Instructions for use

SARS-CoV-2 PLUS ELITe MGB® Kit

reagents for RNA reverse transcription and Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS180ING

UDI 08033891487201

CE 0123

IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
07–R	Expansion for use of the SARS-CoV-2 PLUS products in association with the ELITe InGenius® (ref. INT030) and ELITe BeGenius® (ref. INT040) instruments, the new Assay Protocols with the new extraction method 200:100 and Nasopharingeal Swab (RsS) specimens. Verification of the current LoD against the new WHO reference material and of the impact of the Influenza A H9N2 subtype New graphics and content setting of the IFU.	14/07/25
06–R	Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements. Change of the IVDR classification of SARS-CoV-2 target products from Class D to Class B	06/05/25
05–R	Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements.	17/04/25
04	Update of the paragraph “Warnings and Precautions” with GHS Hazard and Precautions statements applied to the components.	24/01/25
03	Update for the use of the product for Respiratory Swabs matrices in association with «ELITe BeGenius®» instrument (REF INT040). Update of the Limit of detection, Reproducibility and Repeatability. Introduction of the Whole system failure test.	22/09/23
00–02	new product development and succeeding changes	—

NOTE

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

PRODUCT REF.	Lot Number	Expiry date
RTS180ING	U0425-098	30/11/2026
RTS180ING	U0125-209	31/08/2026
RTS180ING	U0425-031	30/10/2025
RTS180ING	U0225-052	31/08/2025
RTS180ING	U1124-042	31/08/2025
RTS180ING	U1024-047	31/08/2025
RTS180ING	U0224-008	31/08/2025

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

TABLE OF CONTENT

1 INTENDED USE

The product **SARS-CoV-2 PLUS ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids reverse transcription and Real-Time PCR assay for the detection and identification of the RNA of **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Influenza A Virus (FluA), Influenza B Virus (FluB), Respiratory Syncytial Virus (RSV)**, extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, reverse transcription, Real-Time PCR and results interpretation, using human specimens of nasopharyngeal swabs.

The product is intended for use as an aid in the screening of Severe Acute Respiratory Syndrome Coronavirus 2 infection in asymptomatic subjects, as an aid in the diagnosis of the Severe Acute Respiratory Syndrome Coronavirus 2, Influenza A Virus, Influenza B Virus, Respiratory Syncytial Virus infections in patients suspected of having a respiratory viral infection and as an aid in the monitoring of Severe Acute Respiratory Syndrome Coronavirus 2 infection in patients with confirmed SARS-CoV-2 infection.

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

2 ASSAY PRINCIPLE

The assay is a multiplex One-Step Reverse Transcription Real-Time PCR detecting SARS-CoV-2, FluA, FluB and RSV RNA isolated from specimens, retro-transcribed and then amplified using a complete reaction mixture, that contains primers and probes with ELITe MGB technology.

The ELITe MGB Kit probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycles (Ct).

The probes are designed with DSQ® technology and labelled with different fluorophores that are hydrolyzed and activated by the DNA polymerase enzyme, after hybridization with the specific product of the amplification reaction. The fluorescence emission is measured and recorded by the instrument. At the end of the 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.

DSQ® (Duplex-Stabilizing Quencher) technology is an evolution of MGB® chemistry. DSQ® is a single molecule that acts as an MGB®and a universal fluorescence quencher at the same time. It was designed by converting a part of the original MGB® molecule into a quencher without compromising the MGB® duplex-stabilizing properties.

3 PRODUCT DESCRIPTION

The **SARS-CoV-2 PLUS ELITe MGB Kit** provides the following components:

· **CoV-2 PLUS PCR Mix,** an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- **SCoV2**, ORF8 and ORF1ab genes, detected in Channel **ScoV2;** the probe is stabilized and quenched by the DSQ group, and labelled by FAM dye.
- **FluA**, matrix protein gene sequence, detected in Channel **FluA**; the probes are stabilized and quenched by the DSQ group, and labelled by AP639 dye.
- **FluB,** matrix protein gene sequence, detected in Channel **FluB**; the probe is stabilized and quenched by the DSQ group, and labelled by AP593 dye.
- **RSV**, matrix protein gene sequence of RSV, detected in Channel **RSV**; the probe is stabilized and quenched by the DSQ group, and labelled by AP690 dye.
- Internal Control (**IC**), human **RNase P** gene sequence as endogenous Internal Control, detected in Channel **IC**; the probe is stabilized and quenched by the DSQ group and labelled by AP525 dye.

The **CoV-2 PLUS PCR Mix** also contains buffer, magnesium chloride, the nucleotide triphosphates, the stabilizers, and the enzyme DNA polymerase with thermic activation (hot start).

· **CoV-2 PLUS RT EnzymeMix,** an optimized and stabilized mixture of enzymes for reverse transcription.

The **SARS-CoV-2 PLUS ELITe MGB Kit** contains sufficient reagents for **96 tests** on **ELITe InGenius** and **ELITe BeGenius**, with 19.3 µL of **CoV-2 PLUS PCR Mix** and 0.7 µL of **CoV-2 PLUS RT EnzymeMix** used per reaction.

4 MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
CoV-2 PLUS PCR Mix ref. RTS180ING	Mixture of reagents for reverse transcription and Real Time PCR in tube with **NATURAL cap**	**2 x 1200 µL**	**-**
CoV-2 PLUS RT EnzymeMix ref. RTS004-RT	Reverse transcription enzymes in tube with cap with **BLACK insert**	**1 x 80 µL**	**-**

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood
- Disposable nitrile powder-free gloves or similar material
- Vortex mixer.
- Bench centrifuge (~5,000 RPM)
- Bench microcentrifuge (~13,000 RPM)
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (volume range: 0.5-1000 µL)
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005)
- 0,5 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.730.005)
- Molecular biology grade water

6 OTHER PRODUCTS REQUIRED

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

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

Table 1

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA, ref. INT030) **ELITe InGenius Software** version 1.3.0.19 (or later) **SARS-CoV-2 PLUS ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **SARS-CoV-2 PLUS ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **SARS-CoV-2 PLUS ELITe_RsS_200_50**, Assay Protocol with parameters for nasopharyngeal swab specimen analysis **SARS-CoV-2 PLUS ELITe_PC_200_100**, Assay Protocol with parameters for Positive Control analysis **SARS-CoV-2 PLUS ELITe_NC_200_100**, Assay Protocol with parameters for Negative Control analysis **SARS-CoV-2 PLUS ELITe_RsS_200_100**, Assay Protocol with parameters for nasopharyngeal swab specimen analysis	**SARS-CoV-2 PLUS - ELITe Positive Control** (EG SpA., ref. CTR180ING) **ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **UTM**® (COPAN Italia S.p.A., ref. 360C or 305C), or an equivalent device, for nasopharyngeal swab specimens only **ELITe InGenius** and **ELITe BeGenius Consumables** (see ELITe InGenius and ELITe BeGenius Instruction for Use)
ELITe BeGenius (EG SpA, ref. INT040) **ELITe BeGenius Software** version 2.3.0 (or later) **SARS-CoV-2 PLUS ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **SARS-CoV-2 PLUS ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **SARS-CoV-2 PLUS ELITe_Be_RsS_200_50**, Assay Protocol with parameters for nasopharyngeal swab specimen analysis **SARS-CoV-2 PLUS ELITe_Be_PC_200_100**, Assay Protocol with parameters for Positive Control analysis **SARS-CoV-2 PLUS ELITe_Be_NC_200_100**, Assay Protocol with parameters for Negative Control analysis **SARS-CoV-2 PLUS ELITe_Be_RsS_200_100**, Assay Protocol with parameters for nasopharyngeal swab specimen analysis	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

Wear suitable protective clothes and gloves and protect eyes and face.

Never pipette solutions by mouth.

Do not eat, drink, smoke, or apply cosmetic products in the work areas.

Carefully wash hands after handling samples and reagents.

Dispose of leftover reagents and waste in compliance with the regulations in force.

Carefully read all the instructions provided before running the assay.

While running the assay, follow the product instructions provided.

Do not use the product after the indicated expiry date.

Only use reagents provided with the product and those recommended by the manufacturer.

Do not use reagents from different batches.

Do not use reagents from other manufacturers.

7.2 Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to sample nucleic acid degradation or sample contamination by PCR products.

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

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA, and RNA.

The extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination.

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

7.3 Warnings and precautions specific for the components

Table 2

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles
CoV-2 PLUS PCR Mix	-20°C or below (protected from light)	one month	up to eight
CoV-2 PLUS RT EnzymeMix	-20°C or below	one month	up to eight times, for up to ten minutes at +2 / +8 °C

The **CoV-2 PLUS PCR Mix** contains hazardous reagents. GHS Hazard and Precautions statements applied to this component are listed below.

Please, note that hazard labeling is not necessary for quantities less than 125 g or 125 mL.

The **CoV-2 PLUS PCR Mix** contains Triton X-100:

Exclamation Mark

H319 - Causes serious eye irritation.
H412 - Harmful to aquatic life with long lasting effects.
P264 - Wash hands, forearms and face thoroughly after use.
P273 - Do not disperse into the environment.
P280 - Wear gloves/protective clothing/eye protection/face protection/hearing protection.
P305+P351+P338 - IN CASE OF CONTACT WITH THE EYES: Rinse thoroughly for several minutes. Remove any contact lenses if it is easy to do so. Continue to rinse.
P337+P313 - If eye irritation persists, consult a physician.
P501 - Dispose of the product/recipient at a hazardous or special waste collection point in accordance with local, regional, national and/or international regulations.

EUH 21: Safety data sheet available on request.

8 SPECIMENS AND CONTROLS

8.1 Specimens and assay protocols

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

Table 3

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ± 10 °C**	**-70 ± 15 °C**
Nasopharyngeal swabs	UTM (COPAN)	≤ 24 hours	≤ 5 days	≤ 1 month	≤ 1 month

NOTE

The equivalence between UTM (COPAN) and other similar collection devices (having the same intended purpose and specifications) has to be demonstrated and validated by the laboratory.

It is recommended to divide the specimens into aliquots before freezing to prevent repeated freeze / thaw cycles. When using frozen samples, thaw the samples just before the extraction to avoid possible nucleic acid degradation.

To perform samples testing on the **ELITe InGenius** and **ELITe BeGenius**, the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB kits and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrix.

Table 4 Assay Protocols for SARS CoV-2 PLUS ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
Nasophar-yngeal swab samples	**ELITe InGenius**	**SARS-CoV-2 PLUS ELITe _RsS_200_50**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 50 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe InGenius	**SARS-CoV-2 PLUS ELITe_RsS_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	**SARS-CoV-2 PLUS ELITe _Be_RsS_200_50**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 50 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	**SARS-CoV-2 PLUS ELITe_Be_RsS_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL

For all protocols, 200 µL of sample must be transferred into Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius).

NOTE

Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the “7 WARNINGS AND PRECAUTIONS page 6” section.

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

Refer to “Potentially Interfering Substances” in the 11 PERFORMANCE CHARACTERISTICS page 20 section to check data concerning interfering substances.

8.2 PCR controls

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

- For the Positive Control, use the product **SARS-CoV-2 PLUS - ELITe Positive Control** (not provided with this kit) with the **SARS-CoV-2 PLUS_PC or SARS-CoV-2 PLUS_PC_200_100** Assay Protocols for **ELITe InGenius** and **SARS-CoV-2 PLUS ELITe_Be_PC or SARS-CoV-2 PLUS ELITe_Be_PC_200_100** Assay Protocols for **ELITe BeGenius**
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **SARS-CoV-2 PLUS ELITe_NC** or **SARS-CoV-2 PLUS ELITe_NC_200_100** Assay Protocols for **ELITe InGenius** and

SARS-CoV-2 PLUS ELITe_Be_NC or **SARS-CoV-2 PLUS ELITe_Be_NC_200_100** Assay Protocols for **ELITe BeGenius.**

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the PCR control validation for each lot of PCR reagent. PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and Negative Controls. The PCR controls must be re-run if any of the following events occur:

- a new lot of reagents is used,
- results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance or service is performed on the **ELITe InGenius** or **ELITe BeGenius**.

8.3 Quality controls

Verification of the extraction and PCR procedure is recommended. Archived samples or certified reference material may be used. External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

9 ELITe InGenius PROCEDURE

The procedure to use the **SARS-CoV-2 PLUS ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

Table 5

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

9.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Controls" menu on the Home page, verify the PCR Controls (**CoV-2 PLUS Positive Control**, **CoV-2 PLUS Negative Control**) are approved and valid (Status) for the **CoV-2 PLUS PCR Mix** lot to be used. If no valid PCR Controls are available for the **CoV-2 PLUS PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "8 SPECIMENS AND CONTROLS page 8").

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

9.2 STEP 2 - Session Setup

The **SARS-CoV-2 PLUS ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

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

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

NOTE

The **ELITe InGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

1. Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **48 tests**. Mix by vortexing at low speed for 10 seconds three times, then spin down the content for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **96 tests**. Mix gently then spin down the content for 5 seconds and keep on ice or cool block.

NOTE

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

3. Prepare one 2 mL tube (Sarstedt, ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and label it with a permanent marker.
4. Calculate the needed volumes of **PCR Mix** and **RT EnzymeMix** for preparing the **complete reaction mixture** on the basis of the number of samples (N) to be analyzed, as described in the table below.

Table 6

Sample Number (N)	CoV-2 PLUS PCR Mix	CoV-2 PLUS RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 19.3 µL	(N + 1) x 0.7 µL
6 ≤ N ≤ 12	(N + 2) x 19.3 µL	(N + 2) x 0.7 µL

5. Prepare the **complete reaction mixture** by transferring in the labeled 2 mL tube the calculated volumes of the two components. 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.

NOTE

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

NOTE

The complete reaction mixture is sensitive to the light, do not expose it to direct light.

To set up one of the three types of run follow the steps below while referring to the GUI:

Table 7

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature. For this assay, 200 µL of pre-treated sample must be transferred in an Extraction tube previously labelled.	**Thaw Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 4 reactions.
2	Not applicable.	Not applicable.	**Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with ELITe InGenius SP 200 Consumable Set.
3	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
4	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 or 100 µL.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 or 100 µL.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 or 100 µL.
5	For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.	Not applicable.
6	**Select the Assay Protocol** in the "Assay" column (see "Specimens and Controls").	**Select the Assay Protocol** in the "Assay" column (see "Specimens and Controls").	**Select the Assay Protocol** in the "Assay" column (see "Specimens and Controls"). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
7	Ensure the "Protocol" displayed is: "Extract + PCR".	Select "PCR Only" in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
8	Select the sample loading position as "Extraction Tube" in the "Sample Position" column.	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
9	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
10	**Load** the **complete reaction mixture** on the "Inventory Block" referring to the "Load List" and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **complete reaction mixture** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **complete reaction mixture** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.
11	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
12	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
13	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
14	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**Load** PCR Cassette and Elution tube with samples extracted	**Load** PCR Cassette, Positive Control and Negative Control tubes.
15	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
16	Close the instrument door.	Close the instrument door.	Close the instrument door.
17	Press "Start".	Press "Start".	Press "Start".

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

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified and stored at -20 ±°C or below for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

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

NOTE

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

NOTE

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

NOTE

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

9.3 STEP 3 - Review and approval of results

The **ELITe InGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen, results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe InGenius** generates results with the **SARS-CoV-2 PLUS ELITe MGB Kit** through the following procedure:

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

9.3.1 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the targets of the Positive Control and Negative Control reactions with the **SARS-CoV-2 PLUS ELITe_PC** or **SARS-CoV-2 PLUS ELITe_PC_200_100** and **SARS-CoV-2 PLUS ELITe_NC** or **SARS-CoV-2 PLUS ELITe_NC_200_100** Assay Protocols parameters. The resulting Ct values are used to verify the system (reagents lot and instrument).

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

The Positive Control and Negative Control results expire **after 15 days**.

The results of the Positive Control and Negative Control amplification are used by the **ELITe InGenius software** to set up the Control Charts monitoring the amplification step performances. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control runs must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same run, the samples can be approved but their results are not validated. In this case, the failed Control(s) and samples must all be repeated.

9.3.2 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (channels **SCoV2**, **FluA**, **FluB** and **RSV**) and the Internal Control (channel **IC**) with the **SARS-CoV-2 PLUS ELITe_RsS_200_50** or **SARS-CoV-2 PLUS ELITe_RsS_200_100** Assay Protocols parameters.

Results are shown in “Results Display” screen.

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

Table 8

1) Positive Control	**Status**
CoV-2 PLUS Positive Control	APPROVED
2) Negative Control	**Status**
CoV-2 PLUS Negative Control	APPROVED

The sample results are automatically interpreted by the **ELITe InGenius software** using Assay Protocol parameters. The possible result messages are listed in the table below.

For each sample the system reports a combination of the following messages specifying if the pathogen RNAs are either detected or not detected.

Table 9

Result of sample run	**Interpretation**
SCoV2:RNA detected.	**SARS-CoV-2 RNA was detected** in the sample.
FluA:RNA detected.	**FluA RNA was detected** in the sample.
FluB:RNA detected.	**FluB RNA was detected** in the sample.
RSV:RNA detected.	**RSV RNA was detected** in the sample.
SCoV2:RNA not detected or below the LoD.	**SARS-CoV-2 RNA was not detected** in the sample. The sample is negative for the SARS-CoV-2 RNA, or its concentration is below the assay Limit of Detection.
FluA:RNA not detected or below the LoD.	**FluA RNA was not detected** in the sample. The sample is negative for the FluA RNA, or its concentration is below the assay Limit of Detection.
FluB:RNA not detected or below the LoD.	**FluB RNA was not detected** in the sample. The sample is for the FluB RNA, or its concentration is below the assay Limit of Detection.

Table 9 (continued)

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

Samples reported as “Invalid - Retest Sample”: in this case, the Internal Control was not efficiently detected, which could be due to problems in sample collection, extraction, reverse transcription or PCR steps (e. g., incorrect sampling, 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) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see “14 TROUBLESHOOTING page 34”).

Samples reported as "Xxx: RNA not detected or below the LoD", are suitable for analysis, but the RNA of the targets was not detected. In this case, the sample may be either negative for the RNA of the targets or the RNA of the targets is present at a concentration below the Limit of Detection of the assay (see “11 PERFORMANCE CHARACTERISTICS page 20”).

SARS CoV 2 carrying a deletion of the ORF8 gene, such as in SARS-CoV-2 Δ382 strain (Y. C. F. Su et al., 2020, B. E. Young et al., 2020), can be correctly detected because the assay detects two pathogen targets and the specific reaction for the ORF1ab region provides a positive result.

NOTE

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

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

9.3.3 Sample result reporting

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

The “Sample Report” shows the results details by selected sample (SID).

The “Track Report” shows the results details by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

10 ELITe BeGenius PROCEDURE

The procedure to use the **SARS-CoV-2 PLUS ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 10

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Positive Control and Negative Control run (PCR Only)

Table 10 (continued)

STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) Sample result reporting

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in "**CLOSED**" mode,
- in the "Controls" menu on the Home page, verify the PCR Controls (**CoV-2 PLUS Positive Control, CoV-2 PLUS Negative Control**) are approved and valid (Status) for the **CoV-2 PLUS PCR Mix** lot to be used. If no valid PCR Controls are available for the **CoV-2 PLUS PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "8 SPECIMENS AND CONTROLS page 8").

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

10.2 STEP 2 - Session Setup

The **SARS-CoV-2 PLUS ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

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

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

NOTE

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

Before to setup a run:

1. Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **48 tests**. Mix by vortexing at low speed for 10 seconds three times, then spin down the contes for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **96 tests**. Mix gently then spin down the content for 5 seconds and keep on ice or cool block.

NOTE

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

3. Prepare one 2 mL tube (Sarstedt, ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and label it with a permanent marker.
4. Calculate the needed volumes of **PCR Mix** and **RT EnzymeMix** for preparing the **complete reaction mixture** on the basis of the number of samples (N) to be analyzed, as described in the table below.

Table 11

Sample Number (N)	PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 19.3 µL	(N + 1) x 0.7 µL
6 ≤ N ≤ 12	(N + 2) x 19.3 µL	(N + 2) x 0.7 µL
13 ≤ N ≤ 24	(N + 4) x 19.3 µL	(N + 4) x 0.7 µL

5. Prepare the **complete reaction mixture** by transferring in the labeled 2 mL tube the calculated volumes of the two components. 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.

NOTE

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

NOTE

The **complete reaction mixture** is sensitive to the light, do not expose it to direct light.

To set up one of the three types of run follow the steps below while referring to the GUI:

Table 12

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature. For this assay, **200 µL of sample** must be transferred in a 2mL Sarstedt Tube previously labelled.	If needed, **thaw the Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes at room temperature for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.
2	Not applicable	Not applicable	Prepare the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an “Elution tube”, provided with the ELITe InGenius SP 200 Consumable Set.
3	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen	Select “**Perform Run**” from the “Home” screen.
4	Remove all the Racks from the “Cooler Unit” and place them on the preparation table.	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) of the “Cooler Unit” and place them on the preparation table	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) from the “Cooler Unit” and place them on the preparation table.
5	Select the “Run mode”: “**Extract + PCR”.**	Select the “Run mode”: “**PCR Only**”.	Select the “Run mode”: “**PCR Only**”.
6	**Load the samples** into the “Sample Rack”. When secondary tubes “2 mL Tubes” are loaded, use the blue adaptors for the “Sample Rack”.	**Load the samples** into the “Elution Rack”.	**Load the Positive Control and Negative Control** tubes into the “Elution Rack”.

Table 12 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
7	**Insert** the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5). Insert the "Sample ID" (SID) for each "Position" used (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3). For each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). For each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
8	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
9	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 50 or 100 µL	Not applicable.	Not applicable.
10	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
11	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
	Note: When more than 12 samples are processed, repeat the procedure from point 6.		Not applicable
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable
13	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3). When more than 12 samples are processed, repeat using "Lane 2" (L2).	Not applicable	Not applicable
14	Click "Next" to continue.	Not applicable	Not applicable
15	**Load** the **complete reaction mixture** into the "Reagent/Elution Rack".	**Load** the **complete reaction mixture** into "Reagent/Elution Rack".	**Load** the **complete reaction mixture** into "Reagent/Elution Rack".
16	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix reagent and / or CPE enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
17	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
18	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
19	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
20	Load the "**PCR Rack**" with "PCR Cassette" in the Inventory Area.	Load the "**PCR Rack**" with "PCR Cassette" in the Inventory Area.	Load the **"PCR Rack**" with "PCR Cassette" in the Inventory Area.

Table 12 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
21	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
22	Load the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables.	Not applicable.	Not applicable.
23	Close the instrument door.	Close the instrument door.	Close the instrument door.
24	Press “Start”.	Press “Start”.	Press “Start”.

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

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified, and stored at -20 °C or below for no longer than one month. Avoid the spilling of the Extracted Sample.

NOTE

The complete reaction mixture has to be freshly prepared for each work session and **cannot** be stored for reuse.

NOTE

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

NOTE

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

NOTE

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

10.3 STEP 3 - Review and approval of results

The **ELITe BeGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe BeGenius** generates the results with the **SARS-CoV-2 PLUS ELITe MGB Kit** through the following procedure:

1. Validation of Positive Control and Negative Control results,

2. Validation of sample results,
3. Sample result reporting.

NOTE

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

11 PERFORMANCE CHARACTERISTICS

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined for the ELITe InGenius instrument, testing a panel of nasopharyngeal swabs collected in UTM (COPAN Italia S. p. A.) spiked with four reference materials of SARS-CoV-2 RNA (WHO International Standard, NIBSC), of FluA (A H1N1pdm, Michigan 45/15 strain, ZeptoMetrix, US), of FluB (Yamagata Lineage, Massachusetts/2/1, ZeptoMetrix, US) and of RSV (RSV A 3/2015 strain, isolate #3, ZeptoMetrix, US). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Table 13 Limit of Detection

Extraction method	Pathogen	LoD (copies / mL or IU/mL)	95% confidence interval limits	
			Lower bound	Upper bound
200_50	**SARS-CoV-2**	673 IU / mL	484 IU / mL	1154 IU / mL
	FluA	189 copies / mL	131 copies / mL	351 copies / mL
	FluB	225 copies / mL	170 copies / mL	386 copies / mL
	RSV	184 copies / mL	133 copies / mL	443 copies / mL
200_100	**SARS-CoV-2**	1328 IU / mL	982 IU / mL	2191 IU / mL
	FluA	189 copies / mL	131 copies / mL	351 copies / mL
	FluB	225 copies / mL	170 copies / mL	386 copies / mL
	RSV	184 copies / mL	133 copies / mL	443 copies / mL

The calculated LoD value were verified, with both extraction method, by testing on ELITe BeGenius and ELITe InGenius a pool of nasopharyngeal swabs collected in UTM, spiked with SARS-CoV-2, FluA, FluB and RSV RNA certified reference material at the claimed concentration.

The results obtained confirmed the claimed concentration for all the targets of the SARS-CoV-2 PLUS ELITe MGB Kit on both ELITe BeGenius and ELITe InGenius and both extraction method.

11.2 Inclusivity: Efficiency of detection

The inclusivity of the assay, as efficiency of detection for different isolates of SARS-CoV-2, FluA, FluB and RSV was evaluated by in silico analysis. The analysis showed sequence conservation and absence of significant mutations except for some strains of Influenza A subtype H9N2. So, an efficient detection for the different isolates of these targets is expected.

The Inclusivity was also verified on ELITe InGenius through the analysis of a panel of different isolates positive for the targets.

The results are reported in the following table.

Table 14 Efficiency of detection (inclusivity)

Virus (Type/Subtype)	Strain	N	Positive	Negative
SARS-CoV-2	Italy-INMI1	3	3	0
SARS-CoV-2 (A Lineage)	Hong Kong/VM20001061/2020	3	3	0
SARS-CoV-2 (A.2 Lineage)	Chile/Santiago_op4d1/2020	3	3	0
SARS-CoV-2 (B.1 Lineage)	New York-PV08410/2020	3	3	0
SARS-CoV-2 (B Lineage)	USA-IL1/2020	3	3	0
SARS-CoV-2 (A Lineage)	USA-CA1/2020	3	3	0
SARS-CoV-2 (A Lineage)	USA-AZ1/2020	3	3	0
SARS-CoV-2 (B.2 Lineage)	USA-CA2/2020	3	3	0
SARS-CoV-2 (B.1.1.7 Lineage)	USA/CA_CDC_5574/2020	3	3	0
SARS-CoV-2 (D614G cDNA)	NA	3	3	0
SARS-CoV-2 (B.1.1.7 cDNA)	NA	3	3	0
SARS-CoV-2 (B.1.351 cDNA)	NA	3	3	0
SARS-CoV-2 (P.1 cDNA)	NA	3	3	0
Influenza A (H1N1pdm09)	A/Dominican Republic/7293/2013	3	3	0
Influenza A (H1N1pdm09)	A/Massachusetts/15/2013	3	3	0
Influenza A (H1N1pdm09)	A/North Carolina/4/2014	3	3	0
Influenza A (H1N1pdm09)	A/Bangladesh/3002/2015	3	3	0
Influenza A (H1N1pdm09)	A/Iowa/53/2015	3	3	0
Influenza A (H1N1pdm09)	A/St. Petersburg/61/2015	3	3	0
Influenza A (H1N1pdm09)	A/Michigan/272/2017	3	3	0
Influenza A (H1N1pdm09)	A/Idaho/07/2018	3	3	0
Influenza A (H1N1pdm09)	A/Wisconsin/505/2018	3	3	0
Influenza A (H1N1pdm09)	A/Hawaii/66/2019	3	3	0
Influenza A (H1N1pdm09)	A/Christ Church/16/2010	3	3	0
Influenza A (H3N2)	A/Guangdong-Maonan/1536/2019	3	3	0
Influenza A (H3N2)	A/California/02/2014	3	3	0
Influenza A (H3N2)	A/Michigan/15/2014	3	3	0
Influenza A (H3N2)	A/Hong Kong/4801/2014	3	3	0
Influenza A (H3N2)	A/Alaska/232/2015	3	3	0
Influenza A (H3N2)	A/Singapore/INFIMH-16-0019/2016	3	3	0
Influenza A (H3N2)	A/Texas/71/2017	3	3	0

Table 14 Efficiency of detection (inclusivity) (continued)

Virus (Type/Subtype)	Strain	N	Positive	Negative
Influenza A (H3N2)	A/Kansas/14/2014	3	3	0
Influenza A (H3N2)	A/Wisconsin/04/2018	3	3	0
Influenza A (H3N2)	A/Arizona/45/2018	3	3	0
Influenza A (H3N2*)	A/Hong Kong/45/2019	3	3	0
Influenza A (H3N2)	A/Perth/16/2009	3	3	0
Influenza A (H3N2)	A/Hong Kong/2671/2019	6	5	1
Influenza B (Victoria)	B/Florida/78/2015	3	3	0
Influenza B (Victoria)	B/Maryland/15/2016	3	3	0
Influenza B (Victoria)	B/Hong Kong/286/2017	3	3	0
Influenza B (Victoria)	B/Hawaii/01/2018 (NA D197N)	3	3	0
Influenza B (Victoria)	B/Missouri/122018 (NA D197E)	3	3	0
Influenza B (Victoria)	B/Michigan/09/2011	3	3	0
Influenza B (Yamagata)	B/Washington/02/2019	3	3	0
Influenza B (Yamagata)	B/Texas/06/2011	3	3	0
Influenza B (Yamagata)	B/Utah/09/2014	3	3	0
Influenza B (Yamagata)	B/Oklahoma/10/2018 (NA D197N)	3	3	0
Influenza B (Yamagata)	B/Indiana/17/2017 (NA I221T)	3	3	0
Influenza B (Yamagata)	B/Wisconsin/10/2016 (NA I221V)	3	3	0
Influenza B (Yamagata)	B/Texas/81/2016	3	3	0
Influenza B (Yamagata)	B/Phuket/3073/2013	3	3	0
RSV-A	1/2015 Isolate 1	3	3	0
RSV-A	12/2014 Isolate #2	3	3	0
RSV-A	2014 Isolate 342	3	3	0
RSV-B	12/2014 Isolate#1	6	5	1
RSV-B	11/2014 Isolate #2	3	3	0
RSV-B	3/2015 Isolate #1	3	3	0

All samples were correctly detected by the SARS-CoV-2 PLUS ELITe MGB Kit.

11.3 Potentially interfering organisms: Cross-reactivity

The potential cross-reactivity of unintended organisms that may be found in clinical specimens was evaluated for the assay by in silico analysis. The analysis showed no significant homology with other unintended organisms (viruses, bacteria, protozoa and fungi). Therefore, no cross-reactivity is expected.

The absence of cross-reactivity with potential interfering organisms was also verified on ELITe InGenius through the analysis of the panel of unintended organisms.

The results are reported in the following tables.

Table 15

Organism	Positive / Replicates				Outcome
	SARS-CoV-2	Flu B	Flu A	RSV	
Adenovirus 1	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Adenovirus type 2	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Adenovirus type 7A	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Bordetella pertussis	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Candida albicans	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Corynebacterium genitalium	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Chlamydophila pneumonia	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Cytomegalovirus	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Coronavirus OC43	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Coronavirus 229E	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Coronavirus NL63	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Haemophilus influenzae	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Human Metapneumovirus 3	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Enterovirus 68	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Epstein-Barr Virus	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Escherichia coli	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Lactobacillus acidophilus	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Lactobacillus jensenii	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Legionella pneumophila	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
MERS Coronavirus	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Moraxella catarrhalis	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Mycobacterium tuberculosis	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Mycoplasma pneumonia	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Neisseria meningitidis	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Parainfluenza virus 1	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Parainfluenza virus 2	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Parainfluenza virus 3	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Parainfluenza virus 4A	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Pneumocystis carninii	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity

Table 15 (continued)

Organism	Positive / Replicates				Outcome
	SARS-CoV-2	Flu B	Flu A	RSV	
Pseudomonas aeruginosa	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Rhinovirus 1A	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Staphylococcus aureus MRSA	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Staphylococcus epidermidis	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Streptococcus pneumonia	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Streptococcus pyogenes	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity
Streptococcus salivarius	0 / 3	0 / 3	0 / 3	0 / 3	No cross-reactivity

The results of the cross-reactivity test by the panel of potential interfering organisms at high concentration are summarized in the following table:

Table 16

Sample	Positive / Replicates	Outcome
	SCoV2	
No Interferent (Reference)	0 / 5	No cross-reactivity
Human coronavirus 229E	0 / 5	No cross-reactivity
Human coronavirus HKU1	0 / 5	No cross-reactivity
Human coronavirus OC43	0 / 5	No cross-reactivity
Human coronavirus NL63	0 / 5	No cross-reactivity
MERS coronavirus	0 / 5	No cross-reactivity
Influenza virus A	0 / 5	No cross-reactivity
Influenza virus B	0 / 5	No cross-reactivity
RSV A	0 / 5	No cross-reactivity
Legionella pneumophila	0 / 5	No cross-reactivity

The results of the cross-reactivity test by the panel of potential interfering organisms at medium concentration are summarized in the following table:

Table 17

Sample	Positive / Replicates	Outcome
	SCoV2	
Human coronavirus 229E	0 / 5	No cross-reactivity
Human coronavirus HKU1	0 / 5	No cross-reactivity
Human coronavirus OC43	0 / 5	No cross-reactivity

Table 17 (continued)

Sample	Positive / Replicates	Outcome
	SCoV2	
Human coronavirus NL63	0 / 5	No cross-reactivity
MERS coronavirus	0 / 5	No cross-reactivity
Influenza virus A	0 / 5	No cross-reactivity
Influenza virus B	0 / 5	No cross-reactivity
RSV A	0 / 5	No cross-reactivity
Legionella pneumophila	0 / 5	No cross-reactivity

All potentially interfering markers tested showed no cross-reactivity for the SARS-CoV-2 target amplification using the SARS-CoV-2 PLUS ELITe MGB Kit.

11.4 Potentially interfering organisms: Inhibition

The potential inhibition of interfering markers that might be found in clinical specimens was evaluated on ELITe InGenius for the assay through the analysis of a panel of unintended organisms by analysis of a panel of markers at relevant concentration in samples of nasopharyngeal swab positive for the targets.

The results are reported in the following table.

Organism	Positive / Replicates				Outcome
	SARS-CoV-2	**Flu B**	**Flu A**	**RSV**	
Adenovirus 1	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Adenovirus type 2	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Adenovirus type 7A	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Bordetella pertussis	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Candida albicans	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Corynebacterium genitalium	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Chlamydophila pneumonia	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Cytomegalovirus	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Coronavirus OC43	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Coronavirus 229E	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Coronavirus NL63	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Haemophilus influenzae	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Human Metapneumovirus 3	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Enterovirus 68	3 / 3	3 / 3	3 / 3	3 / 3	No interference

Epstein-Barr Virus	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Escherichia coli	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Lactobacillus acidophilus	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Lactobacillus jensenii	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Legionella pneumophila	3 / 3	3 / 3	3 / 3	3 / 3	No interference
MERS Coronavirus	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Moraxella catarrhalis	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Mycobacterium tuberculosis	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Mycoplasma pneumonia	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Neisseria meningitidis	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Parainfluenza virus 1	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Parainfluenza virus 2	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Parainfluenza virus 3	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Parainfluenza virus 4A	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Pneumocystis carninii	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Pseudomonas aeruginosa	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Rhinovirus 1A	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Staphylococcus aureus MRSA	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Staphylococcus epidermidis	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Streptococcus pneumonia	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Streptococcus pyogenes	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Streptococcus salivarius	3 / 3	3 / 3	3 / 3	3 / 3	No interference

All potential interfering organisms tested showed no inhibition of all targets detection using the SARS-CoV-2 PLUS ELITe MGB Kit.

11.5 Potential interfering substances: Inhibition

The potential inhibition of interfering substances (endogenous and exogenous) that might be found in clinical nasopharyngeal swab specimens was evaluated for the assay on ELITe InGenius by analysis of a panel of substances at relevant concentration in samples spiked with FluA, FluB, RSV and SARS-CoV-2

The results are reported in the following table.

Table 18

Substance	Positive / Replicates				Outcome
	SARS-CoV-2	Flu B	Flu A	RSV	
Mucin	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Whole Blood	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Benzocaine	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Beclomethasone	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Chloraseptic	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Nasacort	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Zicam	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Fluticasone Propionate	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Mometasone furoate	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Triamcinolone	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Phenylephrine Nasal Spray	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Oxymetazoline	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Ocean Saline Spray	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Zanamivir	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Tobramycin	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Mupirocin	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Budesonide	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Dexamethasone	3 / 3	3 / 3	3 / 3	3 / 3	No interference
Flunisolide	3 / 3	3 / 3	3 / 3	3 / 3	No interference

The test showed that the tested substances do not inhibit the targets detection using the SARS-CoV-2 PLUS ELITe MGB Kit.

11.6 Cross-contamination

The possible **Cross-contamination** during analysis was evaluated for the assay by testing on ELITe InGenius 30 replicates of a negative nasopharyngeal swab specimen alternated to 30 replicates of the same specimen spiked with SARS-COV-2 (NIBSC) at a concentration of 1,000,000 IU / mL in 5 sessions.

The results are reported in the following table.

Table 19

Samples	N	Positive	Negative	%Agreement
Positive	30	30	0	100%
Negative	30	0	30	100%

Cross-contamination was neither detected within sessions nor between sessions with SARS-CoV-2 PLUS ELITe MGB Kit.

11.7 Whole system failure

The Whole system failure rate for the assay was evaluated on ELITe InGenius and ELITe BeGenius with both extraction method, by testing a panel of nasopharyngeal swab specimen spiked by certified reference material of SARS-CoV-2 (WHO International Standard for SARS-CoV-2 RNA reference material, NIBSC, UK) at concentration of 3x LoD (2019 IU / mL).

The results are summarized in the following table.

Table 20

ELITe InGenius					
Extraction method	**Samples**	**N**	**Positive**	**Negative**	**Whole system failure rate**
200_50	Nasopharyngeal swabs spiked by SARS- CoV-2	100	100	0	0%
200_100	Nasopharyngeal swabs spiked by SARS- CoV-2	100	99	1	1%

None of the tested positive samples gave false negative results in association with ELITe InGenius and extraction method 200_50 using SARS-CoV-2 PLUS ELITe MGB Kit. The whole system failure rate was equal to 0%.

One positive sample gave false negative results in association with ELITe InGenius and extraction method 200_ 100 using SARS-CoV-2 PLUS ELITe MGB Kit. The whole system failure rate was equal to 1%.

Table 21

ELITe BeGenius					
Extraction method	**Samples**	**N**	**Positive**	**Negative**	**Whole system failure rate**
200_50	nasopharyngeal swabs spiked by SARS-CoV-2	100	99	1	1%
200_100	nasopharyngeal swabs spiked by SARS-CoV-2	100	99	1	1%

One positive sample gave false negative results in association with ELITe BeGenius and both extraction method using SARS-CoV-2 PLUS ELITe MGB Kit. The whole system failure rate was equal to 1%.

11.8 Repeatability

The Intra – Session and Inter – Session Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius with both extraction method by analysis of a panel of nasopharyngeal swab samples, included one negative sample and one sample spiked at 3x LoD with SARS-CoV-2, FluA, FluB and RSV certified reference material (NIBSC and Zeptometrix).

An example of Intra-Session Repeatability (on one day) results with extraction method 200_50 is shown in the tables below.

Table 22 ELITe InGenius Intra-Session Repeatability (one day)

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD SARS-CoV-2	8	34.35	0.18	0.53	100%
3x LoD FluA	8	35.30	0.34	0.97	100%
3x LoD FluB	8	34.10	0.37	1.10	100%
3x LoD RSV	8	35.40	0.33	0.94	100%
Negative	8	-	-	-	100%

Table 23 ELITe BeGenius Intra-Session Repeatability (one day)

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD SARS-CoV-2	8	35.52	0.38	1.08	100%
3x LoD FluA	8	36.56	0.50	1.38	100%
3x LoD FluB	8	35.07	0.25	0.72	100%
3x LoD RSV	8	36.91	0.24	0.64	100%
Negative	8	-	-	-	100%

An example of Inter-Session Repeatability (on two days) results with extraction method 200_50 is shown in the tables below.

Table 24 ELITe InGenius Inter-Session Repeatability (two days)

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD SARS-CoV-2	16	34.21	0.34	1.00	100%
3x LoD FluA	16	35.10	0.44	1.25	100%
3x LoD FluB	16	34.15	0.36	1.05	100%
3x LoD RSV	16	35.49	0.35	0.99	100%
Negative	16	-	–	-	100%

Table 25 ELITe BeGenius Inter-Session Repeatability (two days)

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD SARS-CoV-2	16	35.44	0.44	1.24	100%
3x LoD FluA	16	36.50	0.39	1.07	100%
3x LoD FluB	16	35.19	0.40	1.13	100%
3x LoD RSV	16	37.05	0.60	1.61	100%
Negative	16	-	-	-	100%

In the Repeatability test, the SARS-CoV-2 PLUS ELITe MGB Kit detected all the targets as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

11.9 Reproducibility

The Inter-Batch and Inter-Instrument Reproducibility of the assay was evaluated with both extraction method on ELITe InGenius and ELITe BeGenius by analysis of a panel of nasopharyngeal swab samples negative or spiked with, SARS-CoV-2, FluA, FluB and RSV certified reference material (NIBSC and Zeptometrix).

An example of Inter-Batch Reproducibility (on two Lots) results with extraction method 200_50 is shown in the tables below.

Table 26 ELITe InGenius Inter-Batch Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD *SARS-CoV-2*	8	34.86	0.66	1.88	100%
3x LoD *FluA*	8	35.97	0.82	2.27	100%
3x LoD *FluB*	8	34.46	0.69	2.01	100%
3x LoD RSV	8	36.16	1.12	3.10	100%
Negative	8	-	-	-	100%

Table 27 ELITe BeGenius Inter-Batch Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD *SARS-CoV-2*	8	35.81	0.84	2.35	100%
3x LoD *FluA*	8	37.83	1.34	3.54	100%
3x LoD *FluB*	8	35.55	0.72	2.02	100%
3x LoD RSV	8	37.62	0.80	2.13	100%
Negative	8	-	-	-	100%

An example of Inter-Instrument Reproducibility (on two instruments) results with extraction method 200_50 is shown in the tables below.

Table 28 ELITe InGenius Inter-Instrument Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD SARS-CoV-2	8	34.79	1.40	4.01	100%
3x LoD FluA	8	35.73	0.53	1.47	100%
3x LoD FluB	8	35.19	1.19	3.39	100%
3x LoD RSV	8	35.99	1.34	3.74	100%
Negative	8	-	-	-	100%

Table 29 ELITe BeGenius Inter-Instrument Reproducibility

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD SARS-CoV-2	8	35.31	0.79	2.23	100%
3x LoD FluA	8	36.61	0.50	1.36	100%
3x LoD FluB	8	35.91	0.77	2.15	100%

Table 29 ELITe BeGenius Inter-Instrument Reproducibility (continued)

Sample	N	Mean Ct	SD	%CV	%Agreement
3x LoD RSV	8	38.17	1.63	4.28	100%
Negative	8	-	-	-	100%

In the Inter - batch and Inter - Instrument Reproducibility test, the SARS-CoV-2 PLUS ELITe MGB Kit detected all the targets as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

11.10 Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with ELITe InGenius and both extraction method, analysing clinical samples of nasopharyngeal swab collected from different subjects, certified negative for the RNA of each target.

As ELITe BeGenius has analytical performances equivalent 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 on ELITe InGenius is also applicable to ELITe BeGenius.

The results are summarized in the table below.

Table 30

Extraction method	Nasopharyngeal swab samples	N	Positive	Nega-tive	Diagnostic Specificity
200_50	SARS-CoV-2 negative	551	1	550	99.8%
	FluA negative	50	0	50	100%
	FluB negative	50	0	50	100%
	RSV negative	49	0	49	100%
200_100	SARS-CoV-2 negative	513	0	513	100%
	FluA negative	51	0	51	100%
	FluB negative	52	0	52	100%
	RSV negative	52	0	52	100%

The IC Ct cut-off is set at 35 for nasopharyngeal swab samples.

11.11 Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with ELITe InGenius and both extraction method, analysing clinical samples of nasopharyngeal swab collected from different subjects, certified positive for the RNA of the targets or spiked with reference materials.

As ELITe BeGenius has analytical performances equivalent 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 on ELITe InGenius is also applicable to ELITe BeGenius.

The results are summarized in the table below.

Table 31

Extraction method	Nasopharyngeal swab samples	N	Positive	Nega-tive	Diagnostic Sensitivity
200_50	SARS-CoV-2 positive	150	146	4	97.7%
	SARS-CoV-2 contrived	24	24	0	
	FluA positive	75	73	2	97.3%
	FluB positive	48	48	0	100%
	FluB contrived	9	9	0	
	RSV positive	72	71	1	98.6%
200_100	SARS-CoV-2 positive	108	107	1	99.2%
	SARS-CoV-2 contrived	24	24	0	
	FluA positive	67	67	0	100%
	FluB positive	38	38	0	100%
	FluB contrived	24	24	0	
	RSV positive	72	70	2	97.2%

The Diagnostic Sensitivity of the SARS-CoV-2 PLUS ELITe MGB Kit in association with nasopharyngeal swabs is equal to 97.7 % for SARS-CoV-2, 97.3 % for FluA, 100 % for FluB and 98.6 % for RSV.

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 "SARS-CoV-2 PLUS ELITe MGB Kit", FTP 180ING.

12 REFERENCES

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13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: Nasopharyngeal swabs.

Currently there are no data available concerning product performance with other clinical samples: sputum, bronco-alveolar lavage (BAL), nasopharyngeal aspirates and cell culture supernatant.

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

The results obtained with this product depend on proper identification, collection, transport storage and processing of the samples. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use provided with the product.

Owing to its high analytical sensitivity, the Real-Time PCR method used in this product is sensitive to contamination from positive clinical samples, positive controls and PCR products. Cross-contamination causes false positive results. The product format is designed to limit cross-contamination. However, cross-contamination can only be avoided by good laboratory practices and following these instructions for use.

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

This product requires the use of personal protective equipment and areas that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques such as extraction, reverse transcription, PCR and detection of nucleic acids.

It is necessary to have separate areas for the preparation of the complete reaction mixture and the extraction / amplification / detection of amplification products to prevent 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 indicates that the target RNA is not detected in the RNA extracted from the sample; however, it cannot be excluded that the target RNA has a lower titer than the product detection limit (see 11 PERFORMANCE CHARACTERISTICS page 20). In this case the result could be a false negative.

In case of co-infections, the sensitivity for one target can be affected by the amplification of a second target (see 11 PERFORMANCE CHARACTERISTICS page 20).

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.

During mix preparation, particular attention must be paid to the addition of the RT enzyme. The human RNase P gene, used as an endogenous internal control, is designed to amplify the endogenous DNA sequence present in the sample. If RT is not properly added to the complete reaction mixture, an RNase P target signal may still be generated, leading to the erroneous validation of the sample.

Possible polymorphisms, insertions or deletions within the region of the RNA targeted 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 in combination with all relevant clinical observations and laboratory results.

As with any other diagnostic medical device, there is a residual risk of obtaining invalid or erroneous results with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient. However, this residual risk associated to the intended use of the product has been weighed against the potential benefits to the patient and it has been assessed acceptable.

14 TROUBLESHOOTING

Table 32

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.
Degradation of complete reaction mixture or of its components.	Do not re-use the complete reaction mixture, prepare it freshly for each work session. Do not leave the PCR Mix 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.
Positive Control degradation.	Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use a new aliquot of Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 33

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 Negative Control	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the complete reaction mixture or of its components.	Prepare again the complete reaction mixture. Use a new aliquot of components.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit.	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 34

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting 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.

Table 34 (continued)

Invalid Sample reaction	
Possible Causes	**Solutions**
Complete reaction mixture degradation or of its components.	Do not re-use the complete reaction mixture, prepare it freshly for each work session. Do not leave the PCR Mix 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.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 35

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If significant amplification is observed in PCR plot select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: - repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a “PCR Only” session - repeat the extraction of the primary sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 36

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

15 SYMBOLS

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

16 NOTICE TO THE USERS

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

17 NOTICE TO PURCHASER: LIMITED LICENSE

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

ELITe MGB ® detection reagents are covered by one or more of U. S. Patent numbers 7319022, 7348146, 7381818, 7541454, 7671218, 7723038, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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

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

Appendix A SARS-CoV-2 PLUS ELITe MGB Kit used in association with Genius series® platforms

CAUTION

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

INTENDED USE

The product **SARS-CoV-2 PLUS ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative multiplex nucleic acids reverse transcription and Real-Time PCR assay for the detection and identification of the RNA of **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Influenza A Virus (FluA), Influenza B Virus (FluB), Respiratory Syncytial Virus (RSV)**, extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, reverse transcription, Real-Time PCR and results interpretation, using human specimens of nasopharyngeal swabs.

The product is intended for use as an aid in the screening of Severe Acute Respiratory Syndrome Coronavirus 2 infection in asymptomatic subjects, as an aid in the diagnosis of the Severe Acute Respiratory Syndrome Coronavirus 2, Influenza A Virus, Influenza B Virus, Respiratory Syncytial Virus infections in patients suspected of having a respiratory viral infection and as an aid in the monitoring of Severe Acute Respiratory Syndrome Coronavirus 2 infection in patients with confirmed SARS-CoV-2 infection.

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

Amplified sequence

Table 37

Sequence	Gene	Fluorophore	Channel
SCoV2	ORF8 and ORF1ab	FAM	SCoV2
FluA	FluA Matrix protein gene	AP639	FluA
FluB	FluB Matrix protein gene	AP593	FluB
RSV	RSV matrix protein gene	AP690	RSV
Internal Control	human RNase P	AP525	Internal Control

Validated matrix

› **Nasopharyngeal Swabs**

Kit content and related products

Table 38

SARS CoV-2 PLUS ELITe MGB Kit (RTS180ING)		SARS CoV-2 PLUS - ELITe Positive Control (CTR180ING)
 X 2	 X 1	 X 3
CoV-2 PLUS PCR Mix 2 tubes of 1200 µL 96 reactions per kit 8 freeze-thaw cycles per tube	**CoV-2 PLUS RT EnzymeMix** 1 tube of 80 µL 96 reactions per kit 8 freeze-thaw cycles per tube	**CoV-2 PLUS Positive Control** 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles
Maximum shelf-life: **18 months**		Maximum shelf-life: **24 months**
Storage temperature: **≤ -20°C**		Storage temperature: ≤ -20°C

Other products required not provided in the kit

Table 39

› ELITe InGenius instrument: INT030. › ELITe BeGenius instrument: INT040. › ELITe InGenius SP 200: INT032SP200.	ELITe InGenius and ELITe BeGenius Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use) UTM® (COPAN Italia S.p.A., ref. 360C or 305C), or an equivalent device, for nasopharyngeal swab specimens only

ELITe InGenius and ELITe BeGenius protocol

Table 40

› Sample volume › Total elution volume › PCR elution input volume › Complete PCR Mix volume	200 µL 50 or 100 µL 10 µL 20 µL	› Frequency of controls	15 days

ELITe InGenius and ELITe BeGenius Performances

Table 41

Matrix	Extraction method	Pathogen	LoD (copies / mL or IU/mL)	Diagnostic Sensitivity	Diagnostic Specificity
Nasopharyng-eal Swab	200_50	**SARS-CoV-2**	673 IU / mL	97.7%	99.8%
		FluA	189 copies / mL	97.3%	100%
		FluB	225 copies / mL	100%	100%
		RSV	184 copies / mL	98.6%	100%
	200_100	**SARS-CoV-2**	1328 IU / mL	99.2%	100%
		FluA	189 copies / mL	100%	100%
		FluB	225 copies / mL	100%	100%
		RSV	184 copies / mL	97.2%	100%

Sample preparation

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

Table 42

Sample type	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2 / +8 °C**	**-20 ±10 °C**	**-70 ±15 °C**
Nasopharyngeal Swab	UTM (COPAN)	≤ 24 hours	≤ 5 days	≤ 1 month	≤ 1 month

ELITe InGenius Procedures

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

Before analysis

Table 43

1. Switch on ELITe InGenius. Log in with username and password Select the mode “CLOSED”	**2.** Verify controls: **CoV-2 PLUS Positive Control** and **CoV-2 PLUS Negative Control** in the “Controls” menu Note: Both must have been run, approved and not expired.	**3.** Thaw the **CoV-2 PLUS PCR Mix** Vortex gently Spin down 5 sec Keep the **RT EnzymeMix** on ice

Table 44

4. Prepare the complete reaction mixture			**5.** Vortex gently
Sample Number (N)	**CoV-2 PLUS PCR Mix**	**CoV-2 PLUS RT EnzymeMix**	Spin down 5 sec Keep the complete reaction mixture in ice. Do not expose to direct light.
1 ≤ N ≤ 5	(N + 1) x 19.3 µL	(N + 1) x 0.7 µL	
6 ≤ N ≤ 12	(N + 2) x 19.3 µL	(N + 2) x 0.7 µL	

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

Table 45

1. Select “Perform Run” on the touch screen	**2.** Verify the extraction volumes: Input: “200 µL”, elution: “50 µL” or “100 µL”	**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID
4. Select the “Assay Protocol” of interest: SARS-CoV-2 PLUS ELITe _RsS_200_50 or SARS-CoV-2 PLUS ELITe_RsS_200_100	**5.** Select the method “Extract + PCR” and the sample position: Extraction Tube	**6.** Load the complete reaction mixture and the Internal Control in the Inventory Block
7. Load: PCR Cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	**8.** Close the door Start the run	**9.** View, approve and store the results

NOTE

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

Procedure 2 - PCR Only (e.g., eluates, controls)

Table 46

1 to 4: Follow the Complete Run procedure described above (select the Assay Protocols: SARS-CoV-2 PLUS_PC or SARS-CoV-2 PLUS_PC_200_100, SARS-CoV-2 PLUS ELITe_NC or SARS-CoV-2 PLUS ELITe_ NC_200_100, or SARS-CoV-2 PLUS ELITe _RsS_200_50 or SARS-CoV-2 PLUS ELITe_RsS_200_100	**5.** Select the method “PCR Only” and the sample position “Elution Tube”	**6.** Load the complete reaction mixture in the Inventory Block
7. Load: PCR Cassette rack and the Elution tube rack with the extracted nucleic acid	**8.** Close the door Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

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

Before analysis

Table 47

1. Switch on ELITe BeGenius. Log in with username and password Select the mode “CLOSED”.	**2.** Verify controls: **CoV-2 PLUS Positive Control** and **CoV-2 PLUS Negative Control** in the “Controls” menu Note: Both must have been run, approved and not expired.	**3.** Thaw the **CoV-2 PLUS PCR Mix** Vortex gently Spin down 5 sec Keep the **RT EnzymeMix** on ice.

Table 48

4. Prepare the complete reaction mixture			**5.** Vortex gently
Sample Number (N)	**CoV-2 PLUS PCR Mix**	**CoV-2 PLUS RT EnzymeMix**	Spin down 5 sec Keep the complete reaction mixture in ice. Do not expose to direct light.
1 ≤ N ≤ 5	(N + 1) x 19.3 µL	(N + 1) x 0.7 µL	
6 ≤ N ≤ 12	(N + 2) x 19.3 µL	(N + 2) x 0.7 µL	
13 ≤ N ≤ 24	(N + 4) x 19.3 µL	(N + 4) x 0.7 µL	

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

Table 49

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “50 µL” or “100 µL”
4. Select the “Assay Protocol” of interest: SARS-CoV-2 PLUS ELITe _Be_RsS_200_50 or SARS-CoV-2 PLUS ELITe_Be_RsS_200_100 **NOTE** If a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit.	**6.** Load the complete reaction mixture in Reagent Rack and insert it in the Cooler Unit.
7. Load “PCR Basket” with “PCR Cassette” and the “Extraction Basket” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

Procedure 2: PCR Only (e.g., eluates, controls)

Table 50

1. Select “Perform Run” on the touch screen and then click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “50 µL” or “100 µL”
4. Select the “Assay protocol” of interest SARS-CoV-2 PLUS_Be_PC or SARS-CoV-2 PLUS_Be_PC_200_100, SARS-CoV-2 PLUS ELITe_Be_NC or SARS-CoV-2 PLUS ELITe_Be_NC_200_100, or SARS-CoV-2 PLUS ELITe _Be_RsS_200_50 or SARS-CoV-2 PLUS ELITe_Be_RsS_200_100)	**5.** Load the complete reaction mixture in the Reagent Rack and insert it in the cooling area Load filter tips and the PCR rack	**6.** Load “PCR Basket” with “PCR Cassette”
7. Close the door. Start the run	**8.** View, approve and store the results	

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