ELITechGroup
EMPOWERING IVD

ELITech Group S.p.A.
C.so Svizzera, 185
10149 Torino ITALY
Offices: Tel. +39-011 976 191 Fax +39-011 936 76 11
E. mail: emd.support@elitechgroup.com
WEB site: www.elitechgroup.com

NOTICE of CHANGE dated 10/04/2026

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HCV ELITe MGB® Kit»
Ref. RTK601ING

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

- *Update of the paragraph "Potential interfering markers: Cross-reactivity".*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

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

PRODUCT REF.	Lot Number	Expiry date
RTK601ING	C0125-009	31/07/2026

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HCV ELITe MGB® Kit

reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

UDI 08033891487072

CE IVD 0123

-20°C

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INTENDED USE

The product **HCV ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids reverse transcription and Real-Time PCR assay for the detection and quantification of the RNA of Hepatitis C Virus (HCV), extracted from clinical specimens.

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

The product is intended for use as an aid in the management of HCV-infected individuals undergoing antiviral therapy.

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

The product is not intended to be used for screening or to detect the presence of or exposure to transmissible agents in blood, blood components, cells, tissues, organs or any of their derivatives in order to assess their suitability for transfusion, transplantation or cell administration. The product is not intended for use as a diagnostic test to confirm the presence of HCV infection.

ASSAY PRINCIPLE

The assay is a quantitative One-Step Reverse Transcription Real-Time PCR detecting HCV RNA isolated from specimens, retro-transcribed and then amplified using a complete reaction mixture that contains primers and probes with ELITe MGB® technology.

The ELITe MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycles (Ct) and the melting temperatures (Tm). The HCV quantity is calculated based on a stored calibration curve.

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.

PRODUCT DESCRIPTION

The **HCV ELITe MGB Kit** provides the following components:

- **HCV ELITe MGB Mix**

  This component provides the following two sub-components:

  - **HCV PCR Mix**, an optimized and stabilized PCR mixture that contains:
    - specific primers and probe for **HCV**, 5' UTR region, detected in Channel **HCV;** the probe is stabilized by MGB®, quenched by Eclipse Dark Quencher®, and labeled by FAM dye.
    - specific primers and probe for Internal Control (**IC**) specific for a region of the phage **MS2** genomic RNA, detected in Channel **IC;** the probe is stabilized by MGB, quenched by Eclipse Dark Quencher, and labelled with AquaPhluor® AP525 dye.
    - buffer, magnesium chloride, nucleotide triphosphates and hot-start DNA Polymerase. Each vial contains **600 µL** of solution and is sufficient for **24 tests**, if processing at least 5 samples per session.
  - **RT EnzymeMix**, an optimized and stabilized mixture of enzymes for reverse transcription. Each vial contains **20 µL** of solution and is sufficient for **48 tests**, if processing at least 5 samples per session.

The **HCV ELITe MGB Mix** contains sufficient reagents for **96 tests** on **ELITe InGenius** and **ELITe BeGenius,** with 20 µL of **HCV PCR Mix** and 0.3 µL of **RT EnzymeMix** used per reaction.

- **HCV ELITe Standard**

This component provides the sub-components **HCV Q-PCR Standard**, four stabilized solutions of plasmid DNA with the amplified HCV 5' UTR region at **known titre.** The **HCV ELITe Standard** must be used with **HCV ELITe MGB Mix** on the **ELITe InGenius** and **ELITe BeGenius**, to calculate the calibration curve of the system (product batch and instrument) for HCV quantification.

The plasmid DNA concentration was determined by UV spectrophotometer as copies / mL, which was correlated to the "WHO International Standard 6th HCV International Standard" (NIBSC, UK, code 18/184) by a conversion factor allowing HCV quantification in International Unit / mL (IU / mL).

The **HCV ELITe Standard** contains sufficient reagents for **2 sessions on ELITe InGenius** and **ELITe BeGenius**, with 20 µL used per reaction.

- **HCV - ELITe Positive Control**

This component contains the sub-component **HCV Positive Control**, a stabilized solution of plasmid DNA with the amplified 5' UTR region of HCV at **known titre**. The **HCV Positive Control** must be used with **HCV ELITe MGB Mix** on **ELITe InGenius** and **ELITe BeGenius**, to construct control plots for the verification of the system (product batch and instrument).

The **HCV Positive Control** contains sufficient reagents for **8 sessions on ELITe InGenius** and **ELITe BeGenius (4 sessions each tube)**, with 20 µL used per reaction.

- **HCV Internal Control**

This component contains the sub-component **HCV CPE** (exogenous Internal Control), a stabilized solution of MS2 genomic RNA. The **HCV CPE** is added to extraction reagents, to validate the results of HCV negative samples.

The **HCV Internal Control** contains sufficient reagents for **96 tests on ELITe InGenius** and **ELITe BeGenius (12 test each tube),** with 10 µL used per extraction.

The **HCV ELITe MGB Kit** can be also used in association with equivalent instruments.

MATERIALS PROVIDED IN THE PRODUCT

Component	Sub-Component	Description	Quantity	Classification of hazards
HCV ELITe MGB Mix ref. RTS601ING	HCV PCR Mix ref. RTS601ING	Mixture of reagents for reverse transcription and real-time PCR in tube with **NATURAL cap**	**4 x 600 µL**	-
	RT EnzymeMix ref. RTS003-RT	Reverse transcription enzymes in tube with **cap with BLACK insert**	**2 x 20 µL**	-
HCV ELITe Standard ref. STD601ING	HCV Q-PCR Standard 10^5 ref. STD601ING-5	Plasmid solution in tube with **RED cap**	**1 x 160 µL**	-
	HCV Q-PCR Standard 10^4 ref. STD601ING-4	Plasmid solution in tube with **BLUE cap**	**1 x 160 µL**	
	HCV Q-PCR Standard 10^3 ref. STD601ING-3	Plasmid solution in tube with **GREEN cap**	**1 x 160 µL**	
	HCV Q-PCR Standard 10^2 ref. STD601ING-2	Plasmid solution in tube with **YELLOW cap**	**1 x 160 µL**	
HCV - ELITe Positive Control ref. CTR601ING	HCV Positive Control ref. CTR601ING	Plasmid solution in tube with **BLACK cap**	**2 x 160 µL**	-
HCV Internal Control ref. CPE601ING	HCV CPE ref. CPE601ING	Solution of plasmid DNAs and MS2 genomic RNA in tube with **NATURAL** cap	**8 x 160 µL**	-

Note: The concentration of the four **Q – PCR Standards** are expressed in copies / reaction (10^5 copies / rxn, 10^4 copies / rxn, 10^3 copies / rxn, 10^2 copies /rxn).

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~3,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample RNA and the consumables are **not** provided with this product. For automated extraction of nucleic acids, reverse transcription, Real-Time PCR and result interpretation of samples, the following products are required.

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030) **ELITe InGenius Software** version 1.3.0.17 (or later) **HCV ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **HCV ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **HCV ELITe_STD,** Assay Protocol with parameters for Calibrators analysis **HCV ELITe_PL_600_50,** Assay Protocol with parameters for Plasma specimen analysis **HCV ELITe_Se_600_50,** Assay Protocol with parameters for Serum specimen analysis	**ELITe InGenius SP 1000** (EG SpA, ref. INT033SP1000) **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS) **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR), **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30000631) with ELITe BeGenius only **ELITe InGenius® Waste Box** (EG SpA, ref. F2102-000)
ELITe BeGenius (EG SpA, ref. INT040) **ELITe BeGenius Software** version 2.1.0. (or later) **HCV ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **HCV ELITe_ Be_NC**, Assay Protocol with parameters for Negative Control analysis **HCV ELITe_ Be_STD,** Assay Protocol with parameters for Calibrators analysis **HCV ELITe_Be_PL_600_50,** Assay Protocol with parameters for Plasma specimen analysis **HCV ELITe_Be_Se_600_50,** Assay Protocol with parameters for Serum specimen analysis	

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with 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.

Warnings and precautions for molecular biology

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

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

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

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

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

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

Warnings and precautions specific for the components

Component (Sub-Component)	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
HCV ELITe MGB Mix (HCV PCR Mix)	-20°C or below (protected from light)	60 days	up to five	not applicable
HCV ELITe MGB Mix (RT EnzymeMix)	-20°C or below	60 days	up to ten times, for up to ten minutes , at +2 / +8 °C	not applicable
HCV ELITe Standard (HCV Q-PCR Standard)	-20°C or below	60 days	up to two	2 separate sessions of 2 hours each
HCV ELITe – Positive Control (HCV Positive Control)	-20°C or below	60 days	up to four	4 separate sessions of 3 hours each
HCV ELITe Internal Control (HCV CPE)	-20°C or below	60 days	up to six	6 separate sessions of 3 hours each

SPECIMENS AND CONTROLS

Specimens

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:

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Plasma	EDTA or ACD	≤ 1 day	≤ 3 days	≤ 1 month	≤ 6 months
Serum	-	≤ 1 day	≤ 5 days	≤ 1 month	≤ 6 months

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 the **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.

Assay Protocols for HCV ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
Plasma	**ELITe InGenius**	**HCV ELITe_PL_600_50**	Positive / copies/mL / IU/mL / Negative	Extraction Input Volume: 600 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1.7 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**HCV ELITe_Be_PL_600_50**	Positive / IU/mL / copies/mL / Negative	Extraction Input Volume: 600 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Serum	**ELITe InGenius**	**HCV ELITe_Se_600_50**	Positive / copies/mL / IU/mL / Negative	Extraction Input Volume: 600 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1.7 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	**HCV ELITe_Be_Se_600_50**	Positive / IU/mL / copies/mL / Negative	Extraction Input Volume: 600 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

For all protocols, 600 µL of sample must be transferred into an Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius).

Note: Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the "Warnings and Precautions" 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 Performance Characteristics section to check data concerning interfering substances.

Do not use plasma collected in heparin, which is a known reverse transcription and PCR inhibitor.

Do not use haemolytic Plasma, as hemoglobin inhibits the PCR reaction.

PCR calibrators and controls

Calibration curve must be generated and approved for each lot of PCR reagent.

- For the calibration curve, use the four levels of the product **HCV ELITe Standard** provided with this kit with the **HCV ELITe_STD** or **HCV ELITe_Be_STD** Assay Protocols,

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

- For the Positive Control, use the product **HCV - ELITe Positive Control** provided with this kit with the **HCV ELITe_PC** or **HCV ELITe_Be_PC** Assay Protocols,
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **HCV ELITe_NC** or **HCV ELITe_Be_NC** Assay Protocols.

Note: The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the calibration curve and PCR control validation for each lot of PCR reagent. Calibration curves expire after **60 days**, at which time it is necessary to re-run the calibration. PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and negative controls.

The Calibrators and PCR controls must be re-run if any of the following events occur:

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

Quality controls

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

ELITe InGenius PROCEDURE

The procedure to use the **HCV ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

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

STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Calibration" menu on the Home page, verify the Calibrators (**HCV Q-PCR Standard**) are approved and valid (Status) for the **HCV PCR Mix** lot to be used. If no valid Calibrators are available for the **HCV PCR Mix** lot, perform calibration as described in the following sections,
- in the "Controls" menu on the Home page, verify the PCR Controls (**HCV Positive Control**, **HCV Negative Control**) are approved and valid (Status) for the **HCV PCR Mix** lot to be used. If no valid PCR Controls are available for the **HCV 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.

STEP 2 - Session Setup

The **HCV ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),
B. Eluted sample run (PCR Only),
C. Calibration run (PCR Only),
D. Positive Control and Negative Control run (PCR Only).

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

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

Before to setup a run:

1. Thaw the needed **HCV PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (5 or more tests per session). Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

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

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **48 tests** in optimized conditions (5 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

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

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

Samples Number (N)	HCV 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

5. Prepare the **complete reaction mixture** by transferring in the labeled 2 mL tube the calculated volumes of the two components. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

Note: The **complete reaction mixture** can be used within **7** hours if kept in a refrigerated block (for 2 sessions of 3 hours each and for the time needed to start a third session). The complete reaction mixture **cannot** be stored for re-use.

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

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

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. For this assay, 600 µL of sample must be transferred in an Extraction tube previously labelled. Exceeding volume will be left in the Extraction tube by ELITe InGenius. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw** the **Elution tube** 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.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 1000 µL and the "Extracted Elute Volume" is 50 µL.	Ensure the "Extraction Input Volume" is 1000 µL and the "Extracted Elute Volume" is 50 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.
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").
6	Ensure the "Protocol" displayed is: "Extract + PCR".	Select "PCR Only" in the "Protocol" column.
7	Select the sample loading position as "Extraction Tube" in the "Sample Position" column. Ensure the "**Dilution factor**" is "**1.7**".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Ensure the "**Dilution factor**" is "**1.7**".
8	Click "Next" to continue.	Click "Next" to continue.
9	**Load CPE** and the **complete reaction mixture** on the "Inventory Block" referring to the "Load List" and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **complete reaction mixture** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.
10	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.
12	Click "Next" to continue.	Click "Next" to continue.
13	**Load** PCR Cassette, ELITe InGenius SP 1000 extraction cartridges, and all required consumables and samples to be extracted.	**Load** PCR Cassette and Elution Tube with samples extracted.
14	Click "Next" to continue.	Click "Next" to continue.
15	Close the instrument door.	Close the instrument door.
16	Press "Start".	Press "Start".

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) at room temperature for 30 minutes. 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. **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 1000 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 1000 µL and the "Extracted Elute Volume" is 50 µL.	Ensure the "Extraction Input Volume" is 1000 µL and the "Extracted Elute Volume" is 50 µL.
4	For the Q-PCR Standard, assign the "Track", **select** the **Assay Protocol** (see "Specimen and Controls") in the "Assay" column and enter the reagent lot number and expiry date.	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.
5	Ensure "PCR Only" is selected in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
6	Ensure the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
7	**Load** the **complete reaction mixture** on the "Inventory Block" referring to the Load List and enter the PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **complete reaction mixture** on the "Inventory Block" referring to the "Load List" and enter the PCR Mix lot number, expiry date and number of reactions for each tube.
8	Click "Next" to continue.	Click "Next" to continue.
9	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.
10	Click "Next" to continue.	Click "Next" to continue.
11	Load the PCR Cassette and the Q-PCR Standard tubes.	Load PCR Cassette, Positive Control and Negative Control.
12	Click "Next" to continue.	Click "Next" to continue.
13	Close the instrument door.	Close the instrument door.
14	Press "Start"	Press "Start".

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

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

Note: The **complete reaction mixture** can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3 hours each and for the time needed to start a third session). Mix gently and spin down the content for 5 seconds before starting the next session. The complete reaction mixture **cannot** be stored for re-use.

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

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: 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.

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 **HCV ELITe MGB Kit** through the following procedure:

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

A. Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the target of the Calibrator reactions with the **HCV ELITe_STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

The Calibration curves, specific for the PCR reagent lot, are recorded in the database (Calibration). They can be viewed and approved by "Administrator" or "Analyst" users, following the GUI instructions.

The Calibration curve expires **after 60 days**.

Note: If the Calibration curve does not meet the acceptance criteria, the "Failed" message is shown on the "Calibration" screen. In this case, the results cannot be approved, and the Calibrator amplification reactions must be repeated. In addition, if samples were included in the run, these are not quantified and must also be repeated to generate quantitative results.

B. Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the target of the Positive Control and Negative Control reactions with the **HCV ELITe_PC** and **HCV ELITe_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and 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 **ELITe InGenius software** processes the Positive Control and Negative Control results and generates Control Charts. Four approved Positive Control and Negative Control results are used to set up the initial Control Chart. For subsequent controls, the results are analyzed by the software to ensure the system performances are within the acceptance criteria, shown in the Control Chart plots. Refer to the instrument manual for more details.

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

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

C. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the target (Channel **HCV**) and the Internal Control (Channel **IC**) with the **HCV ELITe_PL_600_50** and **"HCV ELITe_Se_600_50"** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in the "Results Display" screen.
The sample results can be approved when the three conditions in the table below are true.

1) Calibration Curve	Status
HCV Q-PCR Standard	APPROVED
2) Positive Control	**Status**
HCV Positive Control	APPROVED
3) Negative Control	**Status**
HCV Negative Control	APPROVED

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

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

Result of sample run	Interpretation
HCV:RNA Detected, quantity equal to "XXX" copies / mL or IU / mL	**HCV RNA was detected** in the sample within the assay measurement range, its concentration is shown.
HCV:RNA Detected, quantity below "LLoQ" copies / mL or IU / mL	**HCV RNA was detected** in the sample, its concentration is below the assay Lower Limit of Quantification.
HCV:RNA Detected, quantity beyond "ULoQ" copies / mL or IU / mL	**HCV RNA was detected** in the sample, its concentration is above the assay Upper Limit of Quantification.
HCV:RNA Not detected or below the "LoD" copies / mL or IU / mL	**HCV RNA was not detected** in the sample. The sample is negative for the target RNA, or its concentration is below the assay Limit of Detection.
Invalid-Retest Sample	**Not valid assay result** caused by Internal Control failure (due to e.g., incorrect extraction, inhibitors carry-over). The test should be repeated.

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

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

Samples reported as "HCV:RNA Not Detected or below "LoD" copies / mL or IU / mL" are suitable for analysis but HCV RNA was not detected. In this case, the sample may be either negative for HCV RNA or HCV RNA is present at a concentration below the Limit of Detection of the assay (see "Performance Characteristics").

HCV RNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as "HCV:RNA Detected, quantity below "LLoQ" copies / mL or IU / mL" (see "Performance Characteristics").

HCV RNA positive samples within the Linear Measuring Range (see "Performance characteristics") are detected and are reported as "HCV:RNA Detected, quantity equal to "XXX" copies / mL or IU / mL".

HCV RNA positive samples that are above the Upper Limit of Quantification are reported as "HCV:RNA Detected, quantity beyond "ULoQ" copies / mL or IU / mL" and they are not suitable for quantification. If needed the sample may be diluted before extraction or PCR and retested to yield results within the Linear Measuring Range of the assay.

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

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

D. 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.

ELITe BeGenius PROCEDURE

The procedure to use the **HCV ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

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

STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in "**CLOSED**" mode,
- in the "Calibrations" menu on the Home page, verify that the Calibrators (**HCV Q-PCR Standard**) are approved and valid (Status) for the **HCV PCR Mix** lot to be used. If no valid Calibrators are available for the **HCV PCR Mix** lot, perform calibration as described in the following sections,

  in the "Controls" menu on the Home page, verify the PCR Controls (**HCV Positive Control**, **HCV Negative Control**) are approved and valid (Status) for the **HCV PCR Mix** lot to be used. If no valid PCR Controls are available for the **HCV 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.

STEP 2 – Session Setup

The **HCV ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),
B. Eluted sample run (PCR Only),
C. Calibration run (PCR Only),
D. Positive Control and Negative Control run (PCR Only).

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

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

Before to setup a run:

1. Thaw the needed **HCV PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (5 or more tests per session). Mix by vortexing at low speed for 10 seconds three times then spin down the contents for 5 seconds and keep on ice or cool block.

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

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **48 tests** in optimized conditions (5 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

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

3. Prepare one 2 mL tube (Sarstedt, ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and label it with a permanent marker.

4. Calculate the needed volumes of **HCV PCR Mix** and **RT EnzymeMix** for preparing the **complete reaction mixture** on the basis of the number of samples (N) to be analyzed, as described in the table below.

Samples Number (N)	HCV 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
13 ≤ N ≤ 18	(N + 3) x 20 µL	(N + 3) x 0.3 µL
19 ≤ N ≤ 23	(N + 4) x 20 µL	(N + 4) x 0.3 µL
N = 24	580 µL	8.7 µL

5. Prepare the **complete reaction mixture** by transferring in the labeled 2 mL tube the calculated volumes of the two components. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

Note: The **complete reaction mixture** can be used within **7** hours if kept in a refrigerated block (for 2 sessions of 3 hours each and for the time needed to start a third session). The complete reaction mixture **cannot** be stored for re-use.

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

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

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. For this assay, 600 µL of sample must be transferred in a 2mL Sarstedt Tube (not provided) previously labeled. Exceeding volume will be left in the 2mL Sarstedt Tube by ELITe BeGenius. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block.	If needed, **thaw** the **Elution tube** 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.
2	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.
4	Select the "Run mode": "Extract + PCR".	Select the "Run mode": "PCR Only".
5	**Load** the **samples** into the "Sample Rack". (**Note:** when secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack").	Load the samples into the "Elution Rack".
6	**Insert** the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5). Insert the "Sample ID" (SID) for each "Position" used. (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3) For each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" is 600 µL and the "Extracted Elute Volume" is 50 µL.	Ensure the "Extraction Input Volume" is 600 µL and the "Extracted Elute Volume" is 50 µL.
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").
10	Click "Next" to continue.	Click "Next" to continue.
11	When more than 12 samples are processed, repeat the procedure from point 6 .	When more than 12 samples are processed, repeat the procedure from point 6.
12	**Load** the "**Elution tubes"** into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable.
13	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3). When more than 12 samples are processed, repeat using "Lane 2" (L2).	Not applicable.
15	Click "Next" to continue.	Not applicable.
16	**Load CPE** and the **complete reaction mixture** into the "Reagent/Elution Rack".	**Load** the **complete reaction mixture** into "Reagent/Elution Rack".
17	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix and / or CPE enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
18	Click "Next" to continue.	Click "Next" to continue.
19	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.
20	Click "Next" to continue.	Click "Next" to continue.
21	**Load** the "**PCR Rack"** with "**PCR Cassette**" in the Inventory Area.	**Load** the "**PCR Rack"** with "**PCR Cassette**" in the Inventory Area.
22	Click "Next" to continue.	Click "Next" to continue.
23	**Load** the "**Extraction Rack**" with the "ELITe InGenius SP 1000" extraction cartridges and required extraction consumables.	Not applicable.
24	Close the instrument door.	Close the instrument door.
25	Press "Start".	Press "Start".

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) for 30 minutes at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block	**Thaw the 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. **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 1000 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen
3	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from 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: PCR Only".	Select the "Run mode": "PCR Only"
5	**Load the Q-PCR** Standard tubes into the "Elution Rack".	**Load** the **Positive Control and Negative Control tubes** into the "Elution Rack".
6	**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)	**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)
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" (600 µL) and the "Extracted Elute Volume" (50 µL).	Ensure the "Extraction Input Volume" (600 µL) and the "Extracted Elute Volume" (50 µL).
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").
10	Click "Next" to continue.	Click "Next" to continue.
11	**Load** the **complete reaction mixture** into "Reagent/Elution Rack"	**Load** the **complete reaction mixture** into "Reagent/Elution Rack"
12	**Insert** the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) . For each PCR Mix 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). For each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).)
13	Click "Next" to continue.	Click "Next" to continue.
14	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.
15	Click "Next" to continue.	Click "Next" to continue
16	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area
17	Click "Next" to continue.	Click "Next" to continue.
18	Close the instrument door.	Close the instrument door.
19	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: The **complete reaction mixture** can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3 hours each and for the time needed to start a third session). Mix gently then spin down the content for 5 seconds before starting the next session. The complete reaction mixture **cannot** be stored for re-use.

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

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: 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.

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 **HCV ELITe MGB Kit** through the following procedure:

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

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

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined on the ELITe InGenius instrument, by testing a panel of HCV negative ACD Plasma spiked with certified reference material of HCV (6th WHO International Standard, NIBSC). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following tables.

Limit of Detection (IU/mL) for ACD Plasma samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
HCV	26	19	48

The LoD as copies/mL for ACD Plasma was calculated by applying the specific Conversion factor (2.4 IU / copy). The analytical sensitivity as copies/mL is reported below.

Limit of Detection (copies/mL) for ACD Plasma samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
HCV	11	8	20

The calculated LoD value was verified by testing on ELITe InGenius and ELITe BeGenius a pool of ACD Plasma, a pool of EDTA Plasma and a pool of Serum spiked with HCV certified reference material at the claimed concentration.

The results obtained confirmed the claimed concentration for the target of HCV ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius.

Matrix equivalence: EDTA Plasma versus ACD Plasma and Serum

The Matrix equivalence of the HCV ELITe MGB Kit was verified using paired samples (same donor) of EDTA and ACD Plasma, and EDTA Plasma and Serum on ELITe InGenius.

For 30 samples tested negative for HCV by a CE IVD marked immunoassay, the Negative Percent Agreement (NPA) and the Coefficient of Variation (%CV) of Internal Control Ct values was evaluated.

The results are reported in the following tables.

Sample	N	Positive	Negative	NPA	IC Ct %CV	Whole IC Ct %CV
EDTA Plasma	30	0	30	100%	0.89	0.95
ACD Plasma	30	0	30		1.02	

Sample	N	Positive	Negative	NPA	HCV Ct %CV	Whole HCV Ct %CV
EDTA Plasma	30	30	0	100%	0.90	1.11
Serum	30	30	0		1.28	

For 30 samples spiked with certified reference material (6th WHO HCV International Standard, NIBSC), the Positive Percent Agreement (PPA) and the Coefficient of Variation (%CV) of HCV Ct values was evaluated.

The results are reported in the following tables.

Sample	N	Positive	Negative	PPA	HCV Ct %CV	Whole HCV Ct %CV
EDTA Plasma	30	30	0	100%	1.96	1.79
ACD Plasma	30	30	0		1.60	

Sample	N	Positive	Negative	PPA	HCV Ct %CV	Whole HCV Ct %CV
EDTA Plasma	30	30	0	100%	1.84	1.81
Serum	30	30	0		1.77	

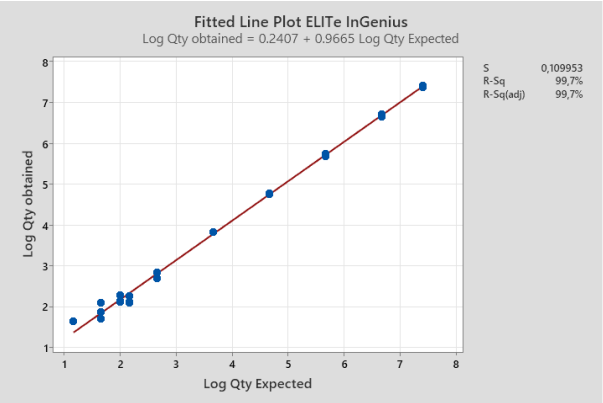
In these tests, both the 30 paired samples of EDTA Plasma and ACD Plasma and the 30 paired samples of EDTA Plasma and Serum showed equivalent performances when analysed by HCV ELITe MGB® Kit in association with ELITe InGenius.

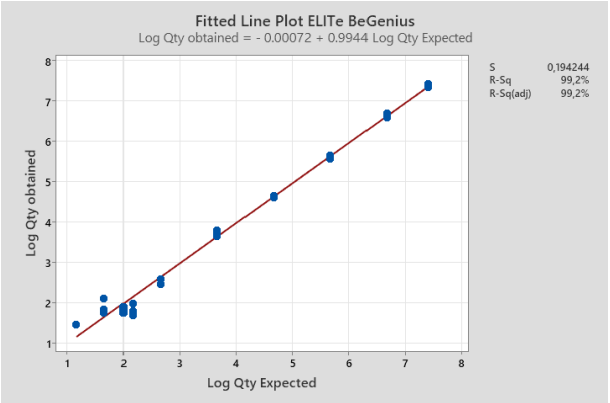
Additional Matrices equivalence testing was performed in the Linear Measuring Range study reported in the following section.

Linear measuring range

The Linear measuring range of the assay was determined in association with Plasma samples on ELITe InGenius and ELITe BeGenius, using a panel of dilutions of HCV reference material (AcroMetrix) in negative EDTA Plasma samples.

The results are reported in the following figures.





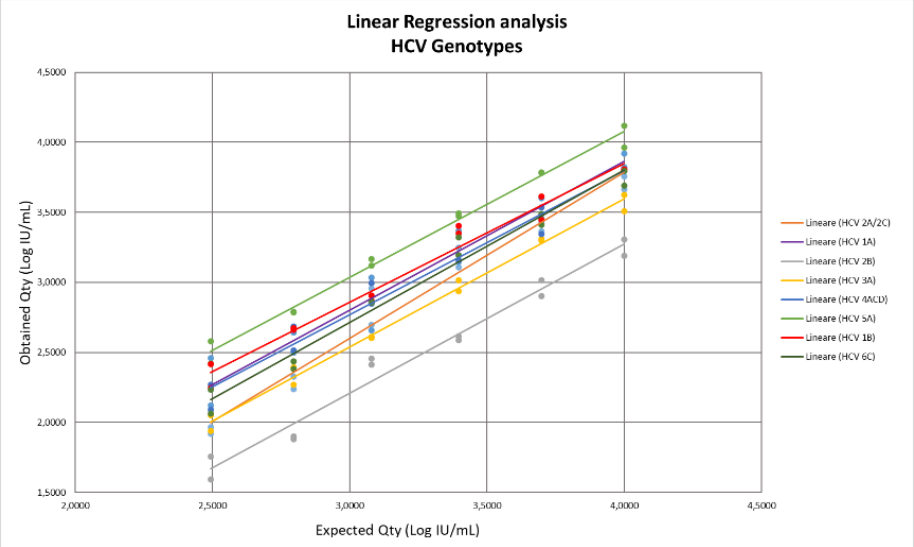
The linear measuring range as copies/mL for EDTA Plasma is calculated by applying the specific conversion factor in the following section.

The final results are summarized in the following table.

Linear measuring range for HCV ELITe MGB Kit and ELITe InGenius and ELITe BeGenius	
Lower Limit	**Upper Limit**
26 IU / mL	25,000,001 IU / mL
11 copies / mL	10,416,667 copies / mL

For the main HCV genotypes (1, 2, 3, 4, 5, 6), the linearity of quantification was verified by analysis of negative EDTA Plasma spiked with HCV reference material (SeraCare).

The results are reported in the following figure.



The linearity of the assay was confirmed for the main HCV genotypes (1, 2, 3, 4, 5, 6): the R2 value ranged from 0.950 to 0.992 and quantitative results fall within ±0.5 Log IU / mL with the exception of HCV genotype 2B that was underestimated of about 0.8 Log IU / mL in comparison with the theoretical value. However, the same samples were underestimated by "cobas® HCV for use on the 6800 Systems" (Roche Diagnostics).

The linearity of the assay was verified by analysis of negative EDTA Plasma, negative ACD Plasma and negative Serum spiked with HCV reference material (6th WHO International Standard NIBSC).

The results are reported in the following figure.

The linearity of the assay was verified for EDTA Plasma, ACD Plasma and Serum giving quantitative results within ±0.5 Log IU / mL and an R2 of 0.950, 0.984 and 0.987 respectively.

Standard Curve Uncertainty

The Uncertainty value of the Standard curve was calculated by combining the random errors (SD) of all level quantifications and multiplying for the Coverage factor k = 2 (Expanded Combined Uncertainty) and is equal to 0.1416 Log copies / reaction.

Standard curve levels	Theoretical Log c/rxn	SD	Expanded Combined Uncertainty
HCV Standard 10^5	5.0000	0.0652	0.1416
HCV Standard 10^4	4.0000	0.0641	
HCV Standard 10^3	3.0000	0.0489	
HCV Standard 10^2	2.0000	0.0964	

Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

The inclusivity of the assay, as efficiency of detection for different genotypes of HCV was evaluated by *in silico* analysis of the sequences available in nucleotide databases. The analysis showed sequence conservation and absence of significant mutations. So, an efficient detection for the different strains or isolates is expected.

The inclusivity of the assay was verified by testing three panels of HVC reference materials (Seracare, AccuTrak, Qnostics) at 3x LoD.

The results are reported in the following tables.

AccuTrak HCV RNA Genotype Performance Panel (SeraCare)		
Sample ID	**Pos. / Rep.**	**Outcome**
HCV 1a	3 / 3	HCV detected
HCV 1b	3 / 3	HCV detected
HCV 2a/2c	3 / 3	HCV detected
HCV 2b	3 / 3	HCV detected
HCV 3a	3 / 3	HCV detected
DHCV 4acd	3 / 3	HCV detected
HCV 5a	3 / 3	HCV detected
HCV 6c	3 / 3	HCV detected
HCV Genotype Evaluation Panel (Qnostics)		
Sample ID	**Pos. / Rep.**	**Outcome**
HCV 1a	3/3	HCV detected
HCV 1b	3/3	HCV detected
HCV 2b	3/3	HCV detected
HCV 3a	3/3	HCV detected
HCV 4a	3/3	HCV detected
HCV 5a	3/3	HCV detected
HCV 6a	3/3	HCV detected
Non WHO Reference Material 4th HCV RNA Genotype Panel (NIBSC)		
Sample ID	**Pos. / Rep.**	**Outcome**
HCV 1a	3/3	HCV detected
HCV 1b	3/3	HCV detected
HCV 2i	3/3	HCV detected
HCV 3a	3/3	HCV detected
HCV 4r	3/3	HCV detected
HCV 5a	3/3	HCV detected
HCV 6l	3/3	HCV detected

All the samples were correctly detected as positive and quantified within ± 0.5 Log by the HCV ELITe MGB® Kit on ELITe InGenius.

Potential interfering markers: Cross-reactivity

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

The absence of cross-reactivity with other organisms was also verified through the analysis of a panel of unintended organisms (ATCC, NIBSC, Vircell and Zeptometrix) at high titre.

The results are reported in the following table.

Sample ID	Pos. / Rep.	Outcome
HIV1	0 / 3	No cross-reactivity
HIV2	0 / 3	No cross-reactivity
HTLVI	0 / 3	No cross-reactivity
HTLVII	0 / 3	No cross-reactivity
CMV	0 / 3	No cross-reactivity
EBV	0 / 3	No cross-reactivity
HAV	0 / 3	No cross-reactivity
HBV	0 / 3	No cross-reactivity
HEV	0 / 3	No cross-reactivity
HSV1	0 / 3	No cross-reactivity
HSV2	0 / 3	No cross-reactivity
HHV6	0 / 3	No cross-reactivity
VZV	0 / 3	No cross-reactivity
Flu A	0 / 3	No cross-reactivity
Flu B	0 / 3	No cross-reactivity
RSV	0 / 3	No cross-reactivity
ADV	0 / 3	No cross-reactivity
EV	0 / 3	No cross-reactivity
PVB19	0 / 3	No cross-reactivity
Staphylococcus aureus	0 / 3	No cross-reactivity
Candida albicans	0 / 3	No cross-reactivity
WNV (NIBSC)	0 / 12	No cross-reactivity
WNV (ATCC)	0 / 3	No cross-reactivity
WNV (Vircell)	0 / 5	No cross-reactivity
DV1	0 / 5	No cross-reactivity
DV2	0 / 5	No cross-reactivity
DV3 (Vircell)	0 / 5	No cross-reactivity
DV3 (ATCC)	0 / 3	No cross-reactivity
DV4	0 / 5	No cross-reactivity
Yellow Fever Virus	0 / 5	No cross-reactivity
Zika Virus Asian	0 / 5	No cross-reactivity
Zika Virus	0 / 5	No cross-reactivity
Chikungunya virus	0 / 5	No cross-reactivity

All potentially interfering markers tested showed no cross-reactivity for the HCV target amplification using the HCV ELITe MGB Kit.

Potential interfering markers: Inhibition

The potential inhibition of unintended organisms that may be found in clinical specimens was evaluated for the assay through the analysis of panels of unintended organisms (ATCC, NIBSC, Zeptometrix) at 3x LoD.

The results are reported in the following table.

Sample ID	HCV Pos. / Rep.	Outcome
HIV1	3 / 3	No interference
HIV2	3 / 3	No interference
HTLVI	3 / 3	No interference
HTLVII	3 / 3	No interference
CMV	3 / 3	No interference
EBV	3 / 3	No interference
HAV	3 / 3	No interference
HBV	3 / 3	No interference
HEV	3 / 3	No interference
HSV1	3 / 3	No interference
HSV2	3 / 3	No interference
HHV6	3 / 3	No interference
VZV	3 / 3	No interference
Flu A	3 / 3	No interference
Flu B	3 / 3	No interference
RSV	3 / 3	No interference
ADV	3 / 3	No interference
WNV	3 / 3	No interference
DV3	3 / 3	No interference
EV	3 / 3	No interference
PVB19	3 / 3	No interference
Staphylococcus aureus	3 / 3	No interference
Candida albicans	3 / 3	No interference

All potential interfering organisms tested showed no inhibition of the HCV target detection and quantification using the HCV ELITe MGB Kit.

Potential interfering substances: Inhibition

The potential inhibition of interfering substances (endogenous and exogenous) that might be found in clinical specimens was evaluated for the assay by analysis of a panel of substances at relevant concentration in samples of Plasma positive for the targets.

The results are reported in the following table.

Sample	HCV Pos. / Rep.	Outcome
Bilirubin	3 / 3	No interference
Triglycerides	3 / 3	No interference
Hemoblogin high	1 / 3	Interference
Hemoblogin medium	3 / 3	Interference
Hemoblogin low	3 / 3	No interference
Heparin	0 / 3	Interference
EDTA	3 / 3	No interference
Ganciclovir	3 / 3	No interference
Azithromycin	3 / 3	No interference
Sofosbuvir	3 / 3	No interference
Pibrentasvir	3 / 3	No interference
Glecaprevir	3 / 3	No interference
Ribavirine	3 / 3	No interference
Velpatasvir	3 / 3	No interference

Most of the tested substances do not interfere with the HCV or Internal Control amplification.

Note: Hemoglobin at medium concentration (1.7 – 2.3 g / dL) is considered interferent as it doesn't quantify for HCV target with mean target Logarithmic Quantity values within the interval requested by the international guidelines after addition or subtraction of SEM value as measurement error.

Heparin and Hemoglobin at high (3.4 – 4.6 g/dL) concentrations were confirmed to be capable of inhibiting the amplification of HCV, however due to the Internal Control Ct cut-off (IC Ct < 31), the samples results were called as "non valid" and not "false negative" in most cases.

Cross-contamination

The possible cross-contamination during analysis was evaluated by testing 30 HCV RNA negative plasma samples alternated to 30 plasma samples spiked by HCV certified reference material (Zeptometrix) at high titre.

The results are reported in the following table.

Samples	N	Positive	Negative
ACD Plasma spiked at 1x10^6 HCV IU/mL	30	30	0
ACD Plasma negative for HCV	30	0	30

In this test HCV ELITe MGB Kit cross-contamination was neither detected within sessions nor among sessions.

Whole system failure rate

The Whole system failure rate for the assay was evaluated on ELITe InGenius and ELITe BeGenius, by testing a panel of samples spiked by certified reference material (6th WHO International Standard, NISBC) at a concentration of 3 x LoD (about 78 IU / mL).

The results are summarized in the following tables.

ELITe InGenius – Whole system failure rate					
Samples	**N**	**Theoretical IU/mL**	**Positive**	**Negative**	**Whole system failure rate**
EDTA Plasma spiked by HCV	100	78	100	0	0%
ACD Plasma spiked by HCV	30	78	30	0	0%
Serum spiked by HCV	30	78	30	0	0%

ELITe BeGenius – Whole system failure rate					
Matrix	**N**	**Theoretical IU/mL**	**Positive**	**Negative**	**Whole system failure rate**
EDTA Plasma spiked by HCV	100	78	100	0	0%

In this test with the HCV ELITe MGB Kit, none of the tested HCV RNA positive samples gave false negative results. In this test the whole system failure rate was equal to 0%.

Repeatability

The Intra-Session and Inter-Session Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Plasma samples, including one negative sample and two samples spiked with HCV certified reference material (6th WHO International Standard, NIBSC).

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

ELITe InGenius Intra-Session Repeatability (Day 1)								
Sample	**N**	**HCV**				**Internal Control**		
		Mean Ct	**Mean Ct**	**Mean Ct**	**% Agreement**	**Mean Ct**	**SD**	**%CV**
Negative	8	-	-	-	100%	27.43	0.21	0.78
3X LOD	8	37.28	0.34	0.92	100%			
10X LOD	8	35.37	0.43	1.23	100%			

ELITe BeGenius Intra-Session Repeatability (Day 1)								
Sample	**N**	**HCV**				**Internal Control**		
		Mean Ct	**Mean Ct**	**Mean Ct**	**% Agreement**	**Mean Ct**	**SD**	**%CV**
Negative	8	-	-	-	100%	27.87	0.30	1.08
3X LOD	8	37.83	1.15	3.05	100%			
10X LOD	8	35.45	0.30	0.85	100%			

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

ELITe InGenius Inter – Session Repeatability (Day1 + Day2)								
Sample	**N**	**HCV**				**Internal Control**		
		Mean Ct	**Mean Ct**	**Mean Ct**	**% Agreement**	**Mean Ct**	**SD**	**%CV**
Negative	16	-	-	-	100%	27.41	0.18	0.66
3X LOD	16	37,37	0,67	1,78	100%			
10X LOD	16	35,40	0,43	1,22	100%			

ELITe BeGenius Inter – Session Repeatability (Day1 + Day2)								
Sample	N	HCV				Internal Control		
		Mean Ct	Mean Ct	Mean Ct	% Agreement	Mean Ct	SD	%CV
Negative	16	-	-	-	100%	27.89	0.31	1.10
3X LOD	16	37.68	0.25	2.31	100%			
10X LOD	16	35.54	0.10	0.96	100%			

In the Repeatability test, the HCV ELITe MGB Kit correctly detected the target and showed a maximum variability of target Ct values as %CV equal to 3.05%.

Reproducibility

The Inter-Site Reproducibility of the assay was evaluated on ELITe InGenius by analysis of a panel of Plasma specimens negative or spiked with HCV (6th WHO International Standard, NIBSC).

A summary of Inter-Site Reproducibility (on two sites) is shown in the tables below.

ELITe InGenius Inter-Site Reproducibility								
Sample	HCV					Internal Control		
	N	Mean Ct	SD	%CV	% Agreement	Mean IC Ct	SD IC Ct	%CV IC Ct
Negative	24	Undet.	-	-	100%	28.15	0.58	2.06
3X LoD	24	36.89	0.55	1.48	100%			
10X LoD	24	35.03	0.46	1.32	100%			

In the Inter- site Reproducibility test, the HCV ELITe MGB Kit detected the target and showed a maximum variability of target Ct values as %CV equal to 1.48 %.

The Reproducibility of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of Plasma specimens negative or spiked with with HCV (6th WHO International Standard, NIBSC).

A summary of Inter-Instrument Reproducibility (on three instruments) is shown in the tables below:

ELITe InGenius Inter – Instrument Reproducibility								
Sample	HCV					Internal Control		
	N	Mean Ct	SD	% CV	% Agreement	Mean Ct	SD	% CV
Negative	24	Undet.	-	-	100%	27.69	0.34	1.24
3x LoD	24	37.03	0.53	1.44	100%			
10x LoD	24	33.97	0.38	1.11	100%			

ELITe BeGenius Inter – Instrument Reproducibility								
Sample	HCV					Internal Control		
	N	Mean Ct	SD	% CV	% Agreement	Mean Ct	SD	% CV
Negative	24	Undet.	-	-	100%	28.52	0.61	2.13
3 x LoD	23	38.00	0.71	1.86	100%			
10 x LoD	24	36.44	0.67	1.84	100%			

A summary of Inter-Batch Reproducibility (on three lots) is shown in the tables below.

ELITe InGenius Inter – batch Reproducibility								
Sample	HCV					Internal Control		
	N	Mean Ct	SD	% CV	% Agreement	Mean Ct	SD	% CV
Negative	48	Undet.	-	-	100%	27.70	0.33	1.20
3x LoD	48	37.32	0.64	1.72	100%			
10x LoD	48	35.44	0.38	1.07	100%			

ELITe BeGenius Inter – batch Reproducibility								
Sample	HCV					Internal Control		
	N	Mean Ct	SD	% CV	% Agreement	Mean Ct	SD	% CV
Negative	48	Undet.	-	-	100%	28.20	0.40	1.41
3 x LoD	48	37.60	0.71	1.88	100%			
10 x LoD	48	35.78	0.48	1.33	100%			

In the Inter - Instrument and Inter - batch Reproducibility test, the HCV ELTe MGB Kit correctly detected all the samples as expected and showed a maximum variability of target Ct values %CV equal to 1.88%.

Conversion factor to International Units

The Conversion factor to report the quantitative results in International Units/mL starting from copies/mL, was calculated using the certified calibrated reference material "6th WHO HCV International Standard" (NIBSC).

The Conversion factor was determined as 2.4 IU / copy.

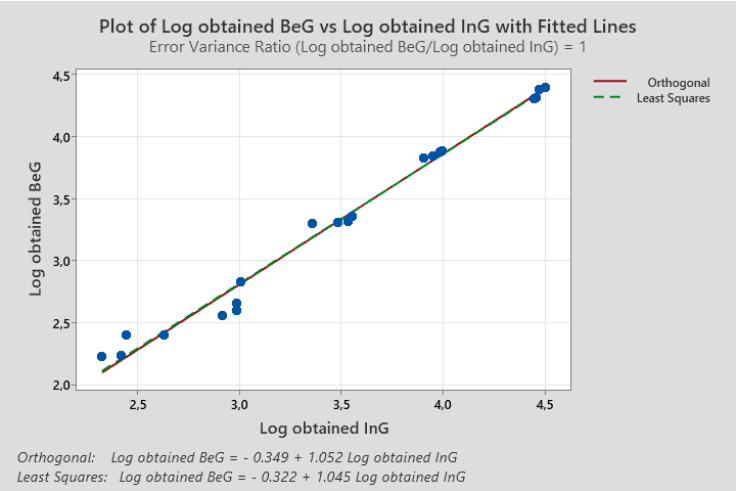
A summary of results is shown in the table below.

Conversion factor to International Units, Fc = 2.4 IU / copy						
Sample			Result			Log difference (ref. - test)
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	
31,623	4.5000	27	13,233	31,254	4.4807	+0.0193
10000	4.0000	27	4,482	10,595	4.0133	+0.0133
3162	3.5000	27	1414	3,342	3.5091	-0.0091
1000	3.0000	27	439	1036	2.9969	+0.0031

As the equivalence between EDTA Plasma, ACD Plasma and Serum was previously demonstrated, the Conversion factor can be applied to the three matrices.

The Conversion factor, to report the quantitative results in International Units / mL starting from copies / mL, was verified on **ELITe BeGenius** and **ELITe InGenius instruments** using the certified calibrated reference material ("6th WHO International Standard, NIBSC).

The results obtained were analysed by orthogonal and linear regression in order to calculate their correlation.



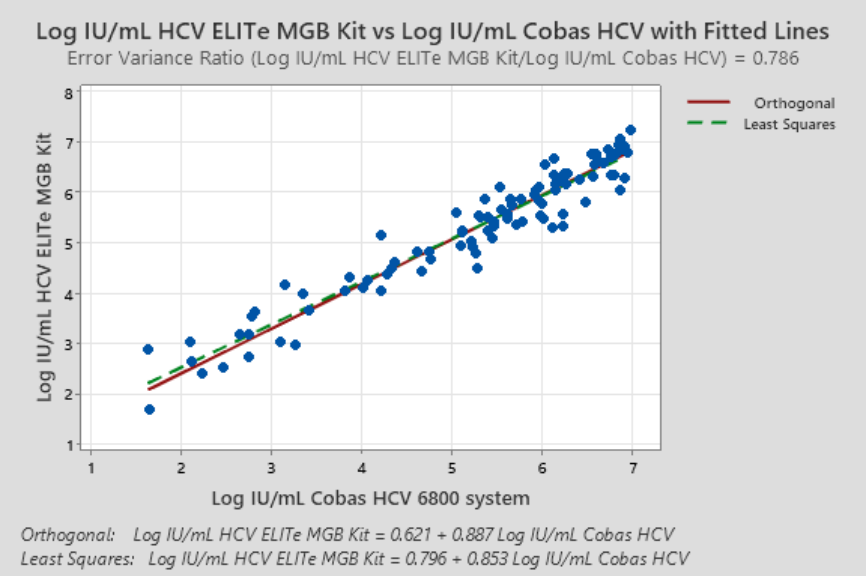
The Orthogonal Regression analysis generated an intercept equal to - 0.3494 (95% CI: - 0.5546; - 0.1442) and a slope equal to 1.0523 (95% CI: 0.9943; 1.1103). The linear regression analysis generated an R^2 of 0.985.

Diagnostic Sensitivity: method correlation

The Diagnostic Sensitivity of the assay, assessed by correlation analysis of different methods, was evaluated at three different sites on **ELITe InGenius** by analysing HCV RNA positive clinical samples from patients undergoing antiviral therapy whose viral load was within the measuring range of the HCV ELITe MBG Kit and of CE IVD marked molecular diagnostic reference methods. The results obtained with the HCV ELITe MBG Kit and the reference method were analysed by Deming and linear regression.

The correlation study was performed at one site on 95 HCV RNA positive clinical samples of plasma collected in EDTA using the "cobas® HCV for use on the 6800 System" as comparator.

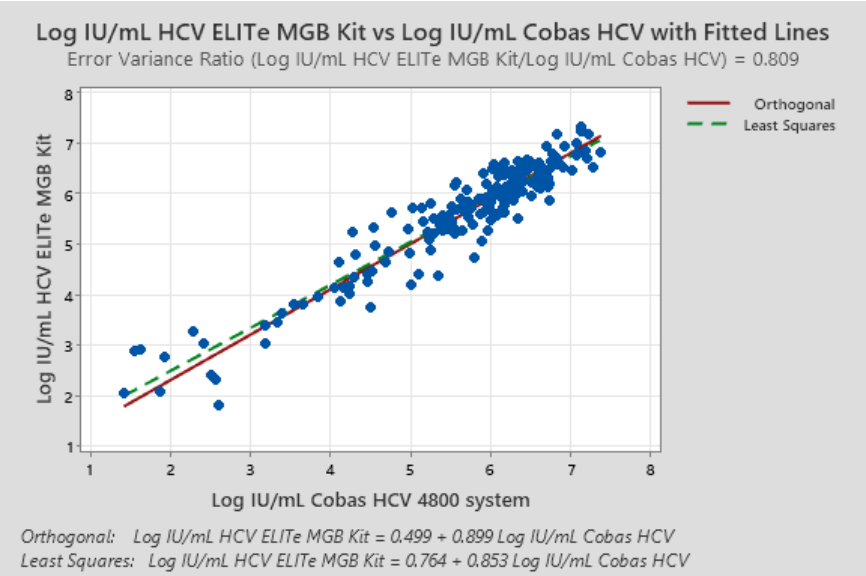
The results are summed up in the following figure.



In this test, the Deming regression analysis generated a slope equal to 0.887 (95% IC: 0.836; 0.938) and an intercept equal to 0.621 (95% CI: 0.345; 0.896). The linear regression analysis generated an R^2 of 0.926.

The correlation study was performed at two other sites on 184 HCV RNA positive clinical samples of plasma collected in EDTA and serum using the “cobas® HCV for use on the 4800 System” as comparator.

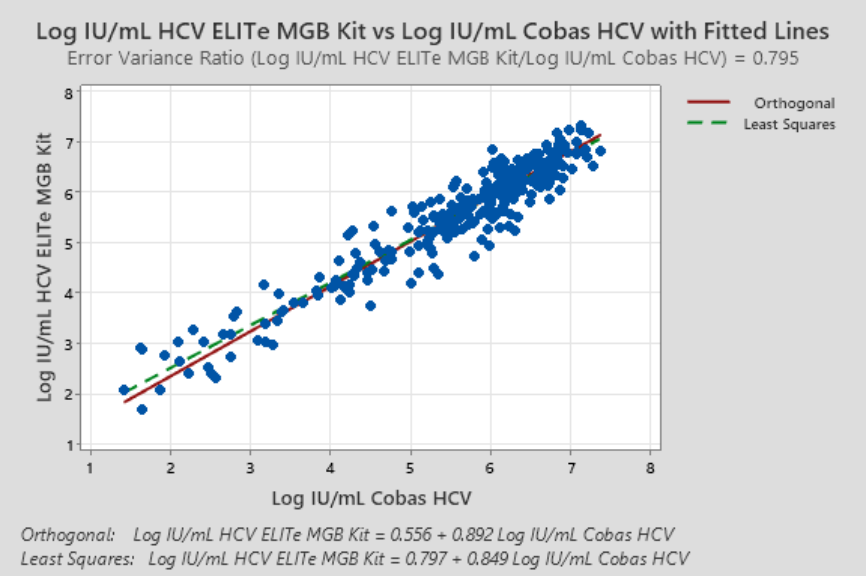
The results are summed up in the following figure.



In this test, the Deming regression analysis generated a slope equal to 0.899 (95% IC: 0.856; 0.943) and an intercept equal to 0.499 (95% CI: 0.245; 0.753). The linear regression analysis generated an R^2 of 0.899.

As the two reference methods (“cobas® HCV for use on the 4800 System” and “cobas® HCV for use on the 6800 System”, Roche Diagnostics) have equivalent performances, the correlation study was also performed on the merged results obtained at the three different sites.

The results are summed up in the following figure.



In this test, the Deming regression analysis generated a slope equal to 0.892 (95% IC: 0.858; 0.926) and an intercept equal to 0.556 (95% CI: 0.363; 0.749). The linear regression analysis generated an R^2 of 0.905.

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

Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, assessed by Negative Percent Agreement, was evaluated at three different sites on **ELITe InGenius** by analysing HCV RNA negative clinical samples tested by CE IVD marked molecular diagnostic reference methods.

The results of the Diagnostic Specificity study, after discrepant analysis, are summarized in the following table, both differentiated by the reference method ("cobas® HCV for use on the 4800 System" and "cobas® HCV for use on the 6800 System", Roche Diagnostics) and merged, as they have equivalent performances.

HCV RNA negative EDTA Plasma and serum samples	N	Positive	Negative	Diagnostic Specificity
Reference: cobas HCV for use on the 6800 System	100	0	100	100%
Reference: cobas HCV for use on the 4800 System	222	5	217	97.7%
Merged results	322	5	317	98.4%

Five samples gave discordant positive results with low titers (4 out of 5 samples having titers below the LoD of the HCV ELITe MBG Kit and of the reference methods), which may randomly generate positive calls.

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

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File for the "HCV ELITe MGB® Kit", FTP601ING.

REFERENCES

P. Halfon et al. (2006) J Clin Microbiology 44: 2507 – 2511

K. Linnet et al. (2004) *Clin. Chem.* 50: 732 - 740.

E. A. Lukhtanov et al. (2007) *Nucleic Acids Res*. 35: e30.

WHO Prequalification of In Vitro Diagnostics Public Report (WHO PQ Public Report), number PQDx 0465-118-00, 03/2021, version 1.0.

https://ltd.aruplab.com (test number 3000572).

https://mayocliniclabs.com (Test ID: HCVQN).

H. H. Kessler et al. (2001) J. Clin. Microb. 39: 1788 - 1790.

K. Sener et al. (2010) J. Clin. Lab. Analysis 24: 134 - 138.

C. Baleriola et al. (2011) J. Clin. Microbiol. 49: 3163 – 3167

WHO (World Health Organization), 2018, Guidelines for the care and treatment of persons diagnosed with chronic hepatitis C virus infection.

CDC (Centers for Disease Control and Prevention), 2020, CDC Recommendations for Hepatitis C Screening Among Adults — United States, 2020.

AASLD (American Association for the study of liver diseases) and IDSA (The Infectious Diseases Society of America), 2022, HCV Guidance: Recommendations for testing, managing and treating Hepatitis C.

EASL (European Association for the Study of the Liver), 2020, EASL recommendations on treatment of Hepatitis C 2020", Journal of Hepatology.

Ministero della Salute, 2022, Linee di indirizzo Nazionali sui percorsi diagnostico terapeutici assistenziali per l'infezione da virus dell'epatite C.

PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: Plasma collected in EDTA or ACD, Serum.

Plasma collected in EDTA or in ACD and Serum shall be obtained from whole blood stored at room temperature or +2 / +8 °C for no longer than 24 hours.

Do not use Plasma collected in heparin with this product: heparin inhibits the amplification reaction of nucleic acids and causes invalid results.

Do not use haemolytic Plasma with this product: haemoglobin inhibits the amplification reaction of nucleic acids and causes invalid results.

Currently there are no data available concerning product performance with other clinical samples such as whole blood.

This product is not intended to be used for screening or to detect the presence or the exposure to transmissible agents in blood, blood components, cells, tissues, organs or any of their derivatives in order to assess their suitability for transfusion, transplantation or cell administration.

The product is not intended for use as a diagnostic test to confirm the presence of HCV infection.

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

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

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

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

This product requires the use of personal protective equipment and instruments dedicated to work

session setup to avoid false positive results.

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

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

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

A negative result obtained with this product indicates that the target RNA is not detected in the RNA extracted from the sample; however, it cannot be excluded that the target RNA has a lower titer than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

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

Possible polymorphisms, insertions or deletions within the region of the RNA targeted by the product primers and probes may impair detection and quantification of target RNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted in combination with all relevant clinical observations and laboratory results.

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

TROUBLESHOOTING

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture, Q-PCR Standards and Positive Control. Check the volumes of complete reaction mixture, Q-PCR Standards and Positive Control.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Degradation of complete reaction mixture or of its components	Do not use the complete reaction mixture 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. 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.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 2 independent sessions (2 hours each in the Extraction Area or in the Cooler Unit). 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 new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

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

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture, Internal Control and sample. Check the volumes of complete reaction mixture, Internal Control 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 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. Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Prepare again the complete reaction mixture. Use a new aliquot of components.
Internal Control template degradation.	Use a new aliquot of Internal Control.
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.

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 Standards or 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.

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.

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

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.

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. At the moment of the current revision of the IFU, no serious incident or recall with impact on product performance and safety of the device has occurred.

A “Summary of Safety and Performance” will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional. 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.

NOTICE TO PURCHASER: LIMITED LICENSE

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ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers 7319022, 7348146, 7381818, 7541454, 7671218, 7718374, 7723038, 7759126, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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

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HCV ELITe MGB® Kit used in association with Genius series®

Ref: RTK601ING

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 **HCV ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids reverse transcription and Real-Time PCR assay for the detection and quantification of the RNA of Hepatitis C Virus (HCV), extracted from clinical specimens.

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

The product is intended for use as an aid in the management of HCV-infected individuals undergoing antiviral therapy.

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

The product is not intended to be used for screening or to detect the presence of or exposure to transmissible agents in blood, blood components, cells, tissues, organs or any of their derivatives in order to assess their suitability for transfusion, transplantation or cell administration. The product is not intended for use as a screening test for the presence of HCV in blood or blood products or as a diagnostic test to confirm the presence of HCV infection.

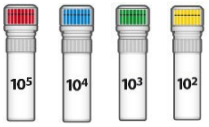
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	HCV 5' UTR region	FAM	HCV
Internal Control	MS2	AP525	IC

Validated matrices

- **Plasma EDTA or Plasma ACD**
- **Serum**

Kit content

HCV ELITe MGB Mix		HCV ELITe Standard	HCV - ELITe Positive Control	HCV Internal Control
 X 4	 X 2	 X 1	 X 2	 X 8
HIV PCR Mix 4 tubes of 600 µL 24 reactions per tube 96 reactions per kit 5 freeze-thaw cycles	RT EnzymeMix 2 tubes of 20 µL 48 reactions per tube 96 reactions per kit 10 freeze-thaw cycles	Ready-to-use Calibrators: 4 levels 1 set of 4 tubes of 160 µL 2 reactions per tube 2 reactions per kit 2 freeze-thaw cycles	Ready-to-use Positive Control 2 tubes of 160 µL 4 reactions per tube 8 reactions per kit 4 freeze-thaw cycles	Ready-to-use Internal Control 8 tubes of 160 µL 12 extraction per tube 96 extractions per kit 6 freeze-thaw cycles

Maximum shelf-life: **18 months**

Storage Temperature: **-20 °C**

Other products required not provided in the kit

- ELITe InGenius instrument: INT030
- ELITe BeGenius instrument: INT040
- ELITe InGenius SP 1000 Extraction Cartridge: INT033SP1000
- ELITe InGenius PCR Cassette: INT035PCR
- ELITe InGenius SP200 Consumable Set: INT032CS
- ELITe InGenius Waste Box: F2102-000
- 300 µL Filter Tips Axigen: TF-350-L-R-S (ELITe InGenius only)
- 1000 µL Filter Tips Tecan: 30000631 (ELITe BeGenius only)

ELITe InGenius and ELITe BeGenius protocol

Sample volume	600 µL	Unit of quantitative result	International Unit: IU/mL Copies: Copies/mL
HCV CPE volume	10 µL	Conversion factor to IU	2.4 IU/copy
Total elution volume	50 µL	Frequency of controls	15 days
PCR elution input volume	20 µL	Frequency of calibration	60 days
Complete PCR Mix volume	20 µL		

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Diagnostic Sensitivity: Method Correlation	Diagnostic Specificity
Plasma / Serum	**26 IU / mL** 11 copies / mL	**R^2 = 0.905** *279 quantified samples*	**98.4%** *317 confirmed samples / 322 tested samples*

reference methods:
"cobas® HCV for use on the 4800 Systems" and
"cobas® HCV for use on the 6800 Systems", Roche Diagnostics.

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.

	Transport/Storage conditions			
Sample type	**+16 / +26 °C (room temperature)**	**+2 / +8 °C**	**-20 ±10 °C**	**-70 ±15 °C**
Plasma collected in EDTA	≤ 1 day	≤ 3 days	≤ 1 month	≤ 6 months
Serum	≤ 1 day	≤ 5 days	≤ 1 month	≤ 6 months

Do not use Plasma collected in heparin to prevent inhibition of amplification reaction and frequent invalid results.
Do not use haemolytic Plasma, as hemoglobin inhibits the PCR reaction.

ELITe InGenius Procedures

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

Before analysis

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

2. Verify calibrators: **Q-PCR Standard** in the "Calibration" menu
Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu.
Note: All must have been run, approved and not expired

3. Thaw the **PCR Mix** and the **CTRCPE** tubes
Vortex gently
Spin down 5 sec
Keep the **RT EnzymeMix** in ice or cool block.

4. Prepare the complete reaction mixture as follows

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

5. Vortex gently
Spin down 5 sec
Keep the complete reaction mixture in ice or cool block. Do not expose to direct light.

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

1. Select "Perform Run" on the touch screen

2. Verify the extraction volumes: Input: "1000 µL", elution: "50 µL"

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

4. Select the "Assay Protocol" of interest: HCV ELITe_PL_600_50 or HCV ELITe_Se_600_50

5. Select the method "Extract + PCR" and the sample position: Extraction Tube

6. Load the complete reaction mixture and the Internal Control in the Inventory Block

7. Load: PCR Cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks

8. Close the door.
Start the run

9. View, approve and store the results

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

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

1 to 4: Follow the Procedure 1 described above (select the Assay Protocols: HCV ELITe_PC and HCV ELITe_NC or HCV ELITe_STD)	**5.** Select the method "PCR Only" and set the sample position "Elution Tube"	**6.** Load the complete reaction mixture in the Inventory Block
7. Load: PCR Cassette rack and the Elution tube rack with the extracted nucleic acids, standards or controls	**8.** Close the door Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe BeGenius software to setup the run. All the steps: extraction, 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 calibrators: **Q-PCR Standard** in the "Calibration" menu Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu *Note*: All must have been run, approved and not expired	**3.** Thaw the **PCR Mix** and the **CTRCPE** tubes Vortex gently Spin down 5 sec Keep the **RT EnzymeMix** in ice or cool block.

4. Prepare the complete reaction mixture as follows

Sample Number (N)	HCV 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
13 ≤ N ≤ 18	(N + 3) x 20 µL	(N + 3) x 0.3 µL
19 ≤ N ≤ 23	(N + 4) x 20 µL	(N + 4) x 0.3 µL
N = 24	580 µL	8.7 µL

5. Vortex gently
Spin down 5 sec
Keep the complete reaction mixture in ice or cool block. Do not expose to direct light.

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: "600 µL", Eluate: "50 µL"
4. Select the "Assay Protocol" of interest: HCV ELITe_Be_PL_600_50 or HCV ELITe_Be_Se_600_50 **Note**: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit.	**6.** Load the complete reaction mixture and the **CPE** in Reagent/Elution Rack and insert it in the Cooler Unit.
7. Load "PCR Rack" with "PCR Cassette" and the "Extraction Rack" with the "ELITe InGenius SP 1000" 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, standards, 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 standards or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit	**3.** Verify the extraction volumes: Input: "600 µL", Eluate: "50 µL"
4. Select the "Assay Protocol" of interest (HCV ELITe_Be_PC and HCV ELITe_Be_NC or HCV ELITe_Be_STD)	**5.** Load the complete reaction mixture in Reagent Rack and insert it in the Coolre Unit	**6.** Load "PCR Rack" with "PCR Cassette".
7. Close the door. Start the run	**8.** View, approve and store the results	