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EMPOWERING IVD

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SARS-CoV-2 Variants ELITe MGB® Kit

reagents for RNA reverse transcription and cDNA Real Time amplification

REF RTS171ING CE IVD -20°C

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INTENDED USE

The «**SARS-CoV-2 Variants ELITe MGB® Kit**» product is part of a qualitative multiplex nucleic acids reverse transcription and amplification and melting curve analysis assay, for the detection and discrimination of the mutations E484K, E484Q and N501Y of the S gene of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in clinical samples from subjects infected by the virus.

The assay is able to detect the mutations associated with the following variants: Alpha variant (UK), lineage B.1.1.7, the Beta variant (South Africa), lineage B.1.351, the Gamma variant (Japanese), lineage P1, the Zeta variant (Brazil), lineage P2, the Eta variant (Nigeria), lineage B.1.525, and Kappa variant (India), lineage B.1617.1.

The assay is validated in association with «**ELITe InGenius®**» system and Respiratory Swab samples (nasopharyngeal, nasal or oropharyngeal swabs).

The product is intended to be used as a reflex test in already diagnosed SARS-CoV-2 positive samples, and to aid in the identification of N501Y, E484K and E484Q mutations in the SARS-Cov-2 S gene.

ASSAY PRINCIPLES

The assay consists of a multiplex reverse transcription and real-time amplification reaction (one-step method) performed by **ELITe InGenius®**, an automated and integrated system for extraction, reverse transcription, amplification, detection, melting analysis of nucleic acids and result interpretation.

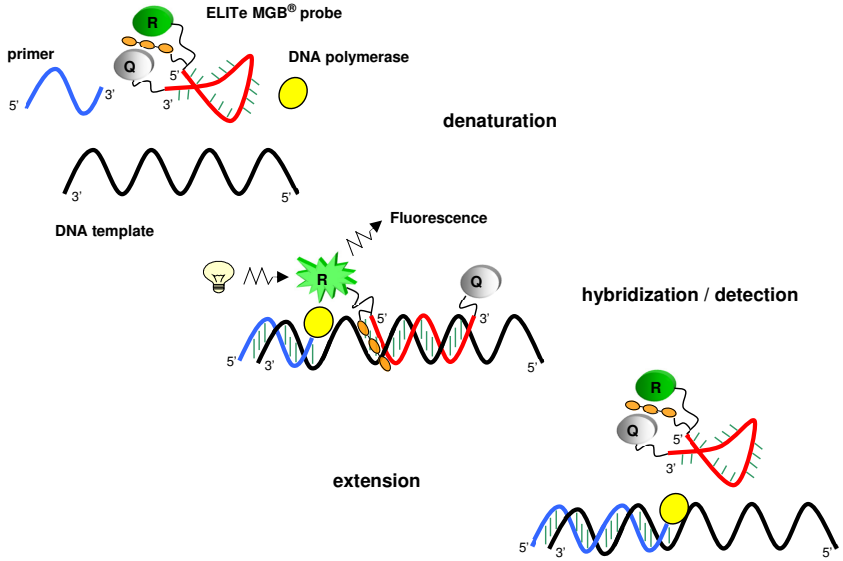
Starting from RNA extracted by **ELITe InGenius®** from sample to be tested, different reactions of reverse transcription and amplification are performed in the PCR Cassette in order to amplify a specific region of the SARS-CoV-2 S gene and to detect the following targets:

- 484 target detected by the specific probe in the **484** channel (Channel 1),
- 501 target detected by the specific probe in the **501** channel (Channel 4).

The complete reaction mixture also amplifies the cellularity, extraction and inhibition control based on human **RNase P** gene as endogenous Internal Control, detected by specific probe in the **IC** Channel (Channel 3).

The probes with ELITe MGB® technology, labelled with different fluorophores, are activated when hybridized with the specific amplification product. The fluorescence emission is measured and recorded by the instrument. At the end of amplification cycle, the dissociation analysis is carried out. The fluorescence plots are analysed to identify the threshold cycles (Ct) and the melting temperatures (Tm). The result interpretation allows to detect the presence of the mutations of interest of the SARS-CoV-2 S gene in the starting sample.

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



PRODUCT DESCRIPTION

The «**SARS-CoV-2 Variants ELITe MGB® Kit**» product provides the following components:

- **CoV-2 Variants PCR Mix**

An optimized and stabilized mixture of oligonucleotides and reagents for reverse transcription and real-time amplification, pre-aliquoted into **two test tubes** (NEUTRAL cap). Each tube contains **1200 µL** of solution, sufficient for **48 tests** (processing at least 5 samples per session) in association with **ELITe InGenius®**.

The CoV-2 Variants PCR Mix contains the specific primers for a region of SARS-CoV-2 S gene and probes for the **484** target and the **501** target:

- the probe **484** is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,
- the probe **501** is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety,

The CoV-2 Variants PCR Mix also contains the specific primers for the human **RNase P** gene as endogenous Internal Control (IC). The probe **IC** is labelled with AP525, stabilized by the MGB® group and quenched by a non-fluorescent moiety.

The CoV-2 Variants PCR Mix also provides the buffer, magnesium chloride, the nucleotide triphosphates, the stabilizers and the hot start DNA polymerase enzyme.

- **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** (processing at least 5 samples per session) in association with **ELITe InGenius®**.

The «**SARS-CoV-2 Variants ELITe MGB® Kit**» product provides components sufficient for **96 tests**, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
CoV-2 Variants PCR Mix	mixture of reagents for reverse transcription and real time amplification **NEUTRAL cap**	**2 x 1200 µL**	**-**
RT EnzymeMix	Reverse transcriptase and RNase inhibitor **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 skirted tube with 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 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., EG SpA, ref. INT030) and the following specific Assay Protocols (EG SpA) are required.

- parameters for positive control amplification «**SARS-CoV-2 VAR ELITe_PC**»,
- parameters for negative control amplification «**SARS-CoV-2 VAR ELITe_NC**»,
- parameters for respiratory swab samples «**SARS-CoV-2 VAR ELITe_RsS_200_100**».

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

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

As template of amplification Positive Control, the specifc product «**SARS-CoV-2 Variants - ELITe Positive Control**» (EG SpA, ref. CTR171ING) is required. The product provides two stabilised solutions containing plasmid DNAs.

In case of multiple testing of different target of the same sample, as template of extraction and inhibition exogenous Internal Control, the generic product «**CPE - Internal Control**» (EG SpA., ref. CTRCPE), should be used. This is a stabilised solution containing two plasmid DNAs and the genomic RNA of MS2 phage.

As collecting 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 in 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 manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to 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 for a maximum of three consecutive sessions in association with ELITe InGenius. 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 extraction products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination.

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

CoV-2 Variants PCR Mix

The **CoV-2 Variants PCR Mix** must be stored at temperature lower than -20 °C in the dark.
The **CoV-2 Variants PCR Mix** can be frozen and thawed for no more than **ten times**: further freezing / thawing cycles may cause a loss of product performances.

RT EnzymeMix

The **RT EnzymeMix** must be stored at temperature lower than -20 °C.
The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than 10 minutes during each use.
The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than **ten times**: further uses 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 Universal Transport Medium (e.g. UTM®, COPAN Italia S.p.A. or an equivalent medium) according to laboratory guidelines, transported and stored at room temperature (+18 / +25 °C) for a maximum of one day 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 Extraction 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.

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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 respiratory swab by the **ELITe InGenius** system and **ELITe InGenius Software** version 1.3.0.16 (or later), use the Assay Protocols **SARS-CoV-2 VAR ELITe_RsS_200_100**. This protocol process 200 µL of sample and elute 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 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 **SARS-CoV-2 Variants – ELITe Positive Control** reagent (not provided with this kit) in association with Assay Protocol **SARS-CoV-2 VAR ELITe_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with Assay Protocol **SARS-CoV-2 VAR ELITe_NC.**

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

- a new lot of amplification reagents is started,
- the results of Quality Control analysis (see following paragraph) are out of specification,
- any major maintenance 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. External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable.

PROCEDURE

The procedure to use the **SARS-CoV-2 Variants 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, **CoV-2 Wild type Positive Control, CoV-2 Mutant Positive Control and CoV-2 Variants Negative Control**) were run, approved and not expired (Status), in association with the amplification reagent lot to be used. If there are not amplification controls approved or valid, run 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 **SARS-CoV-2 Variants ELITe MGB Kit** are described in the table below.

Assay Protocol for SARS-CoV-2 Variants ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
SARS-CoV-2 VAR ELITe_RsS_200_100	Respiratory Swab	Positive / Negative Wild type / Mutated	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: NO 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 **SARS-CoV-2 Variants 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 "Laboratory 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. Thaw for 30 minutes at room temperature (~+25 °C) the **CoV-2 Variants PCR Mix** (NEUTRAL cap) test tubes needed for the session, remembering that the content of each test tube is enough for **48 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 ice or cool block,

Note: Thaw **CoV-2 Variants PCR Mix** in the dark because this reagent is sensitive to the light.

2. Take the **RT EnzymeMix** (cap with BLACK insert) 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 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 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,
4. 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)	CoV-2 Variants PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

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5. Prepare the **complete reaction mixture** by adding into the dedicated 2 mL tube the calculated volumes of the two components.
6. 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 used within **7 hours** if kept on board in the refrigerated block. This time allows to carry out 2 work sessions of 3 hours each and to start a third work session. The complete reaction mixture **cannot** be stored for re-use.

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

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 at room temperature (~+25 °C) the test tubes containing the samples to be analysed and handle according to laboratory guidelines and according to paragraph "Samples and Controls". Remember that 200 µL of sample are needed for the analysis.
2. If required, thaw the **CPE** tubes for the session at room temperature (~+25 °C). Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home" screen.
4. Ensure that the "Extraction Input Volume" is 200 µL and the Extracted Elute Volume is 100 µL.
5. For each Track of interest fill in the "Sample ID" (SID) by typing or by scanning the sample barcode.
6. Select the Assay Protocol to be used in the "Assay" column (e.g. SARS-CoV-2 VAR ELITe_RsS_200_100).
7. Ensure that the "Protocol" displayed is: "Extract + PCR".
8. Select the sample loading position "Extraction tube" in the "Sample Position" column. Click "Next" to continue the setup.
9. Load the CPE (if required) and the complete reaction mixture on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of **CoV-2 Variants PCR Mix**. Click "Next" button to continue the setup.
10. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
11. Load the PCR Cassettes, the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the samples to be extracted 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 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 amplification products, the extraction cartridges and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid spilling the reaction products.

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

B. Amplification run

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

1. Thaw at room temperature (~+25 °C) the test tubes containing the extracted nucleic acid samples to be analysed. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home” screen.
3. 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.
4. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
5. Select the Assay Protocol to be used in the “Assay” column (e.g. SARS-CoV-2 VAR ELITe_RsS_200_100).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure that the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **CoV-2 Variants PCR Mix**. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the **PCR Cassettes** and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue.
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 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 amplification products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid the spilling of the reaction products.

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

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 **CoV-2 Wild Type Positive Control** and the **CoV-2 Mutant Positive Control** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
2. As **Negative Control,** 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. 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.
5. In the Track of interest, select the Assay Protocol to be used in the “Assay” column.

6. For the positive control, select “SARS-CoV-2 VAR ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of **CoV-2 Wild Type Positive Control** and **CoV-2 Mutant Positive Control**.
7. For the negative control, select “SARS-CoV-2 VAR ELITe_NC” in the “Assay” column and fill in the lot number and expiry date of the molecular biology grade water.Click “Next” to continue the setup.
8. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **CoV-2 Variants PCR Mix**. Click “Next” to continue the setup.
9. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the PCR Cassettes, the CoV-2 Wild Type Positive Control and the CoV-2 Mutant Positive Control tubes and the negative control tube following the GUI instruction. Click “Next” to continue.
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 **CoV-2 Wild Type Positive Control** and **CoV-2 Mutant Positive Control** must be removed from the instrument, capped, identified and stored at -20 °C. The remaining Negative Control must be disposed.

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

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

Review and approval of results

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 “Laboratory 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 **SARS-CoV-2 VAR ELITe MGB® Kit** through the following procedure:

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

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes for 484 and 501 targets (Channels **484** and **501**) 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 “SARS-CoV-2 VAR ELITe_PC” and “SARS-CoV-2 VAR 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 calculate the "Control Charts". Four Positive Control and Negative Control results, from four different runs are requested to set up the "Control Chart". After that, the results of Positive control and Negative Control are used for 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 samples can be approved but the results are not validated. In this case, the amplification of all samples must be repeated too.

B. Validation of Sample results

The fluorescence signals emitted by the probes for 484 and 501 targets (Channels **484** and **501**) 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 "SARS-CoV-2 VAR ELITe_RsS_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
CoV-2 Wild Type Positive Control	APPROVED
CoV-2 Mutant Positive Control	APPROVED
2) Negative Control	**Status**
CoV-2 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.

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 targets are either detected or not detected.

Result of sample run	Interpretation
CoV2 484: RNA Detected. 484E wild type.	The RNA of SARS-CoV-2 S gene with codon 484E wild type was detected in the sample.
CoV2 484: RNA Detected. 484K mutant.	The RNA of SARS-CoV-2 S gene with codon 484K mutant was detected in the sample.
CoV2 484: RNA Detected. 484Q mutant.	The RNA of SARS-CoV-2 S gene with codon 484Q mutant was detected in the sample.
CoV2 484: RNA Detected.	The RNA of SARS-CoV-2 S gene was detected in the sample but the analysis of codon 484 was not feasible.
CoV2 501: RNA Detected. 501N wild type.	The RNA of SARS-CoV-2 S gene with codon 501N wild type was detected in the sample.
CoV2 501: RNA Detected. 501Y mutant.	The RNA of SARS-CoV-2 S gene with codon 501Y mutant was detected in the sample.
CoV2 501: RNA Detected.	The RNA of SARS-CoV-2 S gene was detected in the sample but the analysis of codon 501 was not feasible.
CoV2 484: RNA Not Detected or below the LoD.	The RNA of SARS-CoV-2 S gene was not detected by probe 484 in the sample.
CoV2 501: RNA Not Detected or below the LoD.	The RNA of SARS-CoV-2 S gene was not detected by probe 501 in the sample.
Invalid - Retest Sample.	Not valid assay result caused by Internal Control failure (incorrect extraction, inhibitors carry-over). The test should be repeated.

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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 reverse-transcription, amplification or extraction step (degradation or loss of RNA during the extraction or inhibitor carry-over in the eluate) or the number of cells in the sample could be not sufficient due to an incorrect sampling, which may cause incorrect results.

When the eluate volume is sufficient, the extracted sample can be retested as it or diluted, by 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 "RNA Not Detected or below LoD" are suitable for analysis but it was not possible to detect the target RNA. In this case it cannot be excluded that the target RNA is present at a concentration below the limit of detection of the assay (see "Performance characteristics").

Samples reported as "RNA Detected" without any indication about the 484 and 501 codon status are not suitable for genotyping. In this case, the target RNA was detected in the sample, but it was not possible to calculate a Tm or the calculated Tm value was out of the Tm intervals for typing. In this last case could be due to mutations different from the intended ones or to problems in the extraction step (inhibitor carry-over in the eluate).

Note: The results obtained with this assay must be interpreted taking into consideration 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 reporting

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

The "Sample Report" shows the 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

Limit of Detection (LoD)

The Limit of Detection (LoD) of SARS-CoV-2 Variants ELITe MGB Kit was defined in association with respiratory swab samples and ELITe InGenius system.

The LoD was defined by testing a panel of respiratory swabs collected in UTM (COPAN Italia S.p.A.) negative samples spiked with SARS-CoV-2 certified reference material (SARS-CoV-2, Culture Fluid, Zeptometrix) at known titre. Six levels of dilutions were prepared starting from 562 gEq / mL to 32 gEq / mL. Each dilution level was processed in 12 replicates on ELITe InGenius system in "Extract + PCR" mode. The LoD was estimated for 484 target by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call.

Then, the calculated LoD value was verified by testing 20 replicates spiked by SARS-CoV-2 reference material (SARS-CoV-2 wild type and SARS-CoV-2 Variant B.1.1.7, ZeptoMetrix) at the claimed concentrations and processed on ELITe InGenius system in "Extract + PCR" mode. The LoD was confirmed as per CLSI EP17-A guideline (at least 18 positive results out of 20 replicates).

The results are reported in the following tables.

LoD	95% confidence interval	
	Lower bound	Lower bound
470 gEq / mL	296	1156

Limit of Detection for respiratory swab samples and ELITe InGenius					
Sample	Titer	Target	N	Positive	Negative
Swabs collected in UTM	470 gEq / mL	484 target	20	18	2
		501 target	20	18	2

The LoD value for the 484 and 501 targets was confirmed at 470 gEq / mL for respiratory swabs collected in UTM (COPAN Italia S.p.A.).

Inclusivity: Efficiency of detection

The efficiency of amplification and detection for different variants of SARS-CoV-2 was evaluated by *in silico* comparison of the sequences available in the nucleotide database EpiCoV from GISAID.

The analysis of the regions chosen for the hybridization of the primers and of the fluorescent probes in the alignment of the sequences for 484 and 501 regions of SARS-CoV-2 S gene showed sequence conservation and absence of significant mutations, apart from the intended ones, and so efficient amplification and detection of the different variants of SARS-CoV-2 is expected.

The Inclusivity of the assay, as detection efficiency of different variants, was verified by testing the "SARS-CoV-2 ONBOARD Variant Swab kit" from Microbix Biosystems.

Each sample of the panel was diluted in negative UTM (COPAN Italia) and tested on ELITe InGenius system in "Extract + PCR" mode.

The results are reported in the following table.

WHO Label	Pango lineage	Pos. / Rep.	484 outcome	501 outcome
(Wild type)	(Wild type)	3 / 3	484E wild type	501N wild type
Alpha	B.1.1.7	3 / 3	484E wild type	501Y mutated
Beta	B.1.351	3 / 3	484K mutated	501Y mutated
Gamma	P.1	3 / 3	484K mutated	501Y mutated

All the tested SARS-CoV-2 variants were correctly detected as positive and the status of 484 and 501 codons were correctly discriminated when tested by SARS-CoV-2 Variants 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 unintended 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 from ATCC and ZeptoMetrix were analyzed at high concentration in three replicates in association with ELITe InGenius system in "PCR Only" mode. The DNAs and RNAs of each organism were added with 0,4 ng per reaction of human total RNA (InVivoScribe) and 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.

Sample	Outcome
Influenza A virus (H1N1)	No cross-reactivity
Influenza A virus (Hipdm09)	No cross-reactivity
Influenza A virus (H3N2)	No cross-reactivity
Influenza B virus (Florida)	No cross-reactivity
Parainfluenza Virus 1	No cross-reactivity
Parainfluenza Virus 2	No cross-reactivity
Parainfluenza Virus 3	No cross-reactivity
hMPV	No cross-reactivity
Human Coronavirus OC43	No cross-reactivity
Human Coronavirus 229E	No cross-reactivity
SARS-CoV	No cross-reactivity
RSV A	No cross-reactivity
RSV B	No cross-reactivity
Echovirus 4	No cross-reactivity
Rhinovirus 1A	No cross-reactivity
ADV	No cross-reactivity
CMV	No cross-reactivity
Bordetella pertussis	No cross-reactivity
Bordetella parapertussis	No cross-reactivity
Staphylococcus aureus	No cross-reactivity
Haemophilus influenzae	No cross-reactivity
Streptococcus pneumoniae	No cross-reactivity
Legionella pneumophila	No cross-reactivity
Mycoplasma pneumoniae	No cross-reactivity
Chlamydophila pneumoniae	No cross-reactivity
Mycobacterium tuberculosis	No cross-reactivity
Escherichia coli	No cross-reactivity
Aspergillus spp	No cross-reactivity
Candida albicans	No cross-reactivity
Pneumocystis jiroveci	No cross-reactivity

All the tested potential interfering markers showed no cross-reactivity for the 484 and 501 targets detection and typing when tested by SARS-CoV-2 Variants ELITe MGB Kit.

Potential interfering markers: Interference

The absence of interference by other organisms that can be found in clinical samples of respiratory swabs was verified by testing a panel of certified reference materials.

Samples of genomic DNA or RNA from different potentially interfering markers (ATCC and ZeptoMetrix) at high concentration were spiked with SARS-CoV-2 certified reference material (bei Resources) at low concentration (about 30 gEq / reaction). The samples were analyzed in three replicates in association with ELITe InGenius system in "PCR Only" mode. Each sample were added with 0,4 ng per reaction of human total RNA (InVivoScribe) and 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.

Sample	Outcome
Influenza A virus (H1N1)	No interference
Influenza A virus (Hipdm09)	No interference
Influenza A virus (H3N2)	No interference
Influenza B virus (Florida)	No interference
Parainfluenza Virus 1	No interference
Parainfluenza Virus 2	No interference
Parainfluenza Virus 3	No interference
hMPV	No interference
Human Coronavirus OC43	No interference
Human Coronavirus 229E	No interference
SARS-CoV	No interference
RSV A	No interference
RSV B	No interference
Echovirus 4	No interference
Rhinovirus 1A	No interference
ADV	No interference
CMV	No interference
Bordetella pertussis	No interference
Bordetella parapertussis	No interference
Staphylococcus aureus	No interference
Haemophilus influenzae	No interference
Streptococcus pneumoniae	No interference
Legionella pneumophila	No interference
Mycoplasma pneumoniae	No interference
Chlamydophila pneumoniae	No interference
Mycobacterium tuberculosis	No interference
Escherichia coli	No interference
Aspergillus spp	No interference
Candida albicans	No interference
Pneumocystis jiroveci	No interference

All the tested potential interfering organisms showed no significant impact on the 484 and 501 targets detection and typing when tested by SARS-CoV-2 Variants ELITe MGB Kit.

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Potential interfering substances

A panel of potentially interfering substances at relevant concentrations was tested with the product SARS-CoV-2 Variants ELITe MGB Kit.

The substances were individually added to respiratory swab samples spiked by SARS-CoV-2 Variant B.1.1.7 reference material (ZeptoMetrix) at concentration of about 3x LoD. Samples were processed in three replicates on ELITe InGenius system in "Extract + PCR" mode. The Ct values of the 484 and 501 targets and of the Internal Control (reference and test samples) were used to calculate the percentage Coefficient of Variability (%CV) in order to evaluate the possible interference. The same analysis was also carried out for Tm values of the 484 and 501 targets.

The results are reported in the following table.

Sample	484 Ct %CV	501 Ct %CV	IC Ct %CV	484 Tm %CV	501 Tm %CV
Whole blood	2.28	2.62	2.08	0.12	0.09
Mucin	1.52	2.40	2.20	0.08	0.13
Azithromycin	2.02	2.29	0.98	0.16	0.09
Ambroxol	1.83	2.16	1.65	0.06	0.12
Beclometasone	2.83	3.24	0.77	0.11	0.33
Ebastine	1.83	2.58	0.65	0.06	0.09
CPE – Internal Control	1.33	1.46	0.70	0.09	0.12

All the samples resulted correctly detected and typed for the 484 and 501 targets. The percentage %CV of Ct values were lower than 3.5% for Ct values and 0,5% for Tm values.

Inter-Session Repeatability

The Inter-Session Repeatability of results obtained by the product SARS-CoV-2 Variants ELITe MGB Kit in association with the ELITe InGenius system was tested by analyzing a panel of respiratory swab samples. The panel included one negative sample and two samples spiked by SARS-CoV-2 Variant B.1.1.7 reference material (ZeptoMetrix) at concentration of 3x LoD (1,410 gEq / mL) and 5x LoD (2,350 gEq / mL).

The Inter-Session Repeatability was evaluated through the analysis by the same operator of the samples in four replicates, in two runs per day, on two days, using the same lot of product and the same instrument. Samples were processed on ELITe InGenius system in "Extract + PCR" mode. The Ct values of the 484 and 501 targets and of the Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision. The same analysis was also carried out for Tm values of the 484 and 501 targets.

A summary of results is shown in the table below.

484 target				
Sample	**Pos. / Rep.**	**Ct %CV**	**Typing**	**Tm % CV**
3x LoD	16 / 16	2.57	484E	0.29
5x LoD	16 / 16	2.49	484E	0.09
Negative	0 / 16	-	-	-
501 target				
Sample	**Pos. / Rep.**	**Ct %CV**	**Typing**	**Tm %CV**
3x LoD	16 / 16	2.78	501Y	0.21
5x LoD	16 / 16	3.01	501Y	0.17
Negative	0 / 16	-	-	-
Internal Control				
Sample	**Valid / Rep.**	**Ct %CV**		
Whole panel	48 / 48	1.42		

All the samples resulted correctly detected and typed for the 484 and 501 targets. The percentage %CV of Ct values were lower than 3.5% for Ct values and 0,3% for Tm values.

Inter-Batch Reproducibility

The Inter-Batch Reproducibility of results obtained by the product SARS-CoV-2 Variants ELITe MGB Kit in association with the ELITe InGenius system was tested by analyzing a panel of respiratory swab samples. The panel included one negative sample and two samples spiked by SARS-CoV-2 Variant B.1.1.7 reference material (ZeptoMetrix) at concentration of 3x LoD (1,410 gEq / mL) and 5x LoD (2,350 gEq / mL).

The Inter-Batch Reproducibility was evaluated through the analysis of the samples in four replicates, in two runs per day on two days. Three different lots of product were used in six different days, on one instrument by one operator. Samples were processed on ELITe InGenius system in "Extract + PCR" mode. The Ct values of the 484 and 501 targets and of the Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision. The same analysis was also carried out for Tm values of the 484 and 501 targets.

A summary of results is shown in the table below.

484 target				
Sample	**Pos. / Rep.**	**Ct %CV**	**Typing**	**Tm % CV**
3x LoD	48 / 48	2.48	484E	0.25
5x LoD	48 / 48	2.05	484E	0.13
Negative	0 / 48	-	-	-
501 target				
Sample	**Pos. / Rep.**	**Ct %CV**	**Typing**	**Tm %CV**
3x LoD	48 / 48	2.61	501Y	0.19
5x LoD	48 / 48	2.85	501Y	0.21
Negative	0 / 48	-	-	-
Internal Control				
Sample	**Valid / Rep.**	**Ct %CV**		
Whole panel	144 / 144	1.61		

All the samples resulted correctly detected and typed for the 484 and 501 targets. The percentage %CV of Ct values were lower than 3% for Ct values and 0,3% for Tm values.

Inter-Instrument Reproducibility

The Inter-Instrument Reproducibility of results obtained by the product SARS-CoV-2 Variants ELITe MGB Kit in association with the ELITe InGenius system was tested by analyzing a panel of respiratory swab samples. The panel included one negative sample and two samples spiked by SARS-CoV-2 Variant B.1.1.7 reference material (ZeptoMetrix) at concentration of 3x LoD (1,410 gEq / mL) and 5x LoD (2,350 gEq / mL).

The Inter-Instrument Reproducibility was evaluated through the analysis of the samples in four replicates, in one run per day, on two days. Three different lots of product were used in six 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 484 and 501 targets and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision. The same analysis was also carried out for Tm values of the 484 and 501 targets.

A summary of results is shown in the table below.

484 target				
Sample	**Pos. / Rep.**	**Ct %CV**	**Typing**	**Tm % CV**
3x LoD	24 / 24	1.88	484E	0.16
5x LoD	24 / 24	2.11	484E	0.14
Negative	0 / 24	-	-	-
501 target				
Sample	**Pos. / Rep.**	**Ct %CV**	**Typing**	**Tm %CV**
3x LoD	24 / 24	2.29	501Y	0.21
5x LoD	24 / 24	2.27	501Y	0.24
Negative	0 / 24	-	-	-
Internal Control				
Sample	**Valid / Rep.**	**Ct %CV**		
Whole panel	72 / 72	1.73		

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All the samples resulted correctly detected and typed for the 484 and 501 targets. The percentage %CV of Ct values were lower than 2.5% for Ct values and 0,3% for Tm values.

Tets of Proficiency panel

The product SARS-CoV-2 Variants ELITe MGB Kit in association with the ELITe InGenius system was tested by analyzing the proficiency panel "QCMD 2020 Coronavirus Outbreak Preparedness (CVOP) EQA Pilot Study" (QCMD, UK). The panel mimic samples of respiratory swab in transport medium and included SARS-CoV-2 negative samples, samples with interfering organisms and SARS-CoV-2 positive samples.

The samples were processed on ELITe InGenius system in "Extract + PCR" mode. The Ct and Tm values of the 484 and 501 targets were analyzed.

A summary of results is shown in the table below.

Sample ID	Content	Titre (Log c./mL)	484 Ct	484 Tm	501 Ct	501 Tm	Outcome
CPO20S-01	SARS-CoV-2	4.30	31.45	66.4	34.67	56.6	Positive 484 wt / 501 wt
CPO20S-02	Coronavirus NL63	4.64	Undet.	n.a.	Undet.	n.a.	Negative
CPO20S-03	SARS-CoV-2	3.30	34.49	66.7	38.46	56.6	Positive 484 wt / 501 wt
CPO20S-04	Coronavirus OC43	4.03	Undet.	n.a.	Undet.	n.a.	Negative
CPO20S-05	Negative sample	-	Undet.	n.a.	Undet.	n.a.	Negative
CPO20S-06	SARS-CoV-2	4.30	31.86	66.4	34.96	56.6	Positive 484 wt / 501 wt
CPO20S-07	SARS-CoV-2	5.30	28.47	66.6	31.10	56.7	Positive 484 wt / 501 wt
CPO20S-08	SARS-CoV-2	2.30	38.57	67.5	Undet.	n.a.	Positive 484 wt / 501 n.a.

All the samples resulted correctly detected as positive or negative. Th positive sample were typed for the 484 and 501 targets as expected. For sample CPO20S-08, due to the low titre (~200 c. / mL) the 501 target typing was not feasible.

Diagnostic specificity: confirmation of SARS-CoV-2 wild type (484E and 501N) samples

The Diagnostic specificity of the assay, as confirmation of SARS-CoV-2 wild type (484E and 501N) clinical samples, was evaluated by analyzing clinical samples of respiratory swab collected in UTM (COPAN Italia), certified SARS-CoV-2 negative by a CE IVD marked assay and spiked by SARS-CoV-2 Wild Type reference material from ZeptoMetrix (isolate USA-WA1/2020).

The samples were processed on ELITe InGenius system in "Extract + PCR" mode. The Ct and Tm values of the 484 and 501 targets were analyzed.

The results are summarized in the following table.

Wild Type Samples	N	Wild type 484E	Mutant 484K	Mutant 484Q	Negative 484	Invalid
Negative Respiratory Swab samples spiked by WT SARS-CoV-2	48	48	0	0	0	0

Wild Type Samples	N	Wild type 501N	Mutant 501Y	Negative 501	Invalid
Negative Respiratory Swab samples spiked by WT SARS-CoV-2	48	48	0	0	0

All the samples resulted positive for 484 and 501 targets and were detected as Wild Type 484E and 501N. In this test, the assay diagnostic specificity was equal to 100 %.

Diagnostic sensitivity: confirmation of SARS-CoV-2 mutant (E484K, E484Q or N501Y) samples

The Diagnostic sensitivity of the assay, as confirmation of SARS-CoV-2 mutant (E484K, E484Q or N501Y) clinical samples, was evaluated by analyzing clinical samples of respiratory swab collected in UTM (COPAN Italia), certified SARS-CoV-2 negative by a CE IVD marked assay and spiked by:

- Beta Variant SARS-CoV-2 (E484K and N501Y) reference material from ZeptoMetrix (South Africa/KRISP-K005325/2020),
- plasmid DNA with amplified region of the SARS-CoV-2 S gene with mutation E484Q, from SARS-CoV-2 Kappa Variant (Indian), and mutation N501Y,
- Alfa Variant SARS-CoV-2 (484E and N501Y) reference material from ZeptoMetrix (isolate England/204820464/2020).

The samples were processed on ELITe InGenius system in “Extract + PCR” mode. The Ct and Tm values of the 484 and 501 targets were analyzed.

The results are summarized in the following table.

E484K Samples	N	Wild type 484E	Mutant 484K	Mutant 484Q	Negative 484	Invalid
Negative Respiratory Swab samples spiked by Beta Variant SARS-CoV-2	48	1	47	0	0	0

All samples resulted positive for 484 and 501 targets at first test. Forty-seven (47) out of 48 samples were detected as mutant E484K. One resulted Wild Type for position 484 of the S gene (484E) probably due to an error during spiking procedure. In this test, the assay Diagnostic sensitivity for E484K mutation was equal to 97.6%.

E484Q Samples	N	Wild type 484E	Mutant 484K	Mutant 484Q	Negative 484	Invalid
Negative Respiratory Swab samples spiked by plasmid DNA	45	0	0	45	0	0

All samples resulted positive for 484 and 501 targets at first test and were detected as mutant E484Q. In this test, the assay Diagnostic sensitivity for E484Q mutation was equal to 100%.

N501Y Samples	N	Wild type 501N	Mutant 501Y	Negative 501	Invalid
Negative Respiratory Swab samples spiked with Alfa Variant SARS-CoV-2, Beta Variant SARS-CoV-2 or plasmid DNA	96	0	96	0	0

All samples resulted positive for 484 and 501 targets at first test and were detected as mutant N501Y. In this test, the assay Diagnostic sensitivity for N501Y mutation was equal to 100%.

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 Variants ELITe MGB Kit", FTP 171ING.

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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 (nasopharyngeal, nasal or oropharyngeal swabs).

At the moment, there are no data available concerning product performance with the following clinical samples: saliva, broncho-alveolar lavage (BAL), sputum, nasopharyngeal aspirates, cell culture supernatant.

Do not use this product with quantity of extracted 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.

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 separate areas for the molecular biology test and the microbiological culture test to avoid false positive results.

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

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

It is necessary to have separate areas for the 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 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.

Research in the scientific literature do not provide exhaustive information about the performance of the SARS-CoV-2 molecular analysis on the paediatric population.

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

Possible polymorphisms, insertions or deletions within the region of the 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.	Do not use the complete reaction mixture for more than three sessions (7 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.	Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area). 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.
Incorrect session setup on ELITe InGenius	Check the position of reaction mixture or negative control. Check the volumes of reaction mixture or negative control.
Error while setting the instrument	Check the position settings of the samples, negative controls, standards on the instrument
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.
Contamination of the extraction / preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

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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.

Invalid Sample reaction	
Possible Causes	**Solutions**
Session 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.
Complete reaction mixture degradation or of its components.	Do not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). 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.
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.	Use a new aliquot of sample.
Instrument error.	Contact ELITechGroup Technical Service.

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

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 anomalous plot shape.	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.

SARS-CoV-2 Variants ELITe MGB® Kit
reagents for RNA reverse transcription and cDNA Real Time amplification

REF RTS171ING

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.

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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SARS-CoV-2 Variants ELITe MGB® KIT (Ref. RTS171ING)
SARS-CoV-2 Variants - ELITe Positive Control (Ref. CTR171ING)
used in association with ELITe InGenius®

ELITechGroup
EMPOWERING IVD

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

A. Intended use

The «SARS-CoV-2 Variants ELITe MGB® Kit» product is part of a qualitative multiplex nucleic acids reverse transcription and amplification and melting curve analysis assay, for the detection and discrimination of the mutations E484K, E484Q and N501Y of the S gene of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in clinical samples from subjects infected by the virus.
The assay is able to detect the mutations associated with the following variants: Alpha variant (UK), lineage B.1.1.7, the Beta variant (South Africa), lineage B.1.351, the Gamma variant (Japanese), lineage P1, the Zeta variant (Brazil), lineage P2, the Eta variants (Nigeria), lineage B.1.525 and Kappa variant (India), lineage B.1617.1.
The assay is validated in association with «ELITe InGenius®» system and Respiratory Swab samples (nasopharyngeal, nasal or oropharyngeal swabs).
The product is used as a reflex test to identify the possible presence of the N501Y, E484K and E484Q mutations of the SARS-Cov-2 S gene, in samples already diagnosed as positive for SARS-CoV-2.

B. Amplified sequences

Target	Gene	Fluorophore	Channel
484	S gene, 484 target	FAM	484 (Ch1)
501	S gene, 501 target	AP593	501 (Ch4)
Internal Control (endogenous)	RNase P gene	AP525	IC (Ch3)

C. Validated Matrixes

› **Respiratory Swabs**

Note: Transfer 200 µL of sample from the Swab tube into the extraction tube. Do not insert the primary tube directly into the instrument to perform the run.

D. Tube type collection

Copan Ref.	Description
360C or 305C	UTM kit

E. SARS-CoV-2 Variants ELITe MGB® KIT (RTS171ING) content

CoV-2 Variants PCR Mix (Neutral cap)	RT EnzymeMix (Black cap)	
 X 2	 X 2	Maximum shelf-life: 12 Months
2 tubes of 1200 µL 96 reactions per kit 10 freeze-thaw cycles	2 tubes of 20 µL 96 reactions per kit 10 freeze-thaw cycles	Storage temp.: below -20 °C Prepare the complete reaction mixture in a 2 mL Sarstedt tube (Ref. 72694005)

F. SARS-CoV-2 Variants - ELITe Positive Control (CTR171ING) content

CoV-2 Wild Type Positive Control (black cap)	CoV-2 Mutant Positive Control (red cap)	
 X 2	 X 2	Maximum Shelf-life: 24 Months
2 tubes of 160 µL 4 sessions / tube 4 freeze-thaw cycles	2 tubes of 160 µL 4 sessions / tube 4 freeze-thaw cycles	Storage temp.: below -20 °C

G. Material required not provided in the kit

› ELITe InGenius instrument:	INT030
› ELITe InGenius SP200 Extraction Kit:	INT032SP200
› ELITe InGenius PCR Cassette:	INT035PCR
› ELITe InGenius SP200 Consumable Set:	INT032CS
› ELITe InGenius Waste Box:	F2102-000
› 300 µL Filter Tips Axygen:	TF-350-L-R-S

H. ELITe InGenius protocol

Protocol	Volume
Sample	200 µL
Total eluate	100 µL
PCR eluate input	10 µL
Complete PCR Mix	20 µL
Control Frequency	15 days

I. Result Interpretation

Sample results:

Result of sample run	Interpretation
CoV2 484: RNA Detected. 484E wild type.	The RNA of SARS-CoV-2 484E wild type was detected in the sample
CoV2 484: RNA Detected. 484K mutant.	The RNA of SARS-CoV-2 484K mutant was detected in the sample
CoV2 484: RNA Detected. 484Q mutant.	The RNA of SARS-CoV-2 484Q mutant was detected in the sample
CoV2 484: RNA Detected.	The RNA of SARS-CoV-2 was detected in the sample, but 484 typing was not feasible
CoV2 501: RNA Detected. 501N wild type.	The RNA of SARS-CoV-2 501N wild type was detected in the sample
CoV2 501: RNA Detected. 501Y mutant.	The RNA of SARS-CoV-2 501Y mutant was detected in the sample
CoV2 501: RNA Detected.	The RNA of SARS-CoV-2 was detected in the sample, but 501 typing was not feasible
CoV2 484: RNA Not Detected or below the LoD	The RNA of SARS-CoV-2 484 was not detected in the sample
CoV2 501: RNA Not Detected or below the LoD	The RNA of SARS-CoV-2 501 was not detected in the sample
Invalid – Retest sample.	Not valid result caused by Internal Control failure

Sample Ct and Tm range:

Ct 484	Tm 484	Ct 501	Tm 501	Ct RNase P	Result
Det.	65.5 - 68.5	-	-	+/-	SARS-CoV-2 484E wild type
Det.	59.4 - 61.7	-	-	+/-	SARS-CoV-2 mutation E484K detected
Det.	57.1 - 59.3	-	-	+/-	SARS-CoV-2 mutation E484Q detected
-	-	Det.	55.6 - 58.6	+/-	SARS-CoV-2 501N wild type
-	-	Det.	62.6 - 65.6	+/-	SARS-CoV-2 mutation N501Y detected
Undet.	-	Undet.	-	Ct > 35	Invalid

Positive Control Ct and Tm limits:

Controls	Target	Ct	Tm	Result
CoV-2 Wild Type Positive Control	484E WT	Ct < 35	65.5 - 68.5	Positive Control Valid
	501N WT	Ct < 38	55.6 - 58.6	
CoV-2 Mutant Positive Control	484K MUT	Ct < 37	59.4 - 61.7	Positive Control Valid
	501Y MUT	Ct < 36	62.6 - 65.6	
Negative Control	484	Ct > 45	-	Negative Control Valid
	501	Ct > 45	-	

J. Samples

1. Sample inactivation is not required as shown in WHO guideline. The sample can be pretreated with a denaturant solution under a Biosafety cabinet of class II (BSC2). In case of this procedure, dispense 200 µL of sample into the tubes and no more than 200 µL of buffer containing denaturants. Note: This procedure is an off-label protocol and needs to be validated before use as per WHO guidelines
2. Handle and dispose of all biological samples as if they were able to transmit infectious agents even if the sample is inactivated.
3. Eluates obtained with extraction in association with SARS-CoV-2 ELITe MGB KIT (Ref. RTS170ING) or SARS-CoV-2 PLUS ELITe MGB KIT (Ref. RTS180ING) can be used.
4. High-medium viral titre for SARS- CoV-2 allows to obtain good results using this reflex kit.

K. References

- ECDC Reference: *"Rapid increase of a SARS-CoV-2 variant with multiple spike protein mutations observed in the United Kingdom"* December 2020

L. Procedures

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

Before analysis

1. Switch on ELITe InGenius Login with username and password Select the mode "Closed"	**2.** Verify controls in the "Control menu": CoV-2 Wild Type Positive Control, CoV-2 Mutant Positive Control, negative control **Note:** All had to be run, approved and not expired	**3.** Thaw the CoV-2 VAR PCR Mix and RT Enzyme Mix tubes Mix gently and spin down 5 sec Reconstitute the complete reaction mixture in a 2 mL Sarstedt tube (Ref. 72694005) as shown in the table below

Complete reaction mixture preparation:

Reagent	1 Reaction
CoV-2 Variants PCR Mix	20 µL
RT Enzyme Mix	0.3 µL

Samples N	Total reactions
1 to 5	N + 1
6 to 11	N + 2
12	N + 2.5

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", Elute: "100 µL"	**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID
4. Select the "Assay protocol" of interest	**5.** Select the sample position: "Extraction tube" **Note:** 200 µL of sample must be transferred into Extraction tube For samples see **C** and **D**	**6.** Load the complete reaction mixture in the inventory block
7. Load the PCR cassette, Extraction cartridge, Elution tube, Tip and Extraction tube racks	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 2 - PCR only

1 to 4: Follow the Complete Run procedure described above	**5.** Select the mode: "PCR only" Set the sample position: "Elution tube"	**6.** Load the extracted nucleic acid tubes in the Elution tubes rack
7. Load the complete reaction mixture in the inventory block Load the PCR cassette, Elution tube, Tip racks	**8.** Close the door Start the run	**9.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4: Follow the Complete Run procedure described above	**5.** Select the mode: "Extraction Only" Set the sample position: "Extraction tube" **Note:** 200 µL of sample must be transferred into Extraction tube For samples see **C** and **D**	**6.** Load the Extraction cartridge, Elution tube, Tip and Extraction tube racks.
7. Close the door Start the run	**8.** Archive the eluted samples	