + PCR” session.

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

SYMBOLS

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

ASPERGILLUS spp. ELITe MGB® Kit
reagent for DNA Real Time PCR

REF RTS110PLD

NOTICE TO THE USERS

Any serious incident the 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 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.

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 6972339, 7112684, 7319022, 7348146, 7381818, 7541454, 7582739, 7601851, 7671218, 7718374, 7723038, 7759126, 7767834, 7851606, 8008522, 8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1430147, 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 permits the person or legal entity to which this product has been provided to use the product, and the data generated by use of the product, only for human diagnostics. Neither ELITechGroup S.p.A. nor its licensors grant any other licenses, expressed or implied for any other purposes.

ASPERGILLUS *spp.* ELITe MGB® kit used with Genius® series

Ref.: RTS110PLD

ELITechGroup
EMPOWERING IVD

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

A. Intended use

The **ASPERGILLUS spp. ELITe MGB Kit** product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of the *Aspergillus* genus (*Aspergillus spp.*).**

The assay can detect and dose the DNA of the following species of the *Aspergillus* genus: *Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Aspergillus flavus, Aspergillus versicolor, Aspergillus glaucus.*

The assay is CE-IVD validated in combination with the instrument **ELITe InGenius** and **ELITe BeGenius**.

B. Amplified sequence

Target	Gene	Fluorophore
Aspergillus	rDNA 18S gene	FAM
Internal Control	human beta globin gene	AP525

C. Validated matrix

BAL/BA, **Plasma EDTA**

D. Kit content

ASP. Q-PCR Mix
 X 4
Ready-to-use PCR Master Mix 4 tubes of 540 µL 96 reactions per kit 5 freeze-thaw cycles per tube

- **Maximum shelf-life: 24 months**
- **Storage temperature: - 20°C**

E. Material required not provided in the kit

- **ELITe InGenius** instrument: INT030
- **ELITe BeGenius®** instrument**:** INT040
- **ELITe InGenius SP 1000** extraction cartridges: INT033SP1000
- **ELITe InGenius PCR Cassette** amplification cartridges: INT035PCR
- **ELITe InGenius SP 200 Consumable Set** consumables for extraction: INT032CS
- **ASPERGILLUS spp. ELITe Standard:** STD110PLD
- **ASPERGILLUS spp. - ELITe Positive Control:** CTR110PLD
- **CPE – Internal Control:** CTRCPE
- **ELITe InGenius Waste Box:** F2102-000
- **300 µL Filter Tips Axygen:** TF-350-L-R-S
- 1000 µL Filter Tips Tecan: 30180118

F. Protocol

Sample volume	1000 µL
Internal Control volume	10 µL
Total eluate volume	100 µL
PCR eluate input volume	20 µL
ASP. Q-PCR Mix volume	20 µL

Unit of quantitative result	copies/mL
Frequency of controls	15 days
Frequency of calibration	60 days

G. Performance ELITe InGenius® and ELITe BeGenius®

Matrix	Basal level and threshold	Diagnostic Sensitivity	Diagnostic Specificity
BAL/BA	**120 copies / mL**	**100%** 39/39*	**98%** 43/44*
Plasma	**50 copies/mL**	**100%** 32/32*	**98%** 49/50*

**confirmed samples/ tested samples*

I. Sample preparation

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

Sample type	Collection requirements	Transport/Storage conditions			
		RT (~+21 °C)	+2 / +8 °C	~-20 °C	~-70 °C
BAL	Collected without preservatives	Not Tested	≤ 7days	≤ 1 month	≤ 1 year
Plasma	EDTA	Not Tested	≤ 3 days	≤ 1 month	≤ 1 year

Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

BAL Samples must be collected in sterile physiological solution or sterile PBS according to laboratory guidelines

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

L. ELITe InGenius Procedures

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed. Three operational mode are available: complete run, or extraction only, or PCR only.

Before analysis

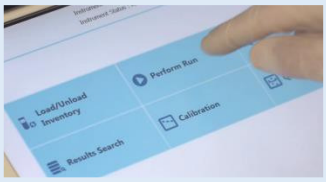
1. Switch on ELITe InGenius
Identification with username and password
Select the mode "Closed"

2. Verify calibrators: ASP. Q-PCR standard in the "Calibration menu"
Verify controls: ASP. pos. and neg. controls in the "Control menu"
NB: Both have been run, approved and not expired

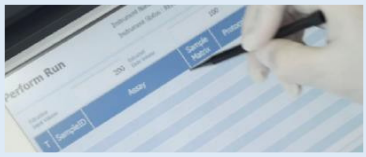
3. Thaw the ASP. Q- PCR-Mix and the CPE tubes
Vortex gently
Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen

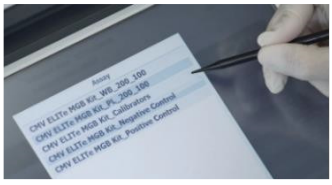


2. Verify the extraction volumes: Input:"1000 µL", eluate: "100 µL"



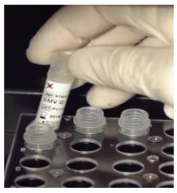
3. Scan the sample barcodes with hand-held barcode reader or type the sample ID

4. Select the "Assay protocol" of interest



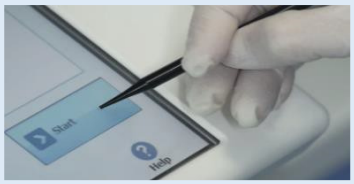
5. Select the sample position: extraction tube

6. Load the Q-PCR-Mix and the Internal Control in the inventory block

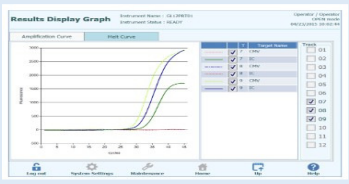


7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, extraction tube racks

8. Close the door
Start the run



9. View, approve and store the results



Procedure 2 - PCR only

1 to 4 : Follow the Complete Run procedure described above

5. Select the protocol "PCR only" and set the sample position "Extra tube"

6. Load the extracted nucleic acid tubes in the rack n°4

7. Load the PCR cassette rack Load the Q-PCR Mix in the inventory block	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” and set the sample position : Extraction tube	**6.** Load the Internal Control in the inventory block
7. Load: Extraction cartridge, Elution tube, Tip cassette, Extraction tube racks	**8.** Close the door Start the run	**9.** Archive the eluate sample

M. ELITe BeGenius Procedures

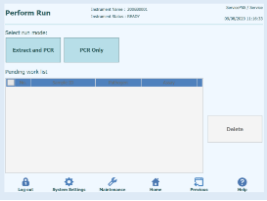
The user is guided step-by-step by the ELITe BeGenius software to prepare the run. All the steps: extraction, amplification and result interpretation are automatically performed. Three operational mode are available: complete run, or extraction only, or PCR only.

Before analysis

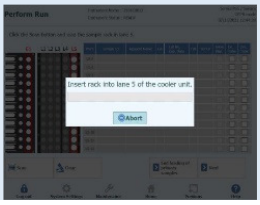
1. Switch on ELITe BeGenius Identification with username and password Select the mode “Closed”	**2.** Verify calibrators: PNEUMOCYSTIS Q-PCR standard in the “Calibration menu” Verify controls: PNEUMOCYSTIS pos. and neg. controls in the “Control menu” *NB:* Both have been run, approved and not expired	**3.** Thaw the PNEUMOCYSTIS Q- PCR-Mix and the CPE Internal Control tubes Vortex gently Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

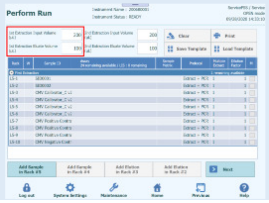
1. Select “Perform Run” on the touch screen and then click on the run mode «Extraction and PCR»



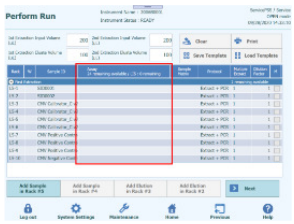
2. Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active



3. Verify the extraction volumes:
Input: “200 µL”, Eluate: “100 µL”

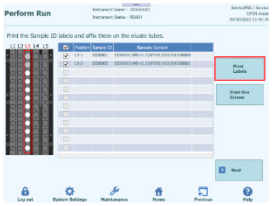


4. Select the “Assay protocol” of interest



Note: if a second extraction is performed repeat steps from 2 to 4

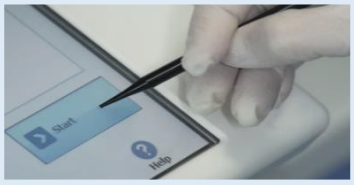
5. Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area



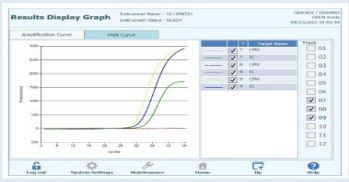
6. Load the Q-PCR-Mix and the CPE Internal Control in Reagent Rack and insert it in the cooling area

7. Load: Filter Tips, Extraction rack, and PCR rack

8. Close the door. Start the run



9. View, approve and store the results



Procedure 2 - PCR only

1. Select “Perform Run” on the touch screen and the click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid barcoded tubes in the Elution Rack and insert it in the cooling area	**3.** Select the “Assay protocol” of interest
4. Load the Q-PCR-Mix in Reagent Rack and insert it in the cooling area Load filter tips and the PCR rack	**5.** Close the door. Start the run	**6.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” in the Assay Protocol selection screen.	**6.** Load the CPE Internal Control in the Elution Rack and insert it in the cooling area
7. Load : Filter Tips and the Extraction Rack	**8.** Close the door Start the run	**9.** Archive the eluate sample

Aspergillus spp. ELITe MGB® Kit used with ABI PCR instrument

Ref.: RTS110PLD

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

A. Intended use

The **«ASPERGILLUS spp. ELITe MGB® Kit»** product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of the *Aspergillus* genus (*Aspergillus spp.*).**

The assay can detect and dose the DNA of the following species of the *Aspergillus* genus: *Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Aspergillus flavus, Aspergillus versicolor, Aspergillus glaucus.*

The assay is CE-IVD validated in combination with **ABI PCR thermal cycler** (Thermo-Fisher) and the following manual extraction kit: **EXTRAblood**.

B. Amplified sequence

Target	Gene	Fluorophore
Aspergillus	rDNA 18S gene	FAM
Internal Control	human beta globin gene	AP525

C. Validated matrix

› **Bronchoalveolar lavage** (BAL)

› **Bronchial aspirate** (BA)

D. Kit content

XXX Q--PCR Mix

X 4

Ready-to-use PCR Master Mix
4 tubes of 540 µL
100 reactions per kit
5 freeze-thaw cycles per tube

› Maximum shelf-life: 24 months
› Storage Temperature: - 20°C

E. Material required not provided in the kit

› **7500 Fast Dx and 7300 PCR Instrument**
› EXTRAblood: **EXTB01**
› EXTRAblood prelysis: **EXTB02**
› **ASPERGILLUS spp. - ELITe Positive Control: C**TR110PLD
ASPERGILLUS spp. ELITe Standard: STD110PLD
› **CPE - Internal Control:** CTRCPE
› › **Molecular biology grade water**

F. Performance

System	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
EXTRAblood - ABI	BAL	-	**100%** (9/9)*	**97%** (38/39)*
	BA		**100%** (2/2)*	**100%** (6/6)*

**confirmed samples/tested samples*

G. Procedure

The procedure below summarized the main steps of the sample analysis with conventional PCR workflow: validated extraction systems, PCR instrument settings, PCR set-up and result interpretation.

Extraction

A pre-treatment with EXTRAblood prelysis is required. Start from 1 mL of sample (a maximum of 1 000 000 cells), add 10 µL of CPE Internal Control for internal control at the beginning of the extraction, eluate the DNA with 100 µL of elution buffer

Amplification - Settings of 7500 Fast Dx and 7300 PCR instruments

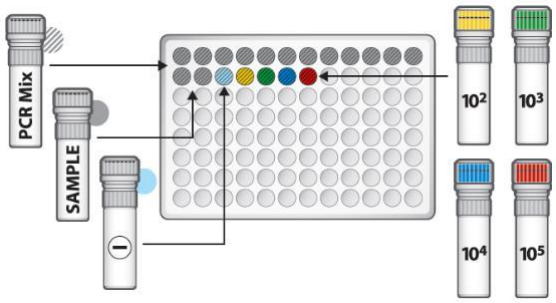
1. Switch on the thermal-cycler
2. Set "ASP" detector with "FAM" and quencher "none"
3. Set "Internal Control" detector with "VIC" and quencher "none"
4. Set passive fluorescence as "Cy5" with 7500 Fast Dx and as "ROX" with 7300 instrument
5. Set up the thermal profile as indicated. Fluorescence acquisition must be set during hybridation step at 60°C

Stage	Temperatures	Timing
Decontamination	50°C	2 min
Denaturation	94°C	2 min
Amplification and detection *45 cycles*	94°C 60°C 72°C	10 sec 30 sec 20 sec

The melt curve analysis is optional, refer to the complete IFU

Amplification - PCR Set-up

1. Thaw ASP Q PCR-Mix and Q-PCR standard tubes or the Positive Control tube
2. Mix gently and spin-down
3. Pipet **20 µL** of Q-PCR-Mix in all microplate wells in use
4. Add, **20 µL** of extracted DNA in sample wells, **20 µL** of molecular grade water in Negative Control well and 20µL of the 4 Q-PCR standards in standard curve wells, if quantitative, 20 µL of the Positive Control, if qualitative. Each one has to be mixed by pipetting 3 times into the reaction mixture
5. Seal the microplate with the amplification sealing sheet
6. Transfer the microplate in the thermocycler and start



Amplification - Threshold for qualitative analysis

Instrument	ASP FAM	Internal Control VIC
7500 Fast Dx Real Time PCR	0.2	0.1
7300 Real Time PCR	0.1	0.05

Interpretation – Qualitative and quantitative results

ASP Ct value	Internal Control Ct value	Interpretation
Determined, Quantity > 1430	Ct ≤ 35	Asp Present and significant
	Ct >35 or Undetermined	Asp Present and significant
Determined, 1170 ≤ Quantity ≤ 1430	Ct ≤ 35	Asp Present but undetermined
	Ct >35 or Undetermined	Invalid*
Determined, Quantity < 1170	Ct ≤ 35	Asp Present but not significant
	Ct >35 or Undetermined	Invalid*
Undetermined	Ct ≤ 35	Asp not present
	Ct >35 or Undetermined	Invalid*

**Repeat the assay starting from the extraction*