Instructions for use

Coagulation ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTSD00ING

UDI 08033891486204

CE 0123

IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
09–R	Correction of a typo in the repeatability and reproducibility test tables and in the introductory text to the tests: the G1691A mutation was incorrectly reported as associated with FII instead of FV, reversing it with the G20210A mutation.	09/03/26
08–R	Change in RTSD00ING product for the detection as singleplex for analytes FII, FV, MTHFR and duplex for FII-FV. Extension to 60 days of the use from first opening. New graphics and content setting of the IFU.	27/08/25
07–R	Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements . Composition and PERFORMANCE CHARACTERISTICS of the product remain unchanged.	20/08/24
05	Extension of use on the ELITe BeGenius instrument. Description of IC cut off value already adopted in the Assay protocol of the product (section “Diagnostic agreement: confirmation of certified sample genotype”)	30/03/23
04	Update of IC Ct value and Tm range of Factor II and Factor V	24/05/22
03	provided more permissive samples storage conditions, in line with available literature and not affecting the safety and performance of the product.	09/03/22
00–02	new product development and succeeding changes	—

TABLE OF CONTENT

1 INTENDED USE

The product **Coagulation ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative nucleic acids Real-Time PCR assay for the allelic discrimination of the following three loci:

- coagulation Factor V gene, single nucleotide polymorphism (SNP) G1691A (Leiden),
- coagulation Factor II gene, SNP G20210A,
- 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, SNP C677T.

in singleplex, duplex (FII and FV) or triplex (FII, FV and MHTFR) in human genomic DNA samples extracted from clinical specimens.

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

The product is intended for use as an aid in assessing the risk of deep vein thrombosis in patients suspected of having coagulation disorders and at risk of deep vein thrombosis.

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

2 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR for the allelic discrimination of coagulation Factor V gene, single nucleotide polymorphism (SNP) G1691A (Leiden), coagulation Factor II gene, SNP G20210A, and 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, SNP C677T, in DNA isolated from specimens and amplified using the assay reagent **52M PCR Mix**, that contains primers and probes with ELITe MGB technology.

The ELITe MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycles (Ct). At the end of amplification cycle, the analysis of the melting curve allows to identify the probes melting temperatures and to detect the presence of wildtype and/or mutated alleles.

In the ELITe MGB probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore.

Note the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

The **Coagulation ELITe MGB Kit** provides the assay reagent **52M PCR Mix,** an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- the coagulation Factor V gene, SNP G1691A (Leiden) region, detected in Channel **FV**; the probe is stabilized by MGB®, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor® 639 (AP639) dye,
- the coagulation Factor II gene, SNP G20210A region. detected in Channel **FII**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by FAM dye,
- the MTHFR gene, SNP C677T region, detected in Channel **MTHFR**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor® 593 (AP593) dye,
- Internal Control (**IC**), based on the human **beta globin** gene sequence, detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labelled by AquaPhluor 525 (AP525) dye.

The **52M PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

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

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
52M PCR Mix ref. RTSD00ING	Mixture of reagents for Real-Time PCR in tube with **NATURAL** cap	**8 x 280 µL**	-

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

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

6 OTHER PRODUCTS REQUIRED

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

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

Table 2

Instruments and software	Products and reagents
ELITe InGenius (ELITechGroup S. p. A., EG SpA, ref. INT030) **ELITe InGenius Software** version 1.3.0.19 (or later) **52M ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **52M ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **52M ELITe_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_FII_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_FV_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_MTHFR_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_FVFII_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis	**Coagulation - ELITe Positive Control** (EG SpA, ref. CTRD00ING) **ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **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) **52M ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **52M ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **52M ELITe_Be_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_FII_Be_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_FV_Be_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_MTHFR_Be_WB_200_200**, Assay Protocol with parameters for Whole Blood specimen analysis **52M ELITe_FVFII_Be_WB_200_200**, Assay Protocol with parameters for Whole Blood 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 to prevent dispersion into the environment and to avoid contamination of the instrument's working area.

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

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
52M PCR Mix	-20°C or below (protected from light)	60 days	up to seven	up to seven separate sessions* of about three hours each or up to 7 consecutive hours (2 sessions of about 3 hours each and the time needed to start a third session)

**with intermediate freezing*

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 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Whole Blood	EDTA	≤ 3 d	≤ 7 d	≤ 30 d	≤ 1 year

EDTA, Ethylenediaminetetraacetic acid; d, days.

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

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

Table 5 Assay Protocols for Coagulation ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
Whole Blood in EDTA	**ELITe InGenius**	**52M ELITe_WB_200_200** **52M ELITe_FII_WB_200_200** **52M ELITe_FV_WB_200_200** **52M ELITe_MTHFR_WB_200_200** **52M ELITe_FVFII_WB_200_200**	Wild-type / heterozy-gous / homozy-gous	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**52M ELITe_Be_WB_200_200** **52M ELITe_FII_Be_WB_200_200** **52M ELITe_FV_Be_WB_200_200** **52M ELITe_MTHFR_Be_WB_200_200** **52M ELITe_FVFII_Be_WB_200_200**	Wild-type / heterozy-gous / homozy-gous	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

NOTE

Verify if the primary tube and the volume of the sample are compatible with ELITe InGenius or ELITe BeGenius, following the Instruction for use of the extraction kit **ELITeInGeniusSP200** (EG SpA, ref. INT032SP200).

The volume of the sample in a primary tube varies according to the type of the tube loaded. Refer to the instructions for use of the extraction kit for more information on how to set up and perform the extraction procedure.

If required, 200 of sample must be transferred into an Extraction tube (for ELITe InGenius) or into a 2 mL Sarstedt Tube (for ELITe BeGeniuss).

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 18 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 **Coagulation - ELITe Positive Control** (not provided with this kit) with the **52M ELITe _PC** or **52M ELITe _Be_PC** Assay Protocols.
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **52M ELITe _NC** or **52M ELITe _Be_NC** Assay Protocols.

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 **Coagulation ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

Table 6

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) 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 (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- 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 “Specimens and Controls”)

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 **Coagulation 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:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **12 tests** in optimized conditions (2 or more tests per session).Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

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

To set up one of the 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. If required, transfer **200 µL of sample** 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.) **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.
2	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.
3	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 200 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 200 µL.	Ensure the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 200 µL.
4	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	Not applicable
5	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”)	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”)	**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.
6	Ensure the “Protocol” displayed is: “Extract + PCR”.	Select “PCR Only” in the “Protocol” column.	Ensure “PCR Only” is selected in the “Protocol” column.

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
7	Select the sample loading position as “Extraction Tube” or “Primary 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)”.
8	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
9	**Load PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.
10	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
11	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.
12	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
13	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**Load** PCR Cassette and Elution tubes with samples extracted	**Load** PCR Cassette, Positive Control and Negative Control tubes.
14	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
15	Close the instrument door.	Close the instrument door.	Close the instrument door.
16	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 ±10 °C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

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

NOTE

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

NOTE

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

9.3 STEP 3 - Review and approval of results

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

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

NOTE

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

The **ELITe InGenius** generates results with the **Coagulation 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 reaction with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct and Tm 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 **FV, FII, MTHFR**) and the Internal Control (channel IC) with the **52M ELITe_WB_200_200, 52M ELITe_FII_WB_200_200, 52M ELITe_FV_WB_200_200, 52M ELITe_MTHFR_WB_200_200** or **52M ELITe_FVFII_WB_200_200** 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
52M Positive Control	APPROVED
2) Negative Control	**Status**
52M 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 target alleles are discriminated, based on the selected Assay Protocol (singleplex, duplex or triplex).

Table 9

Result of sample run	Interpretation
FV:Wild-type for Factor V	Sample has a **wildtype** genotype for Factor V SNP G1691A locus.
FV:Heterozygous for Factor V Leiden	Sample has a **mutated (Leiden) heterozygous** genotype for Factor V SNP G1691A locus.
FV:Homozygous for Factor V Leiden	Sample has a **mutated (Leiden) homozygous** genotype for Factor V SNP G1691A locus.
FII:Wild-type for Factor II	Sample has a **wildtype** genotype for Factor II SNP G20210A locus.
FII:Heterozygous for Factor II 20210A	Sample has a **mutated (20210A) heterozygous** genotype for Factor II SNP G20210A locus.
FII:Homozygous for Factor II 20210A	Sample has a **mutated (20210A) homozygous** genotype for Factor II SNP G20210A locus.
MTHFR:Wild-type for MTHFR	Sample has a **wildtype** genotype for MTHFR SNP C677T locus.
MTHFR:Heterozygous for MTHFR 677T	Sample has a **mutated (677T) heterozygous** genotype for MTHFR SNP C677T locus.
MTHFR:Homozygous for MTHFR 677T	Sample has a **mutated (677T) homozygous** genotype for MTHFR SNP C677T locus.
IC:Valid Sample	Sample has a Ct value of the IC of less than 26.5 and is valid
Inconclusive-Retest Sample	**Inconclusive** assay result due to a sample problem.
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 “Inconclusive–Retest Sample” are not suitable for result interpretation, which it is not been possible to detect the melting temperature (Tm) of wildtype and mutated alleles. In this case, the dissociation curve analysis was not efficiently carried out due to problems with sample (inhibitors carry-over in the eluate or presence of other interfering SNPs), which may cause incorrect results.

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, extraction or PCR steps (e. g., incorrect sampling, degradation or loss of DNA during the extraction or inhibitors in the eluate), which may cause incorrect results.

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see 14 TROUBLESHOOTING page 26).

NOTE

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

NOTE

When approving the assay results, always verify the instrument outcome by checking the melting curve plots in the work session report. The melting temperatures (Tm) of wildtype and/or mutated alleles of each gene in analysis have to correspond to the peaks showed in the melting curve plots.

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 **Coagulation 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)
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 (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- 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 “Specimens and Controls”).

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

10.2 STEP 2 - Session Setup

The **Coagulation 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:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for 12 tests in optimized conditions (2 or more tests per session).Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

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

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

Table 11

	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). If requird, transfer, **200 µL of sample** in a 2 mL Sarstedt tube previously labelled.	**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. Prepare the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen	Select "**Perform Run**" from the "Home" screen.
3	Remove all the Racks from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode": "**Extract + PCR".**	Select the "Run mode": "**PCR Only**".	Select the "Run mode": "**PCR Only**".
5	**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".
6	**Insert** the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert the "Sample ID" (SID) for each "Position" used (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3). If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit", the "Extracted eluate vol." (eluate volume) and the Internal Control.	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
7	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
8	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 200 µL	Not applicable	Not applicable
9	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
10	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
11	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable
12	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
13	Click "Next" to continue.	Not applicable	Not applicable
14	**Load PCR Mix** into the "Reagent/ Elution Rack".	**Load the PCR Mix** into "Reagent/ Elution Rack".	**Load the PCR Mix** into "Reagent/ Elution Rack".
15	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix 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). If needed, for each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
16	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
17	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.
18	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
19	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.
20	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
21	Load the "Extraction Rack" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables.	Not applicable	Not applicable

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
22	Close the instrument door.	Close the instrument door.	Close the instrument door.
23	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 ±10 °C for no longer than one month. Avoid the spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session), mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **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 **Coagulation 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

Analytical sensitivity

The Analytical Sensitivity of the Coagulation ELITe MGB Kit was defined on ELITe InGenius (PCR Only mode) and it was verified on ELITe BeGenius (PCR Only mode) by testing three samples (wild type, heterozygous and homozygous) in 4 replicates at about 70 ng per reaction spiked with panels of human genomic DNA certified for Factor V and Factor II (WHO Reference Reagent Factor V Leiden, Human gDNA, 1st International Genetic International Panel, NIBSC, UK, code 04/224, and WHO Reference Reagent Prothrombin Mutation G20210A, Human gDNA, 1st International Genetic International Panel, NIBSC, UK, code 05/130).

The analytical sensitivity of this assay, allows to identify the presence of about 20,000 molecules of target DNA (corresponding to the genomes of ~ 10,000 cells or ~ 70 ng of human genomic DNA) in 20 µL of extracted DNA in reaction.

Efficiency of detection (Inclusivity)

The Inclusivity of the assay, as efficiency of detection for Factor V, Factor II and MTHFR genes was evaluated by in silico analysis. The analysis showed high level of sequence conservation and absence of significant mutations. So, an efficient amplification and detection is expected.

The fluorescent probes allow to detect the alleles of the Factor V, Factor II and MTHFR genes reported in the following table. The limits of the related Tm intervals for ELITe InGenius and ELITe BeGenius were calculated analysing data obtained in the verification and validation studies and the Tm ranges in use are shown in the following table.

Table 12

Gene	Allele detected	Tm range	
		ELITe InGenius	ELITe BeGenius
Factor V	Allele 1691G (wild type)	53.0 °C – 57.0 °C	52.5 °C – 56.5 °C
	Allele 1691A (mutated, Leiden)	61.0 °C – 65.0 °C	59.5 °C – 63.5 °C
Factor II	Allele 20210G (wild type)	56.0 °C – 61.0 °C	55.0 °C – 60.0 °C
	Allele 20210A (mutated)	64.0 °C – 69.0 °C	63.0 °C – 68.0 °C
MTHFR	Allele MTHFR 677C (wild type)	55.0 °C – 59.0 °C	54.0 °C – 58.0 °C
	Allele MTHFR 677T (mutated)	64.0 °C – 68.0 °C	63.0 °C – 67.0 °C

Analytical Specificity

The Analytical Specificity of this assay, as the ability of not identifying as Factor II mutated for SNP G20210A the gene mutated for SNP C20209T, was verified using a plasmid DNA containing the Factor II amplicon with wildtype nucleotide (G) in position 20210 and the mutant nucleotide (T) in position 20209.

Moreover, the Analytical Specificity was verified for the following uncommon SNPs described in scientific literature. These are rare SNPs that fall in proximity of the SNPs of interest:

Table 13

Gene	rare SNP analysed
Factor V	G1689A, C1690T, A1692C, A1696G
Factor II	A20207C, A20218G, T20219A, C20221T
MTHFR	C678A, G679A, C684G

The Analytical Specificity has been verified using plasmid DNAs containing the amplified region of Factor V, Factor II and MTHFR genes with wildtype nucleotide in the SNPs of interest and the mutant nucleotide in the rare SNPs in analysis.

Simulated samples containing about 80,000 copies of plasmid DNA were tested in 6 replicates (and 24 replicates for SNP C20209T) on ELITe InGenius. The replicates resulted “Inconclusive” or correctly determined. No sample mutated for the SNPs in analysis was called as heterozygous or homozygous mutated for the SNPs of interest.

Rare SNPs falling within the probe hybridization region may interfere with the detection of the SNP of interest and could lead to “false homozygous mutant” result when occurring together with the mutation of the SNP of interest on the other allele.

Potentially interfering organisms: Cross-reactivity

The potential Cross-reactivity with other unintended organisms that might be found in clinical samples of Whole Blood was evaluated by *in silico* analysis of the sequences available in nucleotide databases. The analysis showed absence of significant homologies. So, no cross-reactivity by potential interfering organisms is expected.

Potentially interfering organisms: Inhibition

The potential inhibition by other unintended organisms that might be found in clinical samples of Whole Blood was evaluated by *in silico* analysis of the sequences available in nucleotide databases.

The analysis showed absence of significant homologies. So, no inhibition by potential interfering organisms is expected.

Interfering substances

The effect of potentially interfering substances was evaluated by analyzing a whole blood sample collected in EDTA, heterozygous for the three genes of interest, spiked with the following potential interfering substances at relevant concentration.

The results are summarized in the following table:

Table 14

Sample	Genotype FV het / FII het / MTHFR het	Outcome
Control	3 / 3	No interference
Cyclosporine A	3 / 3	No interference
Ganciclovir	3 / 3	No interference
Heparin	3 / 3	No interference
EDTA	3 / 3	No interference
Ibuprofen	3 / 3	No interference
Triglycerides	3 / 3	No interference
Ampicillin	3 / 3	No interference
Bilirubin	3 / 3	No interference

Repeatability

The Intra-Session and Inter-Session Repeatability of results obtained with the Coagulation ELITe MGB Kit in association with the ELITe InGenius and ELITe BeGenius instruments was tested by analysing a whole blood panel collected in EDTA including one heterozygous genotype sample for each target (FII SNP G20210A, FV SNP G1691A heterozygous (Leiden), and MTHFR SNP C677T).

An example of Intra-Session Repeatability on ELITe InGenius is shown in the tables below.

Table 15 Intra - Session Repeatability on ELITe InGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	8	22.07	0.41
Heterozygous FV SNP G1691A		8	22.91	0.76
Heterozygous MTHFR SNP C677T		8	22.02	1.22

Table 16 Intra - Session Repeatability on ELITe InGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	8	58.19	0.207
	Factor II mutated	8	65.66	0.051
Heterozygous FV SNP G1691A	Factor V wild type	8	55.18	0.048
	Factor V mutated	8	62.31	0.120
Heterozygous MTHFR SNP C677T	MTHFR wild type	8	56.73	0.183
	MTHFR mutated	8	65.90	0.169

An example of Inter-Session Repeatability on ELITe InGenius is shown in the tables below.

Table 17 Inter - Session Repeatability on ELITe InGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	16	22.12	1.12
Heterozygous FV SNP G1691A		16	22.91	0.75
Heterozygous MTHFR SNP C677T		16	22.10	1.36

Table 18 Inter - Session Repeatability on ELITe InGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	16	58.18	0.182
	Factor II mutated	16	65.78	0.258
Heterozygous FV SNP G1691A	Factor V wild type	16	55.18	0.064
	Factor V mutated	16	62.31	0.116
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.73	0.211
	MTHFR mutated	16	65.96	0.257

An example of Intra-Session Repeatability on ELITe BeGenius is shown in the tables below.

Table 19 Intra - Session Repeatability on ELITe BeGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	8	22.35	1.90
Heterozygous FV SNP G1691A		8	22.95	2.20
Heterozygous MTHFR SNP C677T		8	22.40	1.28

Table 20 Intra - Session Repeatability on ELITe BeGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	8	57.64	0.392
	Factor II mutated	8	65.21	0.150
Heterozygous FV SNP G1691A	Factor V wild type	8	54.84	0.120
	Factor V mutated	8	61.56	0.242
Heterozygous MTHFR SNP C677T	MTHFR wild type	8	56.29	0.174
	MTHFR mutated	8	65.30	0.116

An example of Inter-Session Repeatability on ELITe BeGenius is shown in the tables below.

Table 21 Inter - Session Repeatability on ELITe BeGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	16	22.26	1.58
Heterozygous FV SNP G1691A		16	23.10	2.02
Heterozygous MTHFR SNP C677T		16	22.45	1.75

Table 22 Inter - Session Repeatability on ELITe BeGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	16	57.71	0.494
	Factor II mutated	16	65.24	0.119
Heterozygous FV SNP G1691A	Factor V wild type	16	54.82	0.131
	Factor V mutated	16	61.60	0.218
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.29	0.273
	MTHFR mutated	16	65.28	0.099

The Repeatability in association with ELITe InGenius and ELITe BeGenius of the Coagulation ELITe MGB Kit showed a maximum variability of IC Ct and Target Tm values as %CV lower than 5%.

Reproducibility

The Intra-Session and Inter-Session Repeatability of results obtained with the Coagulation ELITe MGB Kit in association with the ELITe InGenius and ELITe BeGenius was tested by analysing a whole blood panel collected in EDTA including one heterozygous genotype sample for each target (FII SNP G20210A, FV SNP G1691A heterozygous (Leiden), and MTHFR SNP C677T).

The Inter-Batch and Inter-Instrument Reproducibility of results obtained with the Coagulation ELITe MGB Kit in association with the ELITe InGenius and ELITe BeGenius instruments was tested by analysing a whole blood panel collected in EDTA including one heterozygous genotype sample for each target (FII SNP G20210A, FV SNP G1691A heterozygous (Leiden) and MTHFR SNP C677T).

The results are summarized in the following tables.

Table 23 Inter – Batch Reproducibility on ELITe InGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	16	22.28	1.28
Heterozygous FV SNP G1691A		16	22.46	2.58
Heterozygous MTHFR SNP C677T		16	23.14	1.47

Table 24 Inter – Batch Reproducibility on ELITe InGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	16	58.1	0.23
	Factor II mutated	16	65.7	0.12
Heterozygous FV SNP G1691A	Factor V wild type	16	55.1	0.10
	Factor V mutated	16	62.3	0.13
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.6	0.32
	MTHFR mutated	16	65.8	0.22

Table 25 Inter - Instrument Reproducibility on ELITe InGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	16	22.38	1.53
Heterozygous FV SNP G1691A		16	22.45	2.34
Heterozygous MTHFR SNP C677T		16	23.13	1.27

Table 26 Inter - Instrument Reproducibility on ELITe InGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	16	58.1	0.27
	Factor II mutated	16	65.6	0.12
Heterozygous FV SNP G1691A	Factor V wild type	16	55.1	0.21
	Factor V mutated	16	62.2	0.16

Table 26 Inter - Instrument Reproducibility on ELITe InGenius (continued)

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.6	0.35
	MTHFR mutated	16	65.7	0.28

Table 27 Inter – Batch Reproducibility on ELITe BeGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	16	22.42	1.72
Heterozygous FV SNP G1691A		16	22.60	1.99
Heterozygous MTHFR SNP C677T		16	22.80	2.20

Table 28 Inter – Batch Reproducibility on ELITe BeGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	16	57.6	0.29
	Factor II mutated	16	65.2	0.11
Heterozygous FV SNP G1691A	Factor V wild type	16	54.8	0.11
	Factor V mutated	16	61.5	0.24
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.2	0.27
	MTHFR mutated	16	65.2	0.28

Table 29 Inter - Instrument Reproducibility on ELITe BeGenius

Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G20210A	IC	16	22.51	1.60
Heterozygous FV SNP G1691A		16	22.63	1.68
Heterozygous MTHFR SNP C677T		16	23.15	1.83

Table 30 Inter - Instrument Reproducibility on ELITe BeGenius

Panel sample	Allele	N	Mean Tm	%CV Ct
Heterozygous FII SNP G20210A	Factor II wild type	16	57.7	0.45
	Factor II mutated	16	65.2	0.13
Heterozygous FV SNP G1691A	Factor V wild type	16	54.8	0.16
	Factor V mutated	16	61.6	0.23
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.2	0.30
	MTHFR mutated	16	65.3	0.17

The Reproducibility in association with ELITe InGenius and ELITe BeGenius of the Coagulation ELITe MGB Kit showed a maximum variability of IC Ct and Target Tm values as %CV lower than 5%.

Robustness: Cross-contamination test

The absence of cross-contamination was verified by analysing 6 replicates of whole blood samples alternated to 6 replicates of molecular biology grade water with the Coagulation ELITe MGB Kit product on ELITe InGenius.

The results are summarized in the following table.

Table 31

Samples	N	Genotype			IC
		FII het	MTHFR wt	FV wt	Ct < 26.5
Whole blood collected in EDTA	30	30/30	30/30	30/30	30/30
Molecular biology grade water	30	NA	NA	NA	0/30

The assay called as a “Invalid” any sample of water tested showing the absence of cross-contamination.

Diagnostic Agreement: confirmation of certified sample genotype

The diagnostic agreement of this assay, as the ability to correctly identify the genotype of certified sample, was evaluated analyzing in association with ELITe InGenius

The results are summarized in the following tables.

Table 32

Sample genotype: Factor V G1691A	N	WT	Het.	Hom.	Diagnostic Agreement	Total Diagnostic Agreement
Wild type	108	108	0	0	100%	100%
Heterozygous	57	0	57	0	100%	
Homozygous mutated	53	0	0	53	100%	

In the allelic discrimination of the Factor V SNP G1691A locus, the assay returned concordant results for all samples tested. In this test the total diagnostic agreement for the Factor V SNP G1691A locus was equal to 100%.

Table 33

Sample genotype: Factor II G20210A	N	WT	Het.	Hom.	Diagnostic Agreement	Total Diagnostic Agreement
Wild type	160	160	0	0	100%	100%
Heterozygous	59	0	59	0	100%	
Homozygous mutated (simulated)	56	0	0	56	100%	

In allelic discrimination of the Factor II SNP G20210A locus, the assay returned concordant results for all samples tested. In this test the total diagnostic agreement for the Factor II G20210A locus was equal to 100%.

Table 34

Sample genotype: MTHFR C677T	N	WT	Het.	Hom.	Diagnostic Agreement	Total Diagnostic Agreement
Wild type	63	63	0	0	100%	100%
Heterozygous	136	0	136	0	100%	
Homozygous mutated	19	0	0	19	100%	
Homozygous mutated (simulated)	36	0	0	36		

In allelic discrimination of the MTHFR SNP C677T locus, the assay returned concordant results for all samples tested. In this test the total diagnostic agreement for the MTHFR C677T locus was equal to 100%.

As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the diagnostic performances of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The Internal Control Ct (IC Ct) cut-off value is set at 26.5 for ELITe InGenius and ELITe BeGenius.

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in Product Technical File of "Coagulation ELITe MGB Kit", FTP RTSD00ING

12 REFERENCES

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Poort, S. R. et al. (1996) Blood 88: 3698 - 3703.

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13 PROCEDURE LIMITATIONS

Use this product only with whole blood clinical samples collected in EDTA.

There are no data available concerning product performance with DNA extracted from the following clinical samples: saliva.

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 clinical samples, Positive Controls and PCR products. Cross-contamination cause false detection for the target allele. 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 results with false detection for the target allele results.

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

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

A result with lack of detection for the target allele obtained with this product indicates that the target DNA is not detected in the DNA extracted from the sample; however, it cannot be excluded that the target DNA has a lower titer than the product detection limit (see 11 PERFORMANCE CHARACTERISTICS page 18). In this case the result could be inconclusive.

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.

An invalid or inconclusive result obtained with this product means that it was not possible to efficiently detect the sample genomic DNA or the dissociation temperatures of alleles. In this case the analysis of sample must be repeated with possible delay in obtaining results.

Possible polymorphisms within the region of the target DNA covered by the product primers and probes may impair detection of target DNA and lead to incorrect results.

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 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 35

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Positive Control. Check the volumes of PCR Mix and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
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 36

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Contamination of the 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 PCR Mix.	Use a new aliquot of PCR Mix.
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 37

Invalid or Inconclusive Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and sample. Check the volumes of PCR Mix and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
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 38

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

Table 39

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 sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 40

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. Use a new tube of PCR Mix.

15 SYMBOLS

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


Consult instructions for use.

CONT Contents.


Keep away from sunlight.


Manufacturer.

16 NOTICE TO THE USERS

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

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

17 NOTICE TO PURCHASER: LIMITED LICENSE

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

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

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

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

Appendix A Coagulation 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 **Coagulation ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative nucleic acids Real-Time PCR assay for the allelic discrimination of the following three loci:

- coagulation Factor V gene, single nucleotide polymorphism (SNP) G1691A (Leiden),
- coagulation Factor II gene, SNP G20210A,
- 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, SNP C677T.

in singleplex, duplex (FII and FV) or triplex (FII, FV and MHTFR) in human genomic DNA samples extracted from clinical specimens.

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

The product is intended for use as an aid in assessing the risk of deep vein thrombosis in patients suspected of having coagulation disorders and at risk of deep vein thrombosis.

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

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	Factor V, SNP G1691A (Leiden)	AP639	FV
Target 2	Factor II, SNP G20210A	FAM	FII
Target 3	MTHFR, SNP C677T	AP593	MTHFR
Internal Control	Human beta globin gene	AP525	IC

Validated matrix

- Whole blood collected in EDTA

Kit content and related products

Coagulation ELITe MGB Kit (RTSD00ING)		Coagulation - ELITe Positive Control (CTRD00ING)	
 X 8		 X 3	
52M PCRMix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles per tube		**52M Positive Control** 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles per tube	
Maximum shelf-life:	**24 months**	Maximum shelf-life	**24 months**
Storage temperature	**≤ -20°C**	Storage temperature	**≤ -20°C**

Other products required not provided in the kit

• ELITe InGenius instrument: INT030. • ELITe BeGenius instrument: INT040. • ELITe InGenius SP 200: INT032SP200.	• **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use)

ELITe InGenius and ELITe BeGenius Protocol

• Sample volume • Total elution volume	200 µL 200 µL	• Eluate PCR input volume • PCR Mix volume • Frequency of controls	20 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Sample genotype: Factor V G1691A	Analytical Sensitivity	Diagnostic Agreement
Wild type	70ng DNA/reaction	100%
Heterozygous	70ng DNA/reaction	100%
Homozygous mutated	70ng DNA/reaction	100%

Sample genotype:Factor II G20210A	Analytical Sensitivity	Diagnostic Agreement
Wild type	70ng DNA/reaction	100%
Heterozygous	70ng DNA/reaction	100%
Homozygous mutated	70ng DNA/reaction	100%

Sample genotype: MTHFR C677T	Analytical Sensitivity	Diagnostic Agreement
Wild type	70ng DNA/reaction	100%
Heterozygous	70ng DNA/reaction	100%
Homozygous mutated	70ng DNA/reaction	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 41

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Whole Blood	EDTA	≤ 3 d	≤ 7 d	≤ 30 d	≤ 1 year

EDTA, Ethylenediaminetetraacetic acid; d, days.

ELITe InGenius Procedures

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

Before analysis

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

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

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "200 µL"	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: 52M ELITe_WB_200_200 52M ELITe_FII_WB_200_200 52M ELITe_FV_WB_200_200 52M ELITe_MTHFR_WB_200_200 52M ELITe_FVFII_WB_200_200	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix and the Internal Control in the Inventory Block
7. Load: PCR Cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	**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)

1. Select “Perform Run” on the touch screen	**2.** Verify the extraction volumes: Input: “200 µL”, Eluate: “200 µL”	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the “Assay Protocol” of interest: 52M ELITe_PC or 52M ELITe_NC or 52M ELITe_WB_200_200 52M ELITe_FII_WB_200_200 52M ELITe_FV_WB_200_200 52M ELITe_MTHFR_WB_200_200 52M ELITe_FVFII_WB_200_200	**5.** Select the method “PCR Only” and the sample position: “Elution Tube”	**6.** Load the PCR Mix in the Inventory Block
7. Load: PCR Cassette rack and Elution tube racks with the extracted nucleic acid	**8.** Close the door. Start the run	View, approve and store the results

ELITe BeGenius Procedures

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

Before analysis

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

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

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “200 µL”
4. Select the “Assay Protocol” of interest: 52M ELITe_Be_WB_200_200 52M ELITe_FII_Be_WB_200_200 52M ELITe_FV_Be_WB_200_200 52M ELITe_MTHFR_Be_WB_200_200 52M ELITe_FVFII_Be_WB_200_200 **Note:** If a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Basket” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

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

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

1. Select “Perform Run” on the touch screen and then click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit	**3.** For Controls: for each “Position” enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions). For eluates: for each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).
4. Select the “Assay Protocol” of interest: 52M ELITe_Be_PC or 52M ELITe_Be_NC or 52M ELITe_Be_WB_200_200 52M ELITe_FII_Be_WB_200_200 52M ELITe_FV_Be_WB_200_200 52M ELITe_MTHFR_Be_WB_200_200 52M ELITe_FVFII_Be_WB_200_200	**5.** Load the PCR-Mix in the Reagent/Elution Rack and insert it in the Cooler Unit	**6.** Load “PCR Rack” with “PCR Cassette”
7. Close the door. Start the run	**8.** View, approve and store the results	

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