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EMPOWERING IVD

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Respiratory Viral PLUS ELITe MGB® Kit

reagents for RNA reverse transcription and cDNA Real Time amplification

REF RTS160ING CE -20°C

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INTENDED USE

The «**Respiratory Viral PLUS ELITe MGB® Kit**» product is part of a qualitative multiplex nucleic acids reverse transcription and amplification assay for the detection and identification of the RNA of **Influenza A Virus (FluA), Influenza B Virus (FluB), Respiratory Syncytial Virus (RSV)** and **human Metapneumovirus (hMPV)** in clinical samples.

The assay is validated in association with **ELITe InGenius®** system and with Respiratory swabs and Broncho-Alveolar Lavage (BAL) samples.

The product is intended for use as an aid in the diagnosis of respiratory infections, in conjunction with the patient's clinical data and other laboratory test results.

ASSAY PRINCIPLES

The assay consists of a multiplex reverse transcription and real-time amplification reaction (one-step method).

Starting from RNA extracted from each sample under test, different reactions of reverse transcription and amplification are performed in the PCR Cassette in order to amplify the following targets:

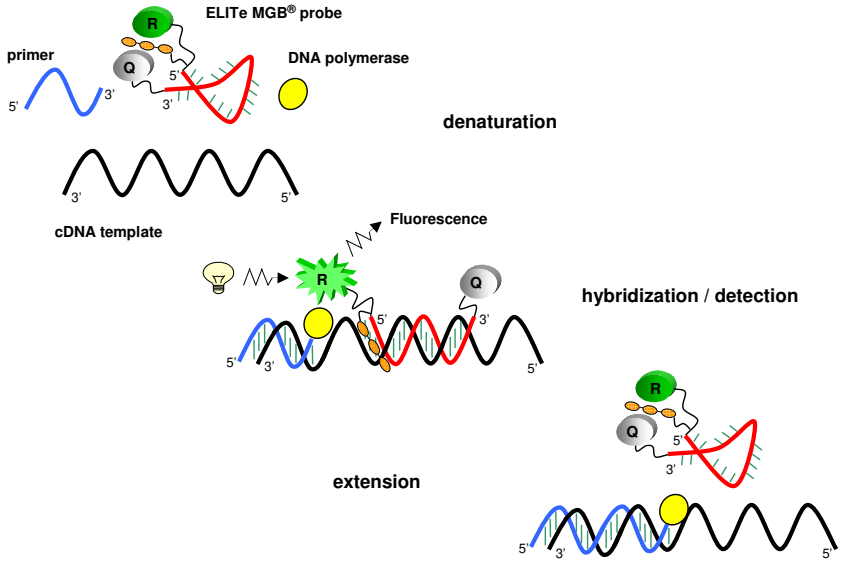
- Matrix protein gene sequence of FluA, detected by the specific probe in Channel **FluA** (Channel 5),
- Matrix protein gene sequence of FluB, detected by the specific probe in Channel **FluB** (Channel 1),
- Matrix protein gene sequence of RSV, detected by the specific probe in Channel **RSV** (Channel 4),
- Fusion protein gene sequence of hMPV, detected by the specific probe in Channel **hMPV** (Channel 6).

The PCR Mix also amplifies the extraction and inhibition Internal Control based on a region of the genomic RNA of MS2 phage and detected by the specific probe in Channel **IC** (Channel 2).

The probes with ELITe MGB® technology, labelled with different fluorophores, are activated when hybridized with the specific product of the amplification reaction. The fluorescence emission is measured and recorded by the instrument. At the end of amplification cycle, the fluorescence plots are analysed to identify the threshold cycles (Ct). The result interpretation allows to detect the presence of the pathogens of interest in the starting sample.

The assay has been validated with **ELITe InGenius**, an automated and integrated system for extraction, amplification and detection of nucleic acids and result interpretation.

In the following picture is shortly shown the mechanism of activation and fluorescence emission of ELITe MGB® technology probe. Note that the probe is not hydrolyzed during the amplification cycle.



PRODUCT DESCRIPTION

The **«Respiratory Viral PLUS ELITe MGB® Kit»** product provides the following components:

- **RV PLUS PCR Mix**

An optimized and stabilized mixture of oligonucleotides and reagents for reverse transcription and real-time amplification, **pre-aliquoted into four test tubes** (WHITE cap). Each tube contains **600 µL** of solution, sufficient for **24 tests** (processing at least 5 samples per session) in association with **ELITe InGenius**.

The RV PLUS PCR Mix contains the specific primers and probe for:

- the **matrix protein** gene sequence of FluA. The probe **FluA** is labelled with AP639 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **matrix protein** gene sequence of FluB. The probe **FluB** is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **matrix protein** gene sequence of RSV. The probe **RSV** is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **fusion protein** gene sequence of hMPV. The probe **hMPV** is labelled with AP690 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the **phage MS2** genomic RNA sequence of exogenous Internal Control (IC). The probe **IC** is labelled with AP525 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety.

The RV PLUS PCR Mix also contains the buffer, magnesium chloride, the nucleotide triphosphates, the stabilizers and the enzyme Taq DNA polymerase with thermic activation (hot start).

- **RT EnzymeMix**

An optimized and stabilized mixture of enzymes for reverse transcription, **pre-aliquoted into two test tubes** (cap with BLACK insert). Each tube contains **20 µL** of solution, sufficient for **48 tests** in association with **ELITe InGenius**.

The product is sufficient for **96 tests in association with ELITe InGenius** system, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
RV PLUS PCR Mix	mixture of reagents for reverse transcription and real time amplification **WHITE cap**	**4 x 600 µL**	**-**
RT EnzymeMix	Reverse transcriptase **cap with BLACK insert**	**2 x 20 µL**	**-**

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- 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).
- Sarstedt 2.0 mL tube skirted screw-cap (Sarstedt Ref. 72.694.005).
- Molecular biology grade water.

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OTHER PRODUCTS REQUIRED

The reagents for the extraction of RNA from the samples to be analyzed, the extraction and inhibition Internal Control, the amplification Positive Control and the consumables are **not** included in this product.

For automatic RNA extraction, reverse transcription, Real Time amplification and result interpretation, the **«ELITe InGenius®»** instrument (ELITechGroup S.p.A., ref. INT030) and the following specific Assay Protocols (ELITechGroup S.p.A.) are required:

- parameters for positive control amplification **«RV PLUS ELITe_PC»**,
- parameters for negative control amplification **«RV PLUS ELITe_NC»**,
- parameters for respiratory swab samples to be analyzed **«RV PLUS ELITe_RsS_200_100»**,
- parameters for BAL samples to be analyzed **«RV PLUS ELITe_BAL_200_100»**.

With the instrument **«ELITe InGenius»** the following generic products are required:

- extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200),
- consumables for extraction **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS),
- amplification cassettes **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR),
- tips **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, ref. TF-350-L-R-S),
- boxes **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000).

As template of extraction and inhibition Internal Control, the generic product **«CPE - Internal Control»** (ELITechGroup S.p.A., ref. CTRCPE), is required. This is a stabilised solution containing plasmid DNAs and phage genomic RNA.

As template of amplification Positive Control, the specifc product **«Respiratory Viral PLUS - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR160ING), is required. This is a stabilised solution containing plasmid DNAs.

As collection device for Respiratory swab samples, the generic product **«UTM® kit»** (COPAN Italia S.p.A., ref. 360C or 305C) or an equivalent device, is required.

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121 °C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. 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 with the product before running the assay.
While running the assay, follow the instructions provided with the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided with the product and those recommended by the manufacturers.
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 the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

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

It is necessary to have available separate areas for the molecular biology test and the microbiological culture test. Never handle the liquid or solid culture into the area designated for extraction / amplification reactions.

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 reagents required for reverse transcription and amplification must be prepared in such a way that they can be used in a single session. 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, free from DNA and RNA.

The PCR Cassettes must be handled in such a way to reduce as much as possible amplification product diffusion into the environment in order to avoid sample and reagent contamination.

Warnings and precautions specific for the components

The **RV PLUS PCR Mix** must be stored at -20 °C in the dark.
The **RV PLUS PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

RT EnzymeMix

The **RT EnzymeMix** must be stored at -20 °C.
The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than 10 minutes. Can be frozen and thawed for no more than **ten times**: further freezing / thawing cycles may cause a loss of product performances.

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Respiratory swab

The Respiratory swab samples for nucleic acid extraction must be collected in UTM medium according to laboratory guidelines, transported and stored at room temperature (+18 / +25 °C) for a maximum of 24 hours or at +2 / +8 °C for a maximum of five days, otherwise they must be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. The 200 µL of medium has to be transferred in the Sonication Tube provided in the «ELITe InGenius SP 200 Consumable Set».

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

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

Note: To carry out the RNA extraction from Respiratory swab by the ELITe InGenius system and ELITe InGenius Software version 1.3 (or later versions), use the Assay protocol **RV PLUS ELITe_RsS_200_100**. This protocol processes 200 µL of sample, adds the **CPE** (Internal Control) at 10 µL per extraction and elutes the nucleic acids in 100 µL.

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Bronchoalveolar Lavage (BAL)

Samples of Broncho-alveolar lavage (BAL), must be collected in sterile physiological solution or sterile PBS according to laboratory guidelines, transported and stored at room temperature (+18 / +25 °C) for a maximum of 24 hours or at +2 / +8 °C for a maximum of five days, otherwise they must be frozen and stored at -20 °C for a maximum of one month or at -70 °C for longer periods. The 200 µL of medium has to be transferred in the Sonication Tube provided in the «ELITe InGenius SP 200 Consumable Set».

It is recommended to split the samples into aliquots before freezing, in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: Pipetting samples from the swab primary tube to the extraction tube might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the "Warning and Precautions" section.

Note: To carry out the RNA extraction from BAL by the **ELITe InGenius** system and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the Assay Protocol **RV PLUS ELITe_BAL_200_100**. This protocol processes 200 µL of sample, adds the **CPE** (Internal Control) at 10 µL per extraction and elutes the nucleic acids in 100 µL.

Interfering substances

Quantities of human genomic DNA and/or RNA higher than 1 µg per reaction could inhibit the reverse transcription reaction and the real-time amplification.

Data available concerning inhibition caused by drugs and other substances are reported in "Interfering substances" paragraph of "Performance characteristics" chapter.

Amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the amplification controls for the amplification reagent lot that will be used in testing:

- as amplification Positive Control, use the **Respiratory Viral PLUS – ELITe Positive Control** reagent (not provided with this kit) in association with Assay Protocol **RV PLUS ELITe_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with Assay Protocol **RV PLUS ELITe_NC**.

Note: The **ELITe InGenius** system requires approved and valid results of amplification controls for each amplification reagent lot stored in its database.
The amplification control results, approved and stored in the database, will expire **after 15 days**. At the expiration date it is necessary to re-run the positive and negative controls in association with the amplification reagent lot in use.
Furthermore, the amplification controls must be re-run when:

- a new lot of amplification reagents is started,
- the results of quality controls (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius** instrument.

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used.

PROCEDURE

The procedure to use the **Respiratory Viral PLUS ELITe MGB® Kit** with the **ELITe InGenius** system consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and export 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** instrument and select the login mode "**CLOSED**",
- verify that the amplification controls (Controls, RV PLUS Positive Control, RV PLUS Negative Control) were run, in association with the amplification reagent lot to be used and the results are approved and valid (Status). If there are not amplification control results approved or valid, generate them as described in the following paragraphs,
- 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, the **ELITe InGenius** instrument and the cited matrix.

The Assay Protocols available for sample testing with the product **Respiratory Viral PLUS ELITe MGB® Kit** is described in the table below.

Assay protocol for Respiratory Viral ELITe MGB® Kit			
Name	**Matrix**	**Report**	**Characteristics**
RV PLUS ELITe_RsS_200_100	Respiratory Swab	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
RV PLUS ELITe_BAL_200_100	BAL	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL

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

Setup of the session

The product **Respiratory Viral PLUS ELITe MGB® Kit** can be used with the **ELITe InGenius** system in order to perform:

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

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

Note: The **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible to load the work session information. Refer to the instrument user's manual for more details.

Before starting the session, it is mandatory to do the following:

1. If needed, thaw at room temperature (+18 / 25 °C) the test tubes containing the samples to be analysed. Mix by vortexing at low speed for 10 seconds, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep in ice,
2. Thaw for 30 minutes at room temperature (+18 / 25 °C) the **RV PLUS PCR Mix** (WHITE cap) test tubes needed for the session, remembering that the content of each test tube is enough for **24 tests**. Mix by vortexing at low speed for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in a cool block,
3. Take the **RT EnzymeMix** (BLACK cap) tubes necessary for the session remembering that the content of each tube is sufficient to set up **48 tests**. Gently shake the tubes, centrifuge for 5 seconds to bring the contents to the bottom and keep in a cool block,

Note: The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

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4. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and mark it in a recognizable manner with a permanent marker,
5. Calculate the volumes of the two components provided by kit that are needed for preparing the **complete reaction mixture** on the basis of the number of samples to be analyzed, as described in the following table.

Note: In order to calculate the volumes of the two components to be used for the **complete reaction mixture** preparation, it is necessary to define the number of samples (N) to be tested in the session and follow the table below.

Sample Number (N)	RV PLUS PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 12	(N + 2) x 20 µL	(N + 2) x 0.3 µL

6. Prepare the **complete reaction mixture** by adding into the dedicated 2 mL tube the calculated volumes of the two components.
7. Mix by **vortexing at low speed** for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep in ice.

Note: The complete reaction mixture has to be freshly prepared for each work session and **cannot** be re-used or stored.

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

A. Integrated run

To setup an integrated run with sample extraction and amplification, carry out the following steps as per the GUI:

1. Thaw the CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
2. Select "Perform Run" from the "Home" screen.
3. Ensure that the "Extraction Input Volume" is 200 µL and the Extracted Elute Volume is 100 µL.
4. For each Track of interest fill in the "Sample ID" (SID) by typing or by scanning the sample barcode.
5. Select the Assay Protocol to be used in the "Assay" column (e.g. RV PLUS ELITe_RsS_200_100).
6. Ensure that the "Protocol" displayed is: "Extract + PCR".
7. Select "Sonication Tube" as sample loading position in the "Sample Position" column. Click "Next" to continue the setup.
8. Load CPE and the **complete reaction mixture** on the "Inventory Block" selected by following the GUI instruction. Click "Next" button to continue the setup.
9. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
10. Load the "PCR Cassettes", the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 7, following the GUI instruction. Click "Next" to continue the setup.
11. Close the instrument door.
12. Press "Start" to start the run.

After process completion, the **ELITe InGenius** system 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 for one month. Avoid spilling the Extracted Sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: At the end of the run, the complete reaction mixture **cannot** be re-used or stored.

B. Amplification run

To set up the amplification run starting from extracted RNA, carry out the following steps as per GUI:

1. Select “Perform Run” from the “Home” screen.
2. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
3. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
4. Select the Assay Protocol to be used in the “Assay” column (e.g. RV PLUS ELITe_RsS_200_100).
5. Select “PCR Only” in the “Protocol” column.
6. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load the “PCR Cassettes” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup.
10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system 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 and stored at -20 °C for one month. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid any spilling of the reaction products.

Note: At the end of the run, the complete reaction mixture **cannot** re-used or be stored.

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C. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, carry out the following steps as per GUI:

1. Thaw the **RV PLUS Positive Control** tube for the session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
2. Transfer at least 50 µL of molecular biology grade water to an “Elution tube”, provided with the ELITe InGenius SP 200 Consumable Set.
3. Select “Perform Run” from the “Home” screen.
4. In the Track of interest, select the Assay Protocol to be used in the “Assay” column.
5. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
6. For the positive control, select “RV PLUS ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of **RV PLUS Positive Control**,
7. For the negative control, select “RV PLUS ELITe_NC” and fill in the lot number and expiry date of the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the “PCR Cassettes”, the **RV PLUS Positive Control** tube and the negative control tube following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows 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 must be removed from the instrument, capped, identified and stored at -20 °C. Avoid spilling the Extracted Sample. The remaining Negative Control must be disposed.

Note: At the end of the run the PCR Cassettes with the reaction products and consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

Note: At the end of the run, the complete reaction mixture **cannot** be re-used or stored.

Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports ("Sample Report" or "Track Report"). Refer to the instrument user's manual for more details.

Note: The **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible send the work session results to the laboratory data centre. Refer to the instrument user's manual for more details.

The **ELITe InGenius** system generates the results with the product **Respiratory Viral PLUS ELITe MGB® Kit** through the following procedure:

A. Validation of amplification Positive Control and Negative Control results,
B. Validation of sample results,
C. Sample results reporting.

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes of pathogen genes (channels **FluA**, **FluB**, **RSV** and **hMPV**) in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols "RV PLUS ELITe_PC" and "RV PLUS ELITe_NC".

The amplification Positive Control and Negative Control results, specific for the lot of amplification reagent used, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire **after 15 days**.

The results of Positive Control and Negative Control amplification runs are used by the instrument software to setup the "Control Charts" monitoring the amplification step performances. Refer to the instrument user's manual for more details.

Note: If the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the "Failed" message is shown on the "Controls" screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction has to be repeated.

Note: If the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

B. Validation of Sample results

The fluorescence signals emitted by the probes of pathogen genes (channels **FluA**, **FluB**, **RSV** and **hMPV**) and by the probe of Internal Control (channel **IC**) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols RV PLUS ELITe_RsS_200_100 and RV PLUS ELITe_BAL_200_100.

Results are shown in the reports generated by the instrument ("Result Display").
The sample run can be approved when the two conditions reported in the table below are met.

1) Positive Control	**Status**
RV PLUS Positive Control	APPROVED
2) Negative Control	**Status**
RV PLUS Negative Control	APPROVED

For each sample, the assay result is automatically interpreted by the system as established by the **ELITe InGenius® Software** algorithm and the Assay Protocol parameters.

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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 RNAs are either detected or not detected.

Result of sample run	**Interpretation**
FluA: RNA detected.	The **RNA of FluA was detected** in the sample.
FluB: RNA detected.	The **RNA of FluB was detected** in the sample.
RSV: RNA detected.	The **RNA of RSV was detected** in the sample.
hMPV: RNA detected.	The **RNA of hMPV was detected** in the sample.
FluA: RNA not detected or below LoD.	The **RNA of FluA was not detected** in the sample. The sample is negative for this pathogen or its concentration is below the Limit of Detection of the assay.
FluB: RNA not detected or below LoD.	The **RNA of FluB was not detected** in the sample. The sample is negative for this pathogen or its concentration is below the Limit of Detection of the assay.
RSV: RNA not detected or below LoD.	The **RNA of RSV was not detected** in the sample. The sample is negative for this pathogen or its concentration is below the Limit of Detection of the assay.
hMPV: RNA not detected or below LoD.	The **RNA of hMPV was not detected** in the sample. The sample is negative for this pathogen or its concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Invalid assay result** caused by Internal Control failure due to incorrect extraction, inhibitors carry-over. The test should be repeated.

Samples reported as "Invalid - Retest Sample" by the **ELITe InGenius Software** are not suitable for result interpretation. In this case, the Internal Control RNA was not efficiently detected due to problems in the amplification or extraction step (degradation of RNA, loss of RNA during the extraction or inhibitor carry-over in the eluate), which may cause incorrect results.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in "PCR Only" mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using "Extract + PCR" mode.

Samples reported as "FluA: RNA not detected or below LoD", "FluB: RNA not detected or below LoD", "RSV: RNA not detected or below LoD" and "hMPV: RNA not detected or below LoD" are suitable for analysis but it was not possible to detect the targets RNA. In this case it cannot be excluded that the target RNAs are present at a concentration below the limit of detection of the assay (see "Performance characteristics").

Note: The results obtained with this assay must be interpreted taking into account all the clinical data and the other laboratory test outcomes concerning the patient.

The sample run results are stored in the database and, if valid, can be approved (Result Display) by personnel qualified as "Administrator" or "Analyst", 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".

C. Sample result export

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

The "Sample Report" shows the details of a work session sorted by selected sample (SID).
The "Track Report" shows the details of a work session by selected Track.
The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: Limit of Detection (LoD)

The Limit of Detection (LoD) of Respiratory Viral PLUS ELITe MGB Kit, was defined in association with Respiratory swabs in UTM, Broncho-Alveolar Lavage (BAL) and ELITe InGenius system.

The LoD was defined by testing a panel of Respiratory swab samples collected in UTM (COPAN Italia S.p.A.) spiked by Influenza A virus (FluA), Influenza B virus (FluB), Respiratory Syncytial Virus (RSV) and human Metapneumovirus (hMPV) reference material (Qnostics and Vircell) at known titre. Six levels of dilutions were prepared starting from a concentration higher than the expected LoD value. Each dilution level was processed in 12 replicates on ELITe InGenius system in "Extract + PCR" mode. The LoD was estimated by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Limit of Detection (copies / mL) for respiratory swab samples and ELITe InGenius

Target	LoD	95% confidence interval	
		Lower bound	Upper bound
FluA	433	318	837
FluB	409	317	725
RSV	359	267	688
hMPV	333	288	496

The calculated LoD value was verified by testing 20 replicates of respiratory swab and of BAL samples spiked with the FluA, FluB, RSV and hMPV reference materials (Qnostics and Vircell) at the claimed concentrations. The results obtained support the claimed concentrations for FluA, FluB, RSV and hMPV for the respiratory swab samples. For the BAL samples, the LoD was confirmed for FluB, RSV and hMPV, for FluA it was verified at 500 copies/mL.

Efficiency of detection (inclusivity)

The efficiency of detection of different strains or isolates of FluA, FluB, RSV and hMPV of the product Respiratory Viral PLUS ELITe MGB Kit was evaluated by in silico analysis of sequences available in the EBI ENA nucleotide database. The regions chosen for the hybridization of the primers and the fluorescent probes were checked on the alignment of the sequences for the Matrix gene (FluA, FluB and RSV) and the Nucleoprotein gene (hMPV). The hybridization regions showed sequence conservation and absence of significant mutations, so an efficient amplification of all the organisms analysed is expected.

The efficiency of detection of different strains or isolates of FluA, FluB, RSV and hMPV was also evaluated through the analysis of a panel of certified materials tested at low concentration (about 100 copies / reaction).

Certified genomic RNA samples from ATCC (USA) and Zeptometrix (USA), were diluted and analyzed in triplicate in association with ELITe InGenius system in "PCR Only" mode.

The final results are reported in the following table.

Inclusivity

Samples	Provider	Pos. / Rep.	Mean Ct	Outcome
FluA H1N1	ATCC	3/3	33.65	Positive
FluA H3N2	ATCC	3/3	33.49	Positive
FluA H1N1 (2009)	Zeptometrix	3/3	30.78	Positive
FluB Florida (Yamagata)	ATCC	3/3	35.92	Positive
FluB Wisconsin (Yamagata)	ATCC	3/3	36.22	Positive
FluB Victoria	Qnostics	3/3	31.07	Positive
RSVA2	ATCC	3/3	35.84	Positive
RSVA	Vircell	3/3	36.12	Positive
RSVB	Vircell	3/3	31.12	Positive
hMPVA	Zeptometrix	3/3	33.39	Positive
hMPVB	Zeptometrix	3/3	32.46	Positive

All the tested samples were detected as positive for the correct pathogen by the Respiratory Viral PLUS ELITe MGB® Kit.

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Potential interfering markers (cross-reactivity)

The potential cross-reactivity with other unintended organisms of the assay was evaluated by *in silico* comparison of sequences available in the nucleotide databases.

The regions chosen for the hybridization of the primers and the fluorescent probes were checked on the alignment of the sequences of other organisms. The analysis of the hybridization regions showed absence of significant homologies and indicated no potential cross-reactivity

The absence of cross-reactivity with other organisms that can be found in clinical samples of respiratory swabs, was also verified by testing a panel of certified reference materials.

Samples of genomic DNA or RNA from different potentially interfering markers (ATCC, ZeptoMetrix) were analyzed in three replicates in association with ELITe InGenius system in "PCR Only" mode. The DNAs and RNAs of each organism were added with 500 ng per reaction of human genomic DNA (Promega) in order to mimic the extracted clinical sample.

The results are reported in the following table.

Potential interfering markers: cross-reactivity

Organisms	Strain	Outcome
Aspergillus fumigatus	ATCC, 118	Negative, no cross-reactivity
Candida albicans	ATCC, 3147	Negative, no cross-reactivity
Staphylococcus aureus	ATCC, Rosenbach	Negative, no cross-reactivity
Escherichia coli	ATCC, H10407	Negative, no cross-reactivity
Bordetella pertussis	Tohama I	Negative, no cross-reactivity
Bordetella parapertussis	12822	Negative, no cross-reactivity
Haemophilus influenzae	ATCC, Rd	Negative, no cross-reactivity
Streptococcus pneumoniae	ATCC, R6	Negative, no cross-reactivity
Legionella pneumophila	ATCC, Philadelphia-1	Negative, no cross-reactivity
Mycoplasma pneumoniae	ATCC, FH	Negative, no cross-reactivity
Chlamydophila pneumoniae	ATCC, AR-39	Negative, no cross-reactivity
Mycobacterium tuberculosis	clinical isolate	Negative, no cross-reactivity
CMV	ATCC, AD-169	Negative, no cross-reactivity
Echovirus 4	ATCC, Pesascek	Negative, no cross-reactivity
ADV	ATCC, Adenoid 6	Negative, no cross-reactivity
P. jirovecii	clinical isolate	Negative, no cross-reactivity
SARS Coronavirus	Zeptometrix	Negative, no cross-reactivity
Coronavirus OC43	Zeptometrix, OC43	Negative, no cross-reactivity
Coronavirus E229	Zeptometrix, E229	Negative, no cross-reactivity
SARS-CoV-2	Zeptometrix	Negative, no cross-reactivity
Rhinovirus 1A	Zeptometrix, 1A	Negative, no cross-reactivity
Parainfluenza Virus 1	Zeptometrix, Type 1	Negative, no cross-reactivity
Parainfluenza Virus 2	Zeptometrix, Type 2	Negative, no cross-reactivity
Parainfluenza Virus 3	Zeptometrix, Type 3	Negative, no cross-reactivity
Coxsackievirus A9	ATCC, A9	Negative, no cross-reactivity

All the tested samples were detected as positive for the correct pathogen by the Respiratory Viral PLUS ELITe MGB Kit.

Potential interfering markers (interference)

The absence of inhibition due to other unintended organisms found in respiratory samples was verified by testing a panel of certified genomic DNA and RNA from ATCC.

Genomic DNA and RNA samples at high concentration were spiked by genomic DNA of FluA, FluB, RSV, hMPV at low concentration (about 100 copies per reaction) and analysed in triplicate for each potentially interfering marker in association with ELITe InGenius system in "PCR Only" mode.

The final results are reported in the following table.

Potential interfering markers: interference		
Organisms	**Strain**	**Outcome**
SARS Coronavirus	Zeptometrix	Positive, no inhibition
Coronavirus OC43	Zeptometrix, OC43	Positive, no inhibition
Coronavirus E229	Zeptometrix, E229	Positive, no inhibition
SARS-Cov-2	Zeptometrix, USA-WA1/2020	Positive, no inhibition
Rhinovirus 1A	Zeptometrix, 1A	Positive, no inhibition
Parainfluenza Virus 1	Zeptometrix, Type 1	Positive, no inhibition
Parainfluenza Virus 2	Zeptometrix, Type 2	Positive, no inhibition
Parainfluenza Virus 3	Zeptometrix, Type 3	Positive, no inhibition
Coxsackievirus A9	ATCC, A9	Positive, no inhibition

All the pathogens of interest were correctly detected in presence of the potential interfering organisms listed above when tested by the Respiratory Viral PLUS ELITe MGB Kit.

Interfering substances

A panel of potentially interfering substances at their highest relevant concentrations was tested with the product Respiratory Viral PLUS ELITe MGB® Kit. The substances tested were Mucin, Human Whole Blood, antibiotic Azithromycin, corticosteroid Beclometasone, antihistaminic Ebastine and mucolytic Ambroxol hydrochloride.

The substances were individually added to Respiratory swab samples spiked by the reference materials of FluA, FluB, RSV and hMPV at concentration of 3x LoD. Samples were processed in 3 replicates on ELITe InGenius® system in “Extraction + PCR” mode.

The results are reported in the following table.

Test of Interfering Substances, %CV for the targets						
Substance	**Concentration**	**Pos. / Rep**	**FluA**	**FluB**	**RSV**	**hMPV**
Whole blood	10% v/v	3/3	2.56	1.92	1.14	3.88
Mucin	1% w/v (10 mg/mL)	3/3	3.94	0.83	0.76	1.17
Azithromycin	0.2 µg/mL	3/3	2.93	1.29	2.17	1.31
Ambroxol	0.6 µg/mL	3/3	2.01	0.62	2.17	0.75
Beclometasone	64 ng/mL	3/3	3.85	1.36	2.22	1.46
Ebastine	0.4 µg/mL	3/3	3.96	0.89	3.20	2.92

All the samples resulted positive for the target of interest as expected. The percentage %CV of Ct values were lower than 4%.

Repeatability

The Repeatability of results obtained by the product Respiratory Viral PLUS ELITe MGB Kit in association with the ELITe InGenius system was tested by analyzing a panel of respiratory swab samples. The panel included samples spiked with FluA, FluB, RSV and hMPV reference material (Qnostics and Vircell) at concentration of about 3xLoD.

The Repeatability was obtained through the analysis of panel samples in three replicates, in two runs per day, with the same lot of product. Three lots of products were used in three different days on the same instrument by the same operator. Samples were processed on ELITe InGenius system in “Extract + PCR” mode. The Ct values of the targets and of the 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.

Inter-Batch Repeatability					
Sample	**Pos. / Rep.**	**Mean Ct**	**SD**	**%CV**	**% Positive**
3X LoD FluA	18/18	33.22	0.59	1.79	100%
3X LoD FluB	18/18	31.98	0.51	1.60	100%
3X LoD RSV	18/18	32.84	0.60	1.84	100%
3X LoD hMPV	18/18	32.38	0.69	2.12	100%
IC	72/72	29.27	0.34	1.17	100%

In the Repeatability test, the assay detected the targets as expected and showed low %CV of Ct values that did not exceed 3%.

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Reproducibility

The Reproducibility of results obtained by the product Respiratory Viral PLUS ELITe MGB Kit in association with the ELITe InGenius system was tested by analyzing a panel of respiratory swab samples. The panel included samples spiked with FluA, FluB, RSV and hMPV reference material (Qnostics and Vircell) at concentration of about 3xLoD.

The Reproducibility was obtained through the analysis of panel samples in three replicates, in two runs per day. Three different lots of product were used in three different days on three different instruments by three different operators. Samples were processed on ELITe InGenius system in “Extract + PCR” mode. The Ct values of the targets 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 tables below.

Reproducibility					
Sample	**Pos. / Rep.**	**Mean Ct**	**SD**	**%CV**	**% Positive**
3X LoD FluA	18/18	32.49	1.06	3.26	100%
3X LoD FluB	18/18	31.64	0.56	1.76	100%
3X LoD RSV	18/18	32.11	0.31	0.95	100%
3X LoD hMPV	17/17	33.64	1.06	3.16	100%
IC	72/72	28.83	0.39	1.36	100%

In the Reproducibility test, the assay detected the targets as expected and showed low %CV of Ct values that did not exceed 4%.

Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated by analyzing clinical samples of respiratory swab collected in UTM (COPAN Italia S.p.A.) and of BAL certified negative by a CE IVD marked assay.

The samples were tested by the assay in association with ELITe InGenius system.

The results are summarized in the following table.

Samples	**N**	**Positive**	**Negative**	**Diagnostic Specificity**
Negative respiratory swab collected in UTM	41	0	41	100%
Negative BAL	40	0	40	100%

Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing clinical samples of respiratory swab collected in UTM (COPAN Italia S.p.A.) and BAL certified positive by a CE IVD marked assay for FluA, FluB, RSV or hMPV.

The Diagnostic sensitivity of the assay was evaluated by analyzing positive clinical samples and negative clinical samples spiked by certified material of FluA, FluB, RSV and hMPV. Contrived samples for FluA, FluB, RSV and hMPV were spiked at a concentration of 3x, 5x, 10x LoD.

The samples were tested by the assay in association with ELITe InGenius system.

The results are summarized in the following table.

Respiratory swab samples collected in UTM	**N**	**Positive**	**Negative**	**Diagnostic Sensitivity**
FluA positive	31	28	3	95.6%
FluA spiked	59	58	1	
FluB positive	30	30	0	100%
FluB spiked	29	29	0	
RSV positive	34	33	1	98.4%
RSV spiked	30	30	0	
hMPV positive	17	17	0	100%
hMPV spiked	27	27	0	

BAL samples	N	Positive	Negative	Diagnostic Sensitivity
FluA positive	28	28	0	96.5%
FluA spiked	29	27	2	
FluB positive	13	12	1	97.7%
FluB spiked	30	30	0	
RSV positive	18	18	0	100%
RSV spiked	30	30	0	
hMPV positive	3	3	0	100%
hMPV spiked	28	28	0	

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrix and instrument are recorded in the Product Technical File "Respiratory Viral PLUS ELITe MGB Kit", FTP 160ING.

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PROCEDURE LIMITATIONS

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

Use this product only with clinical samples of respiratory swab and BAL.

At the moment there are no data available concerning product performance with the following clinical samples: sputum, throat gargle, nasopharyngeal aspirates, cell culture supernatant.

Do not use this product with quantity of extracted DNA or RNA higher than 1 µg: high quantity of nucleic acids may inhibit the reverse transcription and the amplification reactions and may cause invalid results.

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 acid extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the positive 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, cross-contaminations can be avoided only by good laboratory practices and following these instructions for use.

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

This product requires the use of 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 requires the use of special clothing and instruments dedicated to work session setup to avoid false positive results.

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 RNA is not detected in the RNA extracted from the sample. It cannot be excluded that the target RNA has a lower titre than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

In case of co-infections, the sensitivity for one target can be affected by the amplification of a second target.

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

Possible polymorphisms, deletion or insertion within the region of the target RNA covered by the product primers and probes may impair detection of target RNA.

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 Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture and positive control. Check the volumes of complete reaction mixture and positive control.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its components.	Not use the complete reaction mixture for more than one session (3 hours in the Inventory Area). Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Prepare again the complete reaction mixture. Use a new aliquot of components.
Positive control degradation.	Use a new aliquot of positive control.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture and negative control. Check the volumes of complete reaction mixture and negative control.
Contamination of the complete reaction mixture or of its components.	Prepare again the complete reaction mixture. Use a new aliquot of components.
Contamination of the negative control	Use a new aliquot of molecular biology grade water.
Contamination of the extraction area, of Racks or of Inventory Block.	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

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

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Invalid Sample reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of complete reaction mixture and sample. Check the volumes of complete reaction mixture and sample.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its components.	Do not use the complete reaction mixture for more than one session. Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Prepare again the complete reaction mixture. Use a new aliquot of components.
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 primary the sample in a “Extract + PCR” session.
Sample degradation.	Repeat the extraction with a 1:2 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session. Use a new aliquot of sample
Instrument error.	Contact ELITechGroup Technical Service.

Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - selected the track related to the sample and approve manually the result. 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 the primary sample in a “Extract + PCR” session.

TH Error	
Possible Causes	**Solutions**
Sample with too high target concentration.	If significant amplification is observed in PCR plot with negative baseline: - 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 the primary sample in a “Extract + PCR” session.

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.

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NOTICE TO PURCHASER: LIMITED LICENSE

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ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800, 9,169,256 and EP patent numbers 1068358, 1144429, 1232157, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

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