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EMPOWERING IVD

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NOTICE of CHANGE dated 07/06/2024

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:

- *Other products required: note regarding availability of HCV ELITe Standard (Ref STD601ING) and HCV - ELITe Positive Control (Ref CTR601ING) as separate products.*
- *ELITe BeGenius Procedure: detailed instructions for preparing the complete reaction mixture for samples number greater than 12 in association with ELITe BeGenius instrument.*
- *ELITe BeGenius Procedure: typing correction in Assay Protocol description.*
- *Warning and Precautions specific for the components: extension of the period of use of each reagent tube up to 60 days from first opening.*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIE REVIEW VON DIESER IFU IST KOMPATIBLE MIT DER VORIGE VERSION VON DEM TEST-KIT

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HCV ELITe MGB® Kit

reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

CE 0344

IVD

-20°C

TABLE OF CONTENTS

INTENDED USE

The **HCV ELITe MGB® Kit** product is quantitative nucleic acids reverse transcription and amplification assay for the **detection and quantification of the RNA** of Hepatitis C virus **(HCV)** in RNA samples extracted from clinical specimens.

The assay is able to detect the RNA of HCV belonging to 1, 2, 3, 4, 5 and 6 genotypes.

The assay is validated in association with **ELITe InGenius®** and **ELITe BeGenius®** systems starting from human plasma samples collected in EDTA or in ACD and serum samples.

The product is intended for use as an aid in the management of HCV-infected individuals undergoing antiviral therapy, together with patient's clinical data and other laboratory test results.

This 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.

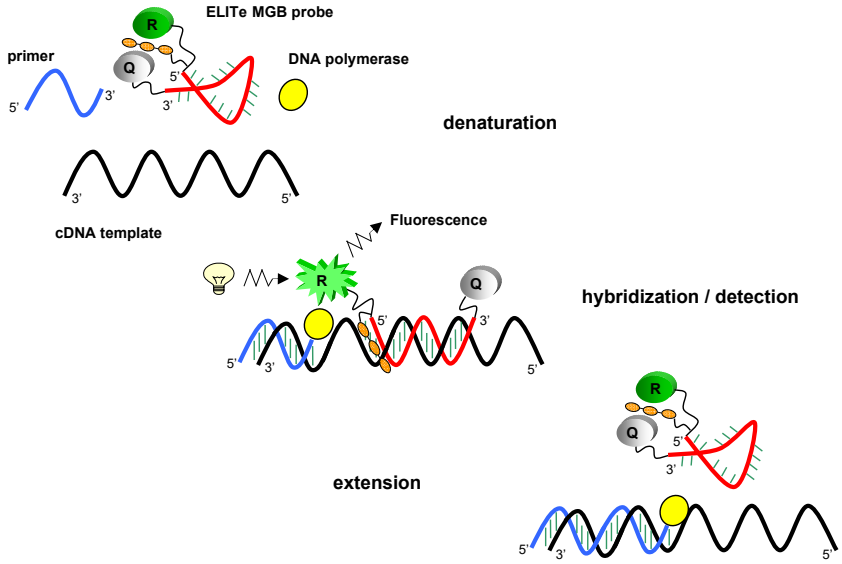
ASSAY PRINCIPLE

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

Starting from RNA extracted by **ELITe InGenius** from sample to be tested, the complete HCV PCR Mix carries out a reaction of reverse transcription and amplification specific for the 5' UTR region of HCV and for a region of the genomic RNA of MS2 phage (exogenous Internal Control of extraction and inhibition).

The HCV specific probe with ELITe MGB® technology, labelled by FAM fluorophore, is activated when hybridized with the specific product of the HCV amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled by AP525 fluorophore, is activated when hybridized with the specific product of Internal Control amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data determines the presence and the titre of HCV RNA in the sample.

In the following picture is synthetically shown the mechanism of activation and fluorescence emission of ELITe MGB technology probe.



PRODUCT DESCRIPTION

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

- **HCV ELITe MGB Mix**

This component provides the following two sub-components:

- "**HCV PCR Mix**", an optimized and stabilized mixture of oligonucleotides and reagents for reverse transcription and real-time amplification, pre-aliquoted into **four test tubes** (WHITE cap). Each tube contains **600 µL** of solution, enough for **24 tests** (processing at least 5 samples per session) in association with **ELITe InGenius** and **ELITe BeGenius.**
  Primers and probes for HCV (stabilized by MGB® group, labelled by FAM fluorophore and quenched by Eclipse non-fluorescent moiety) are specific for the 5' UTR region of HCV. The HCV signal is detected by Channel 1 (HCV) of the **ELITe InGenius** and **ELITe BeGenius**.
  Primers and the probe for Internal Control (stabilized by MGB® group, labelled by AP525 fluorophore and quenched by Eclipse non-fluorescent moiety) are specific for a region of the phage **MS2** genomic RNA. The Internal Control signal is detected by Channel 2 (IC) of the **ELITe InGenius** and **ELITe BeGenius**.
  The reaction mixture provides also the buffer, magnesium chloride, the nucleotide triphosphates and the DNA Polymerase enzyme with hot start capability.
- "**RT EnzymeMix**", an optimized and stabilized mixture of enzymes for reverse transcription, pre-aliquoted into **two test tubes** (cap with BLACK insert). Each tube contains **20 µL** of solution, sufficient for **48 tests** (processing at least 5 samples per session) in association with **ELITe InGenius** and **ELITe BeGenius**.

The two sub-components are sufficient for **96 tests in association with ELITe InGenius** and **ELITe BeGenius**, by using respectively 20 µL and 0.3 µL for reaction.

- **HCV ELITe Standard**

This component provides the sub-components "**HCV Q-PCR Standard**", four stabilized solutions of plasmid DNA at **known titre**, each aliquoted into **one ready to use test tube**. Each tube contains **160 µL** of solution, sufficient for **2 sessions**. The plasmid DNA contains the 5' UTR region of HCV. The detection and quantification of HCV DNA as result of the analysis by **HCV ELITe MGB Mix** component in association with **ELITe InGenius** and **ELITe BeGenius** instruments allows to calculate the standard curve of the system (product batch and instrument) for HCV quantification.

The plasmid DNA concentration in copies / mL was determined through absorbance measurement by spectrophotometer. This plasmid DNA concentration was related to the "WHO International Standard 6th HCV International Standard" (NIBSC, UK, code 18/184) by a conversion factor to allow calculation of concentration in International Unit / mL (IU / mL).

The component is sufficient for **2 separate analytic sessions in association with ELITe InGenius** and **ELITe BeGenius**, by using 20 µL for reaction.

- **HCV - ELITe Positive Control**

This component provides the sub-component "**HCV Positive Control**", a stabilized solution of plasmid DNA at **known titre**, aliquoted into **two ready-to-use test tubes**. Each test tube contains **160 µL** of solution, sufficient for **4 sessions**. The plasmid DNA contains the 5' UTR region of HCV. The detection and quantification of target DNA as result of the analysis with **HCV ELITe MGB Mix** component in association with **ELITe InGenius** and **ELITe BeGenius** instruments allows to validate the system (product batch and instrument) for HCV detection and quantification.

The component is sufficient for **8 separate analytic sessions in association with ELITe InGenius** and **ELITe BeGenius**, by using 20 µL for reaction.

- **HCV Internal Control**

This component provides the sub-component "**HCV CPE**", a stabilized solution of MS2 genomic RNA aliquoted into **eight ready-to-use test tubes**. Each tube contains **160 µL** of solution, sufficient for **12 tests** (processing at least 2 samples per session). The MS2 genomic RNA is used as exogenous Internal Control template. The detection of MS2 cDNA as result of the analysis with **HCV ELITe MGB Mix** component in association with **ELITe InGenius** and **ELITe BeGenius** instruments allows to validate the results of HCV negative samples.

The component is sufficient for **96 tests in association with ELITe InGenius** and **ELITe BeGenius,** by using 10 µL for extraction.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

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 amplification in tube with **WHITE cap**	**4 x 600 µL**	-
	RT EnzymeMix ref. RTS003-RT	Reverse transcriptase 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 **NEUTRAL cap**	**8 x 160 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Sarstedt 2.0 mL tube skirted screw-cap (Sarstedt Ref. 72.694.005).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of RNA from the samples to be analyzed and the consumables are **not** included in this product.

For automatic nucleic acids extraction, reverse transcription, Real Time amplification and result interpretation of samples to be analysed, the instrument **ELITe InGenius** and **ELITe BeGenius** (ELITechGroup S.p.A., EG SpA, ref. INT030) and the following specific **Assay Protocols** (EG SpA) are required:

- parameters for calibrators amplification **«HCV ELITe_STD»**,
- parameters for positive control amplification **«HCV ELITe_PC»**,
- parameters for negative control amplification **«HCV ELITe_NC»**,
- parameters for Plasma samples to be analyzed **«HCV ELITe_PL_600_50»**,
- parameters for Serum samples to be analyzed **«HCV ELITe_Se_600_50»**

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

- extraction cartridges **«ELITe InGenius® SP 1000»** (EG SpA, ref. INT033SP1000),
- consumables for extraction **«ELITe InGenius® SP 200 Consumable Set»** (EG SpA, ref. INT032CS),
- amplification cartridges **«ELITe InGenius® PCR Cassette»** (EG SpA, ref. INT035PCR),
- tips **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S),
- boxes **«ELITe InGenius® Waste Box»** (EG SpA, ref. F2102-000).

For nucleic acid extraction and sample analysis with the **«ELITe BeGenius»** (ELITechGroup S.p.A., ref. INT040) the following **Assay Protocols** (ELITechGroup S.p.A.) are required:

- parameters for calibrators amplification **«HCV ELITe_Be_STD»**,
- parameters for positive control amplification **«HCV ELITe_Be_PC»**,
- parameters for negative control amplification **«HCV ELITe_Be_NC»**,
- parameters for Plasma samples to be analyzed **«HCV ELITe_Be_PL_600_50»**,
- parameters for Serum samples to be analyzed **«HCV ELITe_Be_Se_600_50»**.

With the instrument **ELITe BeGenius** the following generic products are required:

- extraction cartridges **«ELITe InGenius® SP 1000»** (EG SpA, ref. INT033SP1000),
- consumables for extraction **«ELITe InGenius® SP 200 Consumable Set»** (EG SpA, ref. INT032CS),
- amplification cartridges **«ELITe InGenius® PCR Cassette»** (EG SpA, ref. INT035PCR),
- tips **«1000 µL Filter Tips Tecan»** (Tecan, Switzerland, ref. 30180118)
- boxes **«ELITe InGenius® Waste Box»** (EG SpA, ref. F2102-000).

Note: in case of need, the Calibrators and the Positive Control are also available as separate products: **HCV ELITe Standard,** ref. STD601ING, and **HCV - ELITe Positive Control,** ref. CTR601ING.

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121°C before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in the work areas.
Carefully wash hands after handling samples and reagents.
Dispose of leftover reagents and waste in compliance with the regulations in force.
Carefully read all the instructions provided with the product before running the assay.
While running the assay, follow the instructions provided with the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided with the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

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

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.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

The tips used must be sterile, free from DNases and RNases and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The reagents required for the session must be prepared in such a way that they can be used in a single day. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, free from DNA and RNA.

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

The PCR Cassettes must be handled in order to avoid amplification product diffusion into the environment and sample and reagent contamination.

Warnings and precautions specific for the components

- **HCV ELITe MGB Mix**

The **HCV PCR Mix** must be stored at temperature lower than -20 °C in the dark.
The **HCV PCR Mix** must be used within 60 days from the first tube opening.
The **HCV PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.
The **RT EnzymeMix** must be stored at temperature lower than -20 °C.
The **RT EnzymeMix** must be used within 60 days from the first tube opening.
The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than 10 minutes during each use.
The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than **ten times**: further uses may cause a loss of product performances.

- **HCV ELITe Standard**

The **HCV Q-PCR Standard** must be stored at temperature lower than -20°C.
The **HCV Q-PCR Standard** must be used within 60 days from the first tube opening.
The **HCV Q-PCR Standard** can be frozen and thawed for no more than **two times**: further freezing / thawing cycles may cause a loss in titre.
The **HCV Q-PCR Standard** can be kept on board in the **ELITe InGenius** or in the **ELITe BeGenius** instruments up **two independent work sessions of two hours each** ("PCR Only" run mode).

- **HCV - ELITe Positive Control**

The **HCV Positive Control** must be stored at temperature lower than -20 °C.
The **HCV Positive Control** must be used within 60 days from the first tube opening.
The **HCV Positive Control** can be frozen and thawed for no more than **four times**: further freezing / thawing cycles may cause a loss of product performances.
The **HCV Positive Control** can be kept on board in the **ELITe InGenius** or in the **ELITe BeGenius** instruments up **four independent work sessions of three hours each** ("Extraction + PCR Only" run mode).

- **HCV Internal Control**

The **HCV CPE** must be stored at temperature lower than -20 °C.
The **HCV CPE** must be used within 60 days from the first tube opening.
The **HCV CPE** can be frozen and thawed for no more than **twelve times**: further freezing / thawing cycles may cause a loss of product performances.
The **HCV CPE** can be kept on board in the **ELITe InGenius** or in the **ELITe BeGenius** instruments up **six independent work sessions of three hours each** ("Extraction + PCR" run mode).

ELITe InGenius

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Plasma collected in EDTA or ACD

Plasma samples for nucleic acid extraction, must be collected in EDTA or ACD, identified according to laboratory guidelines, transported and stored at room temperature (+18/+25 °C) for a maximum of 24 hours or at +2 / +8 °C for a maximum of 3 days. Otherwise, they must be frozen and stored at -20 °C for a maximum of 1 month or at -70 °C for 6 months.

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

Note: The RNA extraction from plasma collected in EDTA or ACD is carried out with the **ELITe InGenius** system and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), using the Assay protocol **HCV ELITe_PL_600_50**. This protocol processes 600 µL of sample, starting from secondary tube, adds 10 µL per extraction of the **HCV CPE** (Internal Control) and elutes the nucleic acids in 50 µL. Primary tube cannot be used in association with the Assay Protocol.

Purified nucleic acids can be stored at -20 °C for one month.

Serum

Serum samples, intended for RNA extraction, must be collected and identified according to laboratory guidelines, transported and stored at room temperature (~+25 °C) for a maximum of three days or at +2 / +8 °C for a maximum of five days. Otherwise, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for 6 months.

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

Note: The RNA extraction from serum is carried out with the **ELITe InGenius** system and with **ELITe InGenius Software** version 1.3 (or later equivalent versions) using the Assay Protocol **HCV ELITe_Se_600_50**. This protocol processes 600 µL of sample, adds 10 µL per extraction of the **HCV CPE** (Internal Control) and elutes the nucleic acids in 50 µL. Primary tube cannot be used in association with the Assay Protocol.

Purified nucleic acids can be stored at -20 °C for one month.

Other samples

At the moment there are no data available concerning product performance with other clinical samples such as whole blood or CSF.

Interfering substances

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

Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

Do not use haemolytic Plasma in order to prevent inhibition of amplification reaction and frequent invalid results.

Amplification controls

Before analysing any sample, it is absolutely mandatory to generate and to approve the Calibration curve and the amplification controls for each lot of amplification reagent:

- as calibrator set, use the four concentration levels of the **HCV ELITe Standard** component provided with this kit, in association with Assay Protocol **HCV ELITe_STD**,

- as amplification Positive Control, use the **HCV - ELITe Positive Control** component provided with this kit, in association with Assay Protocol **HCV ELITe_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with Assay Protocol **HCV ELITe_NC**.

Note: ELITe InGenius system requires approved and valid results of calibration curve and amplification controls for each lot of amplification reagent stored in its database.
The calibration curves, approved and stored in the database, will expire after **60 days**. At expiration date it is necessary to re-run the Q-PCR Standards in association with the amplification reagent lot.
The amplification control results, approved and stored in the database, will expire after **15 days**. At the expiration date it is necessary to re-run the Positive and Negative Controls in association with the amplification reagent lot.
Furthermore, the calibrators and amplification controls must be re-run when:

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

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used. External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable.

PROCEDURE

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

- Verification of the system readiness,
- Setup of the session,
- Review and export of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the ELITe InGenius and select the login mode “**CLOSED**”,
- 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 below,verify the amplification Controls (**HCV Positive Control**, **HCV Negative Control**) are approved and valid (Status) for the HCV PCR Mix lot to be used. If no valid Controls are available for the **HCV PCR Mix** lot, run the Controls as described below. This can be checked under the “Control” menu on the Home page;choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB Kits and the ELITe InGenius instrument with the indicated matrices.
- The Assay Protocols available for sample testing with the product **HCV ELITe MGB Kit** are described in the table below:

Assay Protocol for HCV ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
HCV ELITe_PL_600_50	Plasma	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

Assay Protocol for HCV ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
HCV ELITe_Se_600_50	Serum	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

IU, international units

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

Session setup

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

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

All 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 InGenius** system can be linked to the "Laboratory Information Server" (LIS) which enables loading the session information. Refer to the instrument manual for more details.

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

1. Thaw the needed **HCV PCR Mix** (WHITE cap) tubes for 30 minutes at room temperature (~+25 °C). Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix by vortexing for 10 seconds three times and spin down the contents for 5 seconds and keep in ice,

Note: Thaw **HCV PCR Mix** in the dark because this reagent is sensitive to the light.

2. Take the needed **RT EnzymeMix** (cap with BLACK insert) tubes. Each tube is sufficient to set up 48 tests. Gently shake the tubes, spin down the contents for 5 seconds and keep in ice,

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

3. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and mark it in a recognizable manner with a permanent marker,

4. Calculate the volumes of the two sub-components that are needed for preparing the **complete reaction mixture** on the basis of the number of samples to be analyzed, as described in the following table.

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

Sample Number (N)	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 adding into the dedicated 2 mL tube the calculated volumes of the two components.

6. Mix by **vortexing at low speed** for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep in ice.

Note: The **complete reaction mixture** should be used within **7** hours if kept on board in the refrigerated block. The complete reaction mixture **cannot** be stored for re-use.

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

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

The main steps for the setup of the three types of runs are described in the following paragraphs.

A. Integrated run

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

1. Thaw at room temperature (~+25 °C) the test tubes containing the samples to be analysed and handle according to laboratory guidelines and according to paragraph "Samples and Controls". Remember that 600 µL of sample are needed for the analysis.
2. Thaw the **HCV CPE** tubes for the session at room temperature (~+25 °C). Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home" screen.
4. Ensure that the "Extraction Input Volume" is 1000 µL (even if 600 µL of sample will be used) and the "Extracted Elute Volume" is 50 µL.
5. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
6. Select the Assay Protocol to be used in the "Assay" column (e.g. HCV ELITe_PL_600_50).
7. Ensure that the "Protocol" displayed is: "Extract + PCR".
8. Select the sample loading position "Extraction Tube" in the "Sample Position" column. Click "Next" to continue the setup.
   For the analysis 600 µL of sample must be transferred in the "Extraction Tube". Any exceeding volume will be left in the "Extraction Tube" by the **ELITe InGenius**.
9. Load **complete reaction mixture** and **HCV CPE** on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of **HCV PCR Mix** and **HCV CPE**. Click "Next" button to continue the setup.
10. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.
11. Load the **PCR Cassettes**, the **ELITe InGenius SP 1000** extraction cartridges, all the required consumables and the samples to be extracted, following the GUI instruction. Click "Next" to continue.
12. Close the instrument door.
13. Press "Start" to start the run.

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

Note: At the end of the run the remaining Extracted Sample in the "Elution tube" must be removed from the instrument, capped, identified and stored at -20 °C for one month. Avoid spilling the Extracted Sample.

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

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

B. Amplification run (PCR only)

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

1. Thaw at room temperature (~+25 °C) the test tubes containing the extracted nucleic acid samples to be analysed. Mix gently, spin down the content for 5 seconds.
2. Select "Perform Run" from the "Home" screen.
3. Even if no extraction will be carried out, ensure that the "Extraction Input Volume" is 1000 µL (even if 600 µL of sample has been used) and the "Extracted Elute Volume" is 50 µL.
4. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
5. Select the Assay Protocol to be used in the "Assay" column (e.g. HCV ELITe_PL_600_50).

6. Select “PCR Only” in the “Protocol” column.
7. Ensure that the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **HCV PCR Mix**. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the **PCR Cassettes** and the extracted nucleic acid samples following the GUI instruction. Click “Next” to continue.
11. Close the instrument door.
12. Press “Start” to start the run.

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

Note: At the end of the run the remaining Extracted Sample in the “Elution tube” must be removed from the instrument, capped and stored at -20 °C for one month. Avoid the spilling of the Extracted Sample.

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

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

C. Calibration run

To set up the Calibration run with Q-PCR Standards, carry out the following steps as per GUI:

1. Thaw **HCV Q-PCR Standard** tubes (Cal1: HCV Q-PCR Standards 10^2, Cal2: HCV Q-PCR Standards 10^3, Cal3: HCV Q-PCR Standards 10^4, Cal4: HCV Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for preparing 4 reactions. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home” screen.
3. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 1000 µL (even if 600 µL of sample will be used) and the “Extracted Elute Volume” is 50 µL.
4. In the Track of interest, select the Assay Protocol “HCV ELITe_STD” in the “Assay” column and fill in the lot number and expiry date of **HCV Q-PCR Standard**. Click “Next” to continue the setup.
5. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **HCV PCR Mix**. Click “Next” to continue the setup.
6. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
7. Load the **PCR Cassettes** and the **HCV Q-PCR Standard** tubes following the GUI instruction. Click “Next” to continue.
8. Close the instrument door.
9. Press “Start” to start the run.

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

Note: At the end of the run the remaining **HCV Q – PCR Standards** must be removed from the instrument, capped and stored at -20 °C.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

Note: The **HCV Q-PCR Standards** can be used for 2 independent work sessions of 2 hours each.

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

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

D. Amplification run for Positive Control and Negative Control

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

1. Thaw **HCV Positive Control** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for preparing 4 reactions. Mix gently, spin down the content for 5 seconds.
2. As **Negative Control,** transfer at least 50 µL of molecular biology grade water to an “Elution tube”, provided with the **ELITe InGenius SP 200 Consumable Set**.
3. Select “Perform Run” from the “Home screen”.
4. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 1000 µL (even if 600 µL of sample will be used) and the “Extracted Elute Volume” is 50 µL.
5. In the Track of interest, select the Assay Protocol to be used in the “Assay” column.
6. For the Positive Control, select the Assay Protocol “HCV ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of **HCV Positive Control**.
7. For the Negative Control, select the Assay Protocol “HCV ELITe_NC” in the “Assay” column and fill in the lot number and expiry date of the molecular biology grade water. Click “Next” to continue the setup.
8. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **HCV PCR Mix**. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the **PCR Cassettes**, the **HCV Positive Control** tube and the **Negative Control** tube following the GUI instruction. Click “Next” to continue.
11. Close the instrument door.
12. Press “Start” to start the run.

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

Note: At the end of the run the remaining **HCV Positive Control** must be removed from the instrument, capped and stored at -20 °C. The remaining **Negative Control** must be disposed.

Note: The **HCV Positive Control** can be used for 4 independent work sessions of 3 hours each.

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

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

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 sample / Standard / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports (“Sample Report” or “Track Report”). Refer to the instrument user’s manual for more details.

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

The **ELITe InGenius** system generates the results with the product **HCV ELITe MGB Kit** through the following procedure.

A. Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the HCV (Channel “HCV”) of the calibrator reactions with the **“HCV ELITe_STD”** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

The Calibration curve, specific for a PCR reagent lot, are recorded in the database (Calibration). They can be viewed and approved by personnel qualified as “Administrator” or “Analyst”, following the GUI instructions. Calibration curve expire **after 60 days**.

Before analysing samples, a Calibration curve must be generated and approved for the lot of PCR reagent used. Calibration curves with “Approved” (Status) are shown in the “Calibration” window of the ELITe InGenius software.

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 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.

Note: if the Calibration curve is run together with samples and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

B. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probe for HCV (Channel “HCV”) in the Positive and Negative Control amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols “**HCV ELITe_PC**” and “**HCV ELITe_NC**”.

The Positive and Negative Control results, specific for the PCR target lot, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as “Administrator” or “Analyst”, following the GUI instructions. The Positive and Negative Control results expire after 15 days.

The ELITe InGenius software processes the Positive Control and Negative Control results and generates the “Control Charts”. Four Positive Control and Negative Control results, from four different runs are requested to set up the “Control Chart”. After that, the results of Positive control and Negative Control are used for monitoring the amplification step performances. Refer to the user’s manual of the instrument for more details.

Note: if the result of Positive Control or Negative Control 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 a Positive Control or Negative Control is run together with samples to be tested and its result is invalid the samples can be approved but the results are not validated. In this case, the amplification of all samples must be repeated too.

C. Validation of Sample results

The fluorescence signals emitted by the probes for HCV (Channel “HCV”) and by the probe of Internal Control (Channel “IC”) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocols “HCV ELITe_PL_600_50” and “HCV ELITe_Se_600_50”. Results are shown in "Result Display" module.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

The sample run can be approved when the three conditions reported in the table below are met.

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

Sample results are automatically interpreted by the **ELITe InGenius software** algorithm and the Assay Protocol parameters.

The possible result messages are listed in the table below.

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 measurement range of the assay, quantity as shown.
HCV: RNA Detected, quantity below LLoQ copies / mL or IU/mL.	**HCV RNA was detected** in the sample below the lower limit of quantification of the assay.
HCV: RNA Detected, quantity beyond ULoQ copies / mL or IU/mL.	**HCV RNA was detected** in the sample beyond the upper limit of quantification of the assay.
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 HCV RNA or its concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Not valid assay result** caused by Internal Control failure (incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as “Invalid - Retest Sample” are not suitable for result interpretation. In this case, the Internal Control RNA was not efficiently detected due to problems in the reverse-transcription amplification or extraction step (degradation or loss of RNA during the extraction or inhibitors carry-over in the eluate), which may cause incorrect results. If sufficient eluate volume remains, the eluate can be retested (as it 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.

Samples reported as “HCV RNA Not Detected or below LoD" are suitable for analysis but it was not possible to detect HCV RNA. In this case may either be negative for HCV RNA is present at a concentration below the limit of detection of the assay (see “Performance characteristics”).

Samples reported as “HCV: RNA Detected, quantity below LLoQ” are not suitable for quantification. The concentration of HCV RNA detected in the sample is below the level at which it can be accurately quantified. If the sample was diluted before extraction or PCR, it can be retested without dilution (see “Performance characteristics”).

Samples reported as “DNA Detected, quantity beyond ULoQ” are not suitable for quantification. The concentration of HCV RNA detected in the sample is above the level at which it can be accurately quantified. The sample may be diluted before extraction or PCR and retested to yield results within the linear range of the assay.

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

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

Sample result reporting

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

The “Sample Report” shows the results 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

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Plasma collected in EDTA or ACD

Plasma samples for nucleic acid extraction, must be collected in EDTA or ACD, identified according to laboratory guidelines, transported and stored at room temperature (+18 / ~+25 °C) for a maximum of three days or at +2 / +8 °C for a maximum of five days. Otherwise, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for 6 months.

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

Note: The RNA extraction from plasma collected in EDTA or ACD is carried out with the **ELITe BeGenius** system and with **ELITe BeGenius Software** version 2.1.0 (or later equivalent versions) using the Assay Protocol **HCV ELITe_Be_PL_600_50**. This protocol processes 600 µL of sample, adds 10 µL per extraction of the **HCV CPE** (Internal Control) and elutes the nucleic acids in 50 µL.

Purified nucleic acids can be stored at ~-20 °C for one month.

Serum

Serum samples, intended for RNA extraction, must be collected and identified according to laboratory guidelines, transported and stored at room temperature (~+25 °C) for a maximum of three days or at +2 / +8 °C for a maximum of five days. Otherwise, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for 6 months.

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

Note: The RNA extraction from serum is carried out with the **ELITe BeGenius** system and with **ELITe BeGenius Software** version 2.1.0 (or later equivalent versions) using the Assay Protocol **HCV ELITe_Be_Se_600_50**. This protocol processes 600 µL of sample, adds 10 µL per extraction of the **HCV CPE** (Internal Control) and elutes the nucleic acids in 50 µL. Primary tube cannot be used in association with the Assay Protocol.

Purified nucleic acids can be stored at -20 °C for one month.

Other samples

At the moment there are no data available concerning product performance with other clinical samples such as whole blood or CSF.

Interfering substances

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

Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

Amplification controls

Before analysing any sample, it is mandatory to generate and to approve the Calibration curve and the amplification controls for each lot of amplification reagent:

- as calibrator set, use the four concentration levels of the **HCV ELITe Standard** product provided with this kit, in association with Assay Protocol **HCV ELITe_Be_STD**,
- as amplification Positive Control, use the **HCV - ELITe Positive Control** product provided with this kit, in association with Assay Protocol **HCV ELITe_Be_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with Assay Protocol **HCV ELITe_Be_NC**.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

Note: ELITe BeGenius system requires approved and valid results of calibration curve and amplification controls for each lot of amplification reagent stored in its database.
The calibration curves, approved and stored in the database, will expire after **60 days**. At expiration date it is necessary to re-run the Q-PCR Standards in association with the amplification reagent lot.
The amplification control results, approved and stored in the database, will expire after **15 days**. At the expiration date it is necessary to re-run the Positive and Negative Controls in association with the amplification reagent lot.
Furthermore, the calibrators and amplification controls must be re-run when:

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

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used. External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable.

PROCEDURE

Using the **HCV ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and export of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the ELITe BeGenius and select the login mode “**CLOSED**”,
- verify that the Calibrators (**HCV Q-PCR Standard**) are approved valid (Status) for the **HCV PCR Mix** lot to be used. If not Calibrators are available for the **HCV PCR Mix** lot, perform calibration as described below,
- verify that the amplification Controls (**HCV Positive Control**, **HCV Negative Control**) are approved and valid (Status) for **HCV PCR Mix** lot to be used. If no valid amplification Controls are available for the **HCV PCR Mix** lot, run the Controls as described below, choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB Kits and the ELITe BeGenius instrument and the cited matrix.
- The Assay Protocols available for sample testing with the product **HCV ELITe MGB Kit** are described in the table below:

Assay Protocol for HCV ELITe MGB Kit and ELITe BeGenius			
Name	**Matrix**	**Report**	**Characteristics**
HCV ELITe_Be_PL_600_50	Plasma	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
HCV ELITe_Be_Se_600_50	Serum	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

IU, international units

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

Setup of the session

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

A. Integrated run (Extract + PCR),
B. Amplification run (PCR only),
C. Calibration run (PCR only),
D. Amplification run for Positive Control and Negative Control (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** system can be connected to the "Location Information Server" (LIS) which enables loading the session information. Refer to the instrument manual for more details.

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

1. Thaw the needed **HCV PCR Mix** (WHITE cap) tubes for 30 minutes at room temperature (~+25 °C) Each tube is sufficient for **24** reactions in optimized conditions (2 or more tests per session). Mix by vortexing for 10 seconds three times and spin down the content for 5 seconds and keep in ice,

Note: Thaw **HCV PCR Mix** in the dark because this reagent is sensitive to the light.

2. Take the needed **RT EnzymeMix** (cap with BLACK insert) Each tube is sufficient to set up **48 tests**. Gently shake the tubes, spin down the contents for 5 seconds and keep in ice,

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

3. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and mark it in a recognizable manner with a permanent marker,

4. Calculate the volumes of the two sub-components that are needed for preparing the **complete reaction mixture** on the basis of the number of samples to be analyzed, as described in the following table.

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

Sample Number (N)	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 adding into the dedicated 2 mL tube the calculated volumes of the two components.

Note: For more than 12 samples:
- prepare a first 2 mL tube with the **complete reaction mixture** for 12 samples;
- prepare a second 2 mL tube with the **complete reaction mixture** by calculating the volumes of the two sub-components on the basis of the number of samples remaining (N-12)

6. Mix by **vortexing at low speed** for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep in ice.

Note: The **complete reaction mixture** should be used within **7** hours if kept on board in the refrigerated block. 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.

The main steps for the setup of the three types of runs are described in the following paragraphs.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

A. Integrated run

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

1. Thaw at room temperature (+18 / 25 °C) the test tubes containing the samples to be analysed and handle according to laboratory guidelines and according to paragraph "Samples and Controls".

   Remember that 600 µL of sample are needed for the analysis.

2. Thaw the **HCV CPE** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.

3. Select "Perform Run" from the "Home screen".

4. Remove the Racks from the "Cooler Unit" and place them on the preparation table.

5. Select the "run mode": "Extract + PCR".

6. Load the samples into the Racks 5 and 4 (start always from Rack 5).

7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.

Note: If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the sample ID.

8. Check the Extraction Input Volume (600 µL) and the Extracted Elute Volume (50 µL).

9. Select the assay protocol to be used in the "Assay" column (i.e. HCV ELITe_Be_PL_600_50). Click "Next" to continue the setup.

10. If used, repeat step 7 to 9 for Rack 4.

11. Load the Elution tubes into the Racks 3 and 2 (start always from Rack 3).

Note: Elution tubes can be labelled to improve traceability.

12. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.

13. If used, repeat steps 11 and 12 for Rack 2.

14. Load **CPE** and **complete reaction mixture** into the Rack 1.

15. Insert the Rack 1 into the "Cooler Unit". Click "Next" to continue the setup.

16. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.

17. Load the Basket with "PCR Cassette" in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.

18. Load the Basket with the "ELITe InGenius SP 1000" extraction cartridges and the required extraction consumables by following the GUI instruction. Click "Next" to continue the setup.

19. Close the instrument door.

20. Press "Start" to start the run.

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

Note: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped, identified and stored at -20 °C. Avoid the spilling of the Extracted Sample.

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

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

B. Amplification run

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

1. Thaw at room temperature (~+25 °C) the test tubes containing the extracted nucleic acid samples to be analysed. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home screen”.
3. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
4. Select the “run mode”: “PCR Only”.
5. Load the samples into the Racks 3 and 2 (start always from Rack 3).
6. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
7. Even if extraction is not performed, check the Extraction Input Volume (600 µL) and the Extracted Elute Volume (50 µL).
8. Select the assay protocol to be used in the “Assay” column (e.g. HCV ELITe_Be_PL_600_50). Click “Next” to continue the setup.
9. If used, repeat step from 5 to 8 for Rack 2.
10. Load the **complete reaction mixture** into the Rack 1.
11. Insert the Rack 1 into the “Cooler Unit”. Click “Next” to continue the setup.
12. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
13. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
14. Close the instrument door.
15. Press “Start” to start the run.

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

Note: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped, identified and stored at -20 °C. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the “PCR Cassette” with the reaction products must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

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

C. Calibration run

To set up the Calibration run with the Q-PCR Standards, carry out the steps below following the GUI:

1. Thaw **HCV Q-PCR Standard** tubes (Cal1: HCV Q-PCR Standards 10^2, Cal2: HCV Q-PCR Standards 10^3, Cal3: HCV Q-PCR Standards 10^4, Cal4: HCV Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for preparing 2 reactions. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home screen”.
3. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
4. Select the “run mode”: “PCR Only”.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

5. Load the Calibrator tubes into the Rack 3.
6. Select the assay protocol to be used in the “Assay” column (HCV ELITe_Be_STD). Click “Next” button to continue the setup.
7. Load the **complete reaction mixture** into the Rack 2.
8. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
10. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

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

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

Note: The **HCV Q-PCR Standards** can be used for 2 independent work sessions of 2 hours each.

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

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

D. Positive Control and Negative Control run

To setup the amplification run with Positive Control and Negative Control, carry out the steps below following the GUI:

1. Thaw **HCV Positive Control** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for preparing 4 reactions. Mix gently, spin down the content for 5 seconds.
2. Transfer at least 50 µL of the molecular biology grade water (as Negative Control) for the sessions in one Elution tube, provided with the ELITe InGenius SP Consumable Set.
3. Select “Perform Run” from the “Home screen”.
4. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
5. Select the “run mode”: “PCR Only”.
6. Load the Positive Control and Negative Control tubes into the Rack 3.
7. Select the assay protocol to be used in the “Assay” column (HCV ELITe_Be_PC and HCV ELITe_Be_NC). Click “Next” button to continue the setup.
8. Load the **complete reaction mixture** into the Rack 2.
9. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
11. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.

12. Close the instrument door.
13. Press “Start” to start the run.

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

Note: At the end of the run the remaining Positive Control can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Positive Controls. The remaining Negative Control must be disposed.

Note: The **HCV Positive Control** can be used for 4 independent work sessions of 3 hours each.

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

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

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 sample / Calibrator / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports (“Sample Report” or “Track Report”). Refer to the instrument user’s manual for more details.

The **ELITe BeGenius** system generates the results using the HCV ELITe MGB Kit through the following procedure:

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

Note: please, refer to the same **ELITe InGenius** chapters for the details.

PERFORMANCE CHARACTERISTICS
ELITe InGenius and ELITe BeGenius

Limit of Detection (LoD)

The Limit of Detection (LoD) of HCV ELITe MGB Kit was defined in association with Plasma samples and ELITe InGenius system.

The LoD was defined by testing a panel of HCV negative Plasma collected in ACD spiked by HCV certified reference material (6th WHO International Standard, NIBSC) at known titre. Six levels of dilutions were prepared starting from 100 IU / mL to 6 IU / mL. Each dilution level was processed in 24 replicates on ELITe InGenius system in “Extract + PCR” mode. The LoD was estimated by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

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

The LoD as copies / mL for Plasma collected in ACD 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 Plasma collected in ACD 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 30 replicates of Plasma collected in ACD, 30 replicates of Plasma collected in EDTA and 30 replicates of Serum spiked by HCV certified reference material (6th WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 27 out of 30 replicates give a positive result as per CLSI standard EP17-A.

The results are reported in the following table.

Verification of Limit of Detection for Plasma and Serum and ELITe InGenius					
Sample	Titer	Target	N	Positive	Negative
Plasma collected in ACD	26 IU / mL	HCV	30	29	1
Plasma collected in EDTA	26 IU / mL	HCV	30	27	3
Serum	26 IU / mL	HCV	30	27	3

The LoD value for HCV target was confirmed at 26 IU / mL for Plasma collected in ACD, Plasma collected in EDTA and Serum.

The calculated LoD value was verified in association with **ELITe BeGenius** by testing 30 replicates of Plasma collected in ACD 30 replicates of Plasma collected in EDTA and 30 replicates of Serum spiked by HCV certified reference material (6th WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 27 out of 30 replicates give a positive result as per K. Linnet et al. 2004.

The results are reported in the following table.

Verification of Limit of Detection for Plasma, Serum and ELITe BeGenius					
Sample	Titer	Target	N	Positive	Negative
Plasma collected in EDTA	26 IU / mL	HCV	30	30	0
Plasma collected in ACD	26 IU / mL	HCV	30	27	3
Serum	26 IU / mL	HCV	30	27	3

The LoD value for HCV target was confirmed at 26 IU / mL for Plasma collected in ACD, Plasma collected in EDTA and Serum.

Matrix equivalence: Plasma EDTA versus Plasma ACD and Serum

The Equivalence of performances of HCV ELITe MGB Kit was verified using samples of Plasma collected in ACD, Plasma collected in EDTA and Serum in association with ELITe InGenius system.

A test was carried out on 30 samples of Plasma collected in EDTA and 30 samples of Plasma collected in ACD from the same 30 different donors (paired samples), tested negative for HCV by a CE IVD marked immunoassay. The samples were tested on ELITe InGenius system in “Extract + PCR” mode. The percentage negative agreement was evaluated. The percentage Coefficient of Variability (%CV) of Ct values of the Internal Control was calculated in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

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

The same test was carried out on 30 samples of Plasma collected in EDTA and 30 samples of Serum from the same 30 different donors (paired samples), tested negative for HCV by a CE IVD marked immunoassay.

The results are reported in the following table.

Sample	N	Positive	Negative	% Negative agreement	IC Ct %CV	Whole IC Ct %CV
Plasma collected in EDTA	30	0	30	100%	0.90	1.11
Serum	30	0	30		1.28	

A test was carried out on 30 samples of Plasma collected in EDTA and 30 samples of Plasma collected in ACD from the same 30 different donors (paired samples), tested negative for HCV by a CE IVD marked immunoassay and spiked using certified reference material (6th WHO HCV International Standard, NIBSC) at a concentration of 3 x LoD (about 78 IU / mL). The samples were tested on ELITe InGenius system in “Extract + PCR” mode. The percentage positive agreement was evaluated. The percentage Coefficient of Variability (%CV) of Ct values of the HCV target was calculated in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

Sample	N	Positive	Negative	% Positive agreement	HCV Ct %CV	Whole HCV Ct %CV	Bias (Log IU/mL)
Plasma collected in EDTA	30	30	0	100%	1.96	1.79	0.0190
Plasma collected in ACD	30	30	0		1.60		

The same test was carried out on 30 samples of Plasma collected in EDTA and 30 samples of Serum from the same 30 different donors (paired samples), tested negative for HCV by a CE IVD marked immunoassay and spiked using certified reference material (6th WHO HCV International Standard, NIBSC) at a concentration of 3 x LoD (about 78 IU / mL).

The results are reported in the following table.

Sample	N	Positive	Negative	% Positive agreement	HCV Ct %CV	Whole HCV Ct %CV	Bias (Log IU/mL)
Plasma collected in EDTA	30	30	0	100%	1.84	1.81	0.0636
Serum	30	30	0		1.77		

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

Further tests about matrices equivalence were performed during the Linear Measuring Range study.

Linear measuring range

The Linear measuring range of HCV ELITe MGB Kit was determined in association with Plasma samples and ELITe InGenius system.

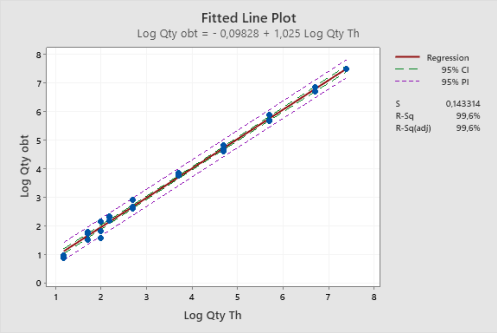
The Linear measuring range was determined using a panel of HCV reference material at known titre (AcroMetrix™ HCV-S Panel) in Plasma collected in EDTA. The panel consisted of ten dilution points from $2.5x10^7$ IU / mL to $1.5x10^1$ IU / mL. Each sample of the panel was tested in 3 replicates on **ELITe InGenius** system in “Extract + PCR” mode.

The analysis of the obtained data, performed by polynomial regression and linear regression, demonstrated that the assay shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.996.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

The results are reported in the following figure.



The Lower Limit of Quantification (LLoQ) was set at the LoD concentration that gives quantitative results precise (Standard Deviation = 0.2787 Log IU / mL) and accurate (Bias = 0.3641 Log IU / mL) within ±0.5 Log IU / mL: 26 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration that gives quantitative results precise (Standard Deviation = 0.0089 Log IU / mL) and accurate (Bias = 0.0794 Log IU / mL) within ±0.5 Log IU / mL: 25,000,001 IU / mL.

The linear measuring range as copy / mL for Plasma EDTA is calculated by applying the specific conversion factor (2.4 IU / copy).

The final results are summarized in the following table.

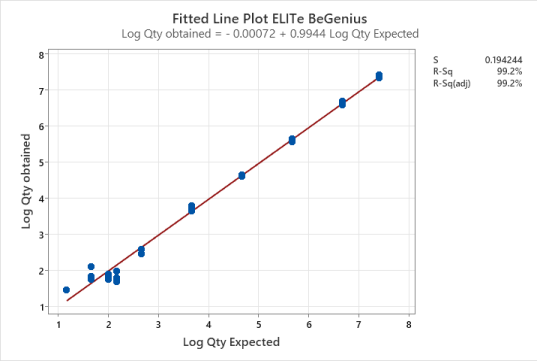
Linear measuring range for Plasma samples and ELITe InGenius	
Lower Limit	Upper Limit
26 IU / mL	25,000,001 IU / mL
11 copies / mL	10.416.667 copies / mL

Note: The product performances in association to plasma EDTA, plasma ACD and serum were demonstrated to be equivalent.

The Linear measuring range of HCV ELITe MGB Kit was verified in association with Plasma samples and **ELITe BeGenius** system using a panel of dilutions of HCV (AcroMetrix™ HCV-S Panel AcroMetrix) in HCV negative samples of Plasma collected in ACD. The panel consisted of ten dilution points from about $2.5x10^7$ IU / mL to 15 IU / mL. Each sample of the panel was tested in 3 replicates on **ELITe BeGenius** system in “Extract + PCR” mode.

The analysis of the obtained data, performed by polynomial regression and linear regression, demonstrated that the assay shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.992.

The results are reported in the following figure.



The Lower Limit of Quantification (LLoQ) was set at the LoD concentration that gives quantitative results precise (Standard Deviation = 0,3762 Log IU / mL) and accurate (Bias = 0.3583 Log IU / mL) within ±0.5 Log IU / mL: 26 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration that gives quantitative results precise (Standard Deviation = 0.0405 Log IU / mL) and accurate (Bias = 0.0064 Log IU / mL) within ±0.5 Log IU / mL: $2.5x10^7$ IU / mL.

The linear measuring range as copy / mL for Plasma ACD is calculated by applying the specific conversion factor (2.4 IU / copy).

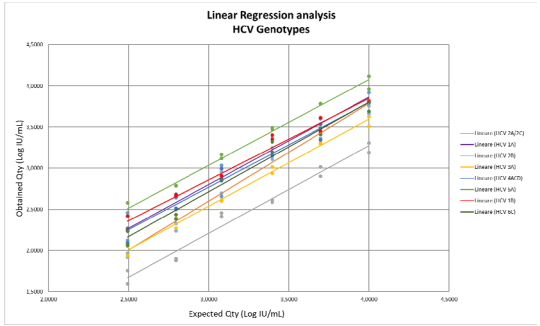
The final results are summarized in the following table.

Linear measuring range for Plasma samples and ELITe BeGenius	
Lower Limit	Upper Limit
26 IU / mL	25,000,001 IU / mL
11 copies / mL	10,416,667 copies / mL

Note: The product performances in association to plasma EDTA, plasma ACD and serum were demonstrated to be equivalent.

The linearity of quantification was verified by analysis of negative Plasma collected in EDTA spiked by HCV reference material (SeraCare) for main HCV genotypes (1, 2, 3, 4, 5, 6). Each HCV genotype was tested in a panel of 6 dilution levels. Each dilution level was tested in 2 replicates on ELITe InGenius system in "Extract + PCR" mode.

The results are reported in the following figure.

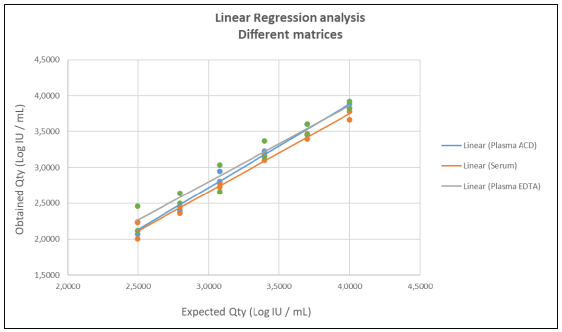


HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

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 the quantitative results fall within ±0.5 Log IU / mL with the exception of HCV genotype 2b that was under-estimated of about 0.8 Log IU / mL in comparison with the theoretical value. However, this sample was also underestimated by "cobas® HCV for use on the 6800 Systems" (Roche Diagnostics).

The linearity of quantification was verified by analysis of negative Plasma collected in ACD and Serum spiked by HCV WHO reference material (NIBSC). Each matrix was tested in a panel of 6 dilution levels. Each dilution level was tested in 2 replicates on ELITe InGenius system in "Extract + PCR" mode. Corresponding results of test with Plasma collected in EDTA were reported as reference.

The results are reported in the following figure.



The linearity of the assay was confirmed for Plasma collected in ACD and Serum giving an R2 respectively of 0.9838 and 0.9870 and the quantitative results within ±0.5 Log IU / mL.

Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

The efficiency of detection for different genotypes of HCV was evaluated by *in silico* comparison of the sequences available in the nucleotide databases.

The analysis of the regions chosen for the hybridization of the primers and of the probe in the alignment of the sequences of the HCV 5' UTR region available in the database showed sequence conservation and absence of significant mismatches in the HCV genotypes (1, 2, 3, 4, 5, 6, 7 and 8). So, an efficient amplification and detection of all the HCV genotypes is expected.

The Inclusivity of the assay, as detection and quantification efficiency on different genotypes, was verified by testing three reference material panels:

- "AccuTrak HCV RNA Genotype Performance Panel" (Seracare), an 8-member validation panel of diverse collection of HCV genotypes 1, 2, 3, 4, 5 and 6.
- HCV Genotype Evaluation Panel 01 (Qnostics).
- Non WHO Reference Material 4th HCV RNA Genotype Panel for Nucleic Acid Amplification Techniques (NIBSC).

Each sample of the panel was diluted at the concentration of 3 x LoD (about 78 IU / mL) in negative samples of Plasma collected in ACD and tested on ELITe InGenius system in "Extract + PCR" mode.
The results are reported in the following table.

AccuTrak HCV RNA Genotype Performance Panel				
Genotype	**Theoretical IU/mL**	**Pos. / Rep.**	**Mean Ct**	**Mean IU/mL**
HCV 1A	78	3 / 3	37.16	74
HCV 1B	78	3 / 3	37.74	48
HCV 2A/2C	78	3 / 3	37.64	56
HCV 2B	78	3 / 3	38.14	36
HCV 3A	78	3 / 3	39.70	14
HCV 4ACD	78	3 / 3	38.18	38
HCV 5A	78	3 / 3	37.20	67
HCV 6C	78	3 / 3	38.07	39

HCV Genotype Evaluation Panel				
Genotype	**Theoretical IU/mL**	**Pos. / Rep.**	**Mean Ct**	**Mean IU/mL**
HCV 1a	78	3 / 3	36.22	133
HCV 1b	78	3 / 3	34.11	615
HCV 2b	78	3 / 3	38.24	33
HCV 3a	78	3 / 3	35.46	225
HCV 4a	78	3 / 3	36.09	144
HCV 5a	78	3 / 3	37.22	69
HCV 6a	78	3 / 3	37.56	56

Non WHO Reference Material 4th HCV RNA Genotype Panel				
Genotype	**Theoretical IU/mL**	**Pos. / Rep.**	**Mean Ct**	**Mean IU/mL**
HCV 1a	78	3 / 3	38.27	40
HCV 1b	78	3 / 3	37.12	160
HCV 2i	78	3 / 3	38.01	71
HCV 3a	78	3 / 3	39.49	19
HCV 4r	78	3 / 3	40.08	18
HCV 5a	78	3 / 3	38.92	39
HCV 6l	78	2 / 3	38.24	8

All the samples of the three panels were correctly detected. In the "Non WHO Reference Material 4th HCV RNA Genotype Panel" (NIBSC) one out of three replicates of HCV genotype 6l was not detected. Most of the samples were also quantified within the theoretical titer ±0.5 Log IU / mL (25 – 247 IU / mL) by the HCV ELITe MGB® Kit in association with the ELITe InGenius instrument. In the "Non WHO Reference Material 4th HCV RNA Genotype Panel" (NIBSC) the HCV genotype 6l was also underestimated. However, this genotype was correctly detected and quantified in the other two panels.

Potential interfering markers: cross-reactivity

The Potential cross-reactivity with other organisms that can be found in clinical samples of Plasma and Serum was evaluated by *in silico* comparison of sequences available in the nucleotide databases.

The regions chosen for the hybridization of the primers and the probe were checked on the alignment of the sequences of other organisms available in the databases. The analysis of the hybridization regions showed absence of significant homologies with the unintended organisms.

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

Samples of genomic DNA or RNA from different potentially interfering markers (ATCC, NIBSC, ZeptoMetrix) were analyzed at high concentration (at least 10^5 copies / reaction) in three replicates in association with ELITe InGenius system in "PCR Only" mode. The genomic DNA or RNA of each organism were added with 80,000 Internal Control copies per reaction in order to mimic the extracted clinical sample.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

The results are reported in the following table.

Sample ID	HCV Pos. / Rep.	Outcome
HIV1	0 / 3	No cross-reactivity
HIV2	0 / 3	No cross-reactivity
HTLV1	0 / 3	No cross-reactivity
HTLV2	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
WNV	0 / 3	No cross-reactivity
DV3	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

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

Potential interfering markers: Interference

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

Samples of genomic DNA or RNA from different potentially interfering markers (ATCC, NIBSC, ZeptoMetrix) at high concentration (at least 10^5 copies / reaction) were spiked by HCV RNA reference material (form 6th WHO International Standard, NIBSC) at low concentration (about 10 copies / reaction). The samples were analyzed in three replicates in association with ELITe InGenius system in "PCR Only" mode. Each sample were added with 80,000 Internal Control copies per reaction in order to mimic the extracted clinical sample.
The results are reported in the following table.

Sample ID	HCV Pos. / Rep.	Outcome
HIV1	3 / 3	No interference
HIV2	3 / 3	No interference
HTLV1	3 / 3	No interference
HTLV2	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

Sample ID	HCV Pos. / Rep.	Outcome
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

The presence of the tested potential interfering organisms showed no inhibition of the amplification of the HCV target using HCV ELITe MGB® Kit.

Potential interfering substances

The effect of Potential interfering substances was evaluated by analyzing the panel "AcroMetrix® Inhibition Panel" (Thermo Fisher Scientific Inc.) containing endogenous substances, resulting from haemolysis, icterus and lipemia, and exogenous substances, EDTA and Heparin.

The samples of the inhibition panel were spiked with HCV HCV certified reference material (6th WHO HCV International Standard, NIBSC) at a concentration of 3 x LoD (about 78 IU / mL).

In addition, other 7 potential interfering substances were tested at relevant concentration: Ganciclovir, Azithromycin, Glecaprevir, Ribavirine, Sofosbuvir, Pibrentasvir, Velpatasvir.

The substances were individually added to HCV negative plasma collected in ACD spiked by HCV certified reference material (NIBSC) at a concentration of 3 x LoD (about 78 IU / mL).

The samples were processed in three replicates on ELITe InGenius system in “Extract + PCR” mode. The Ct values (reference and test samples) of the HCV target and the Internal Control were used to calculate the percentage Coefficient of Variability (%CV) in order to evaluate the possible interference.

The results are reported in the following tables.

Sample	HCV Pos. / Rep.	HCV Ct %CV	IC Ct %CV	Outcome
Bilirubin	3 / 3	1.12	0.91	No interference
Triglycerides	3 / 3	2.20	0.83	No interference
Hemoblogin	1 / 3	N.A.	10,80	Interference
Heparin	0 / 3	N.A.	7.68	Interference
EDTA	3 / 3	1.36	1.28	No interference
Ganciclovir	3 / 3	1.94	0.94	No interference
Azithromycin	3 / 3	2.22	1.69	No interference
Sofosbuvir	3 / 3	2.03	0.93	No interference

Sample	HCV Pos. / Rep.	HCV Ct %CV	IC Ct %CV	Outcome
Pibrentasvir	3 / 3	2.81	0.92	No interference
Glecaprevir	3 / 3	2.00	1.09	No interference
Ribavirine	3 / 3	1.72	1.03	No interference
Velpatasvir	3 / 3	2.01	0.84	No interference
Reference	3 / 3	1.12	0.91	No interference

Most of the tested substances do not interfere with the HCV or Internal Control amplification. The percentage %CV of Ct values were lower than 2.5%.

Heparin and Hemoglobin at high concentration (3.4 – 4.6 g/dL) were confirmed to inhibit the amplification of HCV but, due to the Internal Control Ct cut-off (IC Ct < 31), the samples result "invalid" and not "false negative" in most of cases.

Absence of cross-contamination

The Absence of cross-contamination was tested analyzing the results of five sessions in which HCV RNA negative plasma samples were alternated with plasma samples spiked by HCV certified reference material (ZeptoMetrix) at a concentration of $1x10^6$ IU/mL.

Five series of samples, alternating six positive samples with six negative samples, were tested on ELITe InGenius system in “Extract + PCR” mode.

The results are reported in the following table.

Samples	N	Negative	Positive
Positive	30	0	30
Negative	30	30	0

None of the tested HCV negative samples gave false positive results. In this test no cross-contamination was detected intra-session and inter sessions.

Whole system failure rate

The Whole system failure rate, leading to false negative results, was verified in association with **ELITe InGenius** by analysing a panel of samples spiked for HCV RNA at low titre.

100 different samples of plasma collected in EDTA, 30 different samples of Plasma collected in ACD, and 30 different samples of Serum, tested negative for HCV RNA were spiked with certified reference material (WHO) at a concentration of 3x LoD (about 78 IU / mL). The samples were tested on ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	N	Negative	Positive	Mean HCV IU/mL
Plasma collected in EDTA	100	0	100	55
Plasma collected in ACD	30	0	30	62
Serum	30	0	30	52

None of the tested HCV positive samples gave false negative results. In this test the whole system failure rate was equal to 0%.

The Whole system failure rate, leading to false negative results, was verified in association with **ELITe BeGenius** by analysing a panel of samples spiked for HCV RNA at low titre.

100 different samples of plasma collected in EDTA, tested negative for HCV RNA were spiked with certified reference material (WHO) at a concentration of 3 x LoD (about 78 IU / mL). The samples were tested on ELITe BeGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	N	Negative	Positive	Mean HCV IU/mL
Plasma collected in EDTA spiked by HCV	100	0	100	77

None of the tested HCV positive samples gave false negative results. In this test the whole system failure rate was equal to 0%.

Repeatability

The Repeatability of results obtained by the product HCV ELITe MGB Kit in association with the ELITe InGenius system was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked by HCV certified reference material (WHO) at concentration of 3x LoD (about 78 IU / mL) and of 10x LoD (about 260 IU / mL).

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

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability								
Sample	HCV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	Undet.	-	-	24 / 24	28.41	0.36	1.27
3x LoD	8 / 8	38.02	0.83	2.19				
10x LoD	8 / 8	35.91	0.59	1.65				

Inter – Session Repeatability								
Sample	HCV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	Undet.	-	-	48 / 48	28.47	0.38	1.33
3x LoD	16 / 16	37.89	0.84	2.21				
10x LoD	16 / 16	35.95	0.47	1.32				

Inter – Batch Repeatability								
Sample	HCV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 48	Undet.	-	-	144 / 144	28.52	0.46	1.61
3x LoD	48 / 48	38.25	0.36	0.95				
10x LoD	48 / 48	36.56	0.55	1.51				

In the Repeatability test, the assay detected the HCV target as expected and showed low %CV of Ct values that did not exceed 2.19% for HCV and 1.61% for Internal Control.

The Repeatability of results obtained by the product HCV ELITe MGB Kit in association with the **ELITe BeGenius** system was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked by HCV certified reference material (6th WHO International Standard, NIBSC) at concentration of 3x LoD (about 78 IU / mL) and of 10x LoD (about 260 IU / mL).

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

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability								
Sample	HCV				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 8	Undet.	-	-	24 / 24	28.09	0.28	0.99
3x LoD	8 / 8	37.65	0.50	1.33				
10x LoD	8 / 8	35.97	0.43	1.19				

Inter – Session Repeatability								
Sample	HCV				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 16	Undet.	-	-	48 / 48	28.05	0.29	1.03
3x LoD	16 / 16	37.80	0.72	1.91				
10x LoD	16 / 16	35.93	0.47	1.30				

In the Repeatability test, the assay detected the HCV target as expected and showed Ct values with %CV below 5 % for HCV and Internal Control.

Reproducibility

The Reproducibility of results obtained by the product HCV ELITe MGB Kit in association with the ELITe InGenius system was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HCV certified reference material (WHO) at concentration of 3x LoD (about 78 IU / mL) and of 10x LoD (about 260 IU / mL).

The Reproducibility was obtained through the analysis of panel samples in four replicates, in one run per day, in two days per site. Three different lots of product were used in three different sites with three different instruments by three different operators. Samples were processed in randomized positions on ELITe InGenius system in “Extract + PCR” mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Site Reproducibility								
Sample	HCV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 24	Undet.	-	-	72 / 72	28.18	0.59	2.09
3x LoD	24 / 24	36.84	0.59	1.60				
10x LoD	24 / 24	34.94	0.42	1.20				

In the Reproducibility test, the assay detected the HCV target as expected and showed low %CV of Ct values that did not exceed 1.6% for HCV and 2.09% for Internal Control.

The Reproducibility of results obtained by the product HCV ELITe MGB Kit in association with the **ELITe BeGenius** system was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HCV certified reference material (6th WHO International Standard, NIBSC) at concentration of 3 x LoD (about 78 IU / mL) and of 10 x LoD (about 260 IU / mL).

The Reproducibility was obtained through the analysis of panel samples in four replicates, in one run per day, in two days per instrument. Three different lots of product were used with three different instruments by three different operators. Samples were processed in randomized positions on ELITe BeGenius® system in “Extract + PCR” mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Instrument Reproducibility								
Sample	HCV				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 24	Undet.	-	-	72 / 72	28.57	0.5529	1.94
3 x LoD	23 / 23	37.99	0.6797	1.79				
10 x LoD	24 / 24	36.54	0.6210	1.70				

Inter – Batch Reproducibility								
Sample	HCV				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 24	Undet.	-	-	72 / 72	28.43	0.41	1.45
3x LoD	24 / 24	37.95	0.75	1.97				
10x LoD	24 / 24	36.35	0.62	1.71				

In the Reproducibility test, the assay detected the HCV target as expected and showed Ct values with %CV below 5 % for HCV and Internal Control.

Conversion factor to International Units

The Conversion factor, to express the quantitative results in International Units / mL starting from copies / mL, was calculated using a panel of four dilutions (0.5 Log between dilutions) of the certified calibrated reference material "6th WHO HCV International Standard” (NIBSC) in Plasma collected in EDTA tested negative for HCV RNA.

Each point of the panel was tested in 27 replicates using three different lots of product on three different instruments in three different days. Samples were processed in randomized positions on ELITe InGenius system in “Extract + PCR” mode.

The Conversion factor was calculated by the analysis of the logarithmic concentration difference between the reference titre in IU / mL and the obtained results in copies / mL and it is equal to 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	
31623	4.5000	27	13233	31254	4.4807	+0.0193
10000	4.0000	27	4482	10595	4.0133	-0.0133
3162	3.5000	27	1414	3342	3.5091	-0.0091
1000	3.0000	27	439	1036	2.9969	+0.0031

As the equivalence between Plasma collected in EDTA, Plasma ACD and Serum was demonstrated (see Matrix Equivalence and Linear Measuring Range), the Conversion factor can be applied to the three matrices.

The Conversion factor, to express the quantitative results in International Units / mL starting from copies / mL, was verified on **ELITe BeGenius** and **ELITe InGenius** systems using a panel of dilutions (0.5 Log between dilutions) of the certified calibrated reference material (6th WHO International Standard, NIBSC)

in Plasma collected in EDTA tested negative for HCV RNA. The panel consisted of five dilution points from about 4,5 Log IU/mL to 2.5 Log IU/mL. Each point of the panel was tested in 4 replicates.

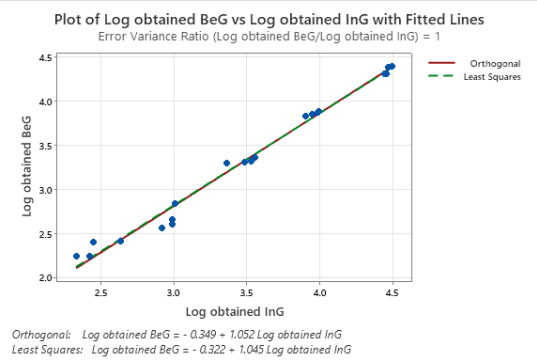
The target quantification precision, as standard deviation of Log IU/mL, was lower than 0.5 log.

The target quantification accuracy, as difference between Theoretical and Measured concentrations in Log IU/mL, was lower than 0.5 Log.

These results confirmed the Conversion factor calculated for Plasma samples with **ELITe InGenius.**

The results obtained by **ELITe InGenius** and **ELITe BeGenius** were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



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 a R2 of 0.985.

Reproducibility with Reference Material

The Reproducibility of the assay results compared with results obtained using other methods in different laboratories has been verified by testing the proficiency study panel "QCMD 2020 Hepatitis C Virus RNA EQA Programme" (Qnostics).

Each point of the panel was tested on ELITe InGenius system in "Extract + PCR" mode.
The quantity values of the consensus of commercial real time amplification systems were compared with the results of the assay in order to evaluate the accuracy as bias.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR
REF RTK601ING

The results are reported in the following table.

QCMD 2020 HCV panel		Consensus results	Test results	Difference
Sample ID	Sample Content	Log IU/mL	Log IU/mL	(cons. - test)
HCVRNA101S-01	HCV genotype 3a	3.3040	3.5794	-0.2754
HCVRNA101S-02	HCV genotype 3a	2.3130	2.2375	0.0755
HCVRNA101S-03	HCV genotype 1b	2.8600	3.0532	-0.1932
HCVRNA101S-04	HCV genotype 1b	2.8370	2.7850	0.0520
HCVRNA101S-05	HCV negative	not detected	not detected	N.A.
HCVRNA101S-06	HCV genotype 1b	3.8500	3.8352	0.0148
HCVRNA101S-07	HCV genotype 1b	3.3730	3.4432	-0.0702
HCVRNA101S-08	HCV genotype 1b	1.7880	1.6812	0.1068

In this test, the assay correctly detected all the panel members. The seven positive samples were quantified within the range of consensus ± 0.5 Log IU / mL.

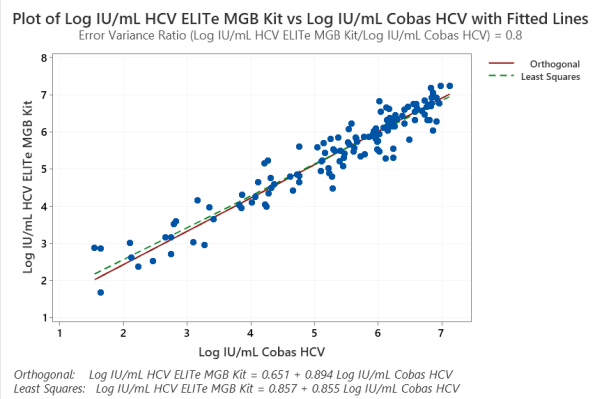
Diagnostic Sensitivity: method correlation

The diagnostic sensitivity of the assay, as correlation of results obtained with different methods, was evaluated by analysing HCV RNA positive clinical patients undergoing antiviral therapy and within the measuring range of the HCV ELITe MBG Kit and of a CE IVD marked molecular diagnostic reference methods ("cobas® HCV for use on the 4800 Systems" and "cobas® HCV for use on the 6800 Systems", Roche Diagnostics, Cobas HCV). As **ELITe BeGenius** has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The correlation study was performed in two different sites on the following 128 samples of Plasma collected in EDTA:

- site 1: 96 HCV RNA positive clinical samples of Plasma collected in EDTA,
- site 2: 32 HCV RNA positive clinical samples of Plasma collected in EDTA,

Each sample was tested carrying out the whole analysis procedure, extraction, reverse transcription, amplification, detection and result interpretation by the ELITechGroup S.p.A. products and by the reference methods. The results obtained by the HCV ELITe MBG Kit and the reference methods were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.
The results are summed up in the following figure.



In this test, the Deming regression analysis generated a slope equal to 0.894 (95% IC: 0.846 – 0.941).and an intercept equal to 0.651 (95% CI: 0.391 – 0.911). The linear regression analysis generated an R2 of 0.916.

Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as percentage negative agreement of results obtained with different methods, was evaluated by analysing HCV RNA negative clinical samples tested by a CE IVD marked molecular diagnostic reference methods ("cobas® HCV for use on the 4800 Systems" and "cobas® HCV for use on the 6800 Systems", Roche Diagnostics, Cobas HCV). As **ELITe BeGenius** has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The Diagnostic Specificity study was performed in two different sites on the following 135 samples of Plasma collected in EDTA:

- site 1:100 HCV RNA negative clinical samples of Plasma collected in EDTA,
- site 2: 35 HCV RNA negative clinical samples of Plasma collected in EDTA.

Each sample was tested carrying out the whole analysis procedure, extraction, reverse transcription, amplification, detection and result interpretation by the ELITechGroup S.p.A. products. The results obtained by the HCV ELITe MBG Kit were analysed in order to calculate the percentage negative agreement with the reference methods.

The results, after discrepant analysis, are summed up in the following table.

Samples	N	Positive	Negative	Invalid	Diagnostic Specificity
HCV RNA negative Plasma collected in EDTA	135	0	135	0	100%

In this test, all samples were confirmed negative. The Diagnostic Specificity of the HCV ELITe MBG Kit was equal to 100 %.

The Internal Control Ct (IC Ct) cut-off value is set at 31.

Note: The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instrument are recorded in Section 7 of the Product Technical File for the "HCV ELITe MGB Kit", FTP 601ING.

REFERENCES

P. Halfon et al. (2006) *J Clin Microbiology* 44: 2507 – 2511
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res*. 35: e30

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

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

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 may be obtained from whole blood stored at +2 / +25 °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.

At the moment there are no data available concerning product performances with other clinical samples such as whole blood.

This 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.

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

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

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

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

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

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

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

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

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

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, insertion or deletions within the region of the target RNA covered 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 taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, false positive and false negative results obtained with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

TROUBLESHOOTING

Invalid Q-PCR Standard reaction or Positive Control reaction **Invalid Standard curve**	
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.
Complete reaction mixture degradation or of its sub-components	Do not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Use a new aliquot of sub-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). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area). 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 complete reaction mixture or of its sub-components	Prepare again the complete reaction mixture. Use a new aliquot of components.
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 extraction area, of Racks or of Inventory Block.	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

HCV ELITe MGB® Kit
reagents for RNA reverse transcription and Real Time PCR

REF RTK601ING

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture and sample. Check the volumes of complete reaction mixture and sample.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its sub-components.	Do not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Prepare again the complete reaction mixture. Use a new aliquot of sub-components.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to sample interfering substances.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in a “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - selected the track related to the sample and approve manually the result. If a Ct value is required: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session.

TH Error, SDM Error, Ct Error	
Possible Causes	**Solutions**
Sample with anomalous plot shape.	If significant amplification is observed in PCR plot generating error: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session.

SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE 0344	Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device. Certification released by DEKRA Certification B.V., the Netherland.
	Contains sufficient for "N" tests.
	Attention, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

NOTICE TO PURCHASER: LIMITED LICENSE

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

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

ELITe InGenius and ELITe BeGenius technology is covered by patents and requests for patents.

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

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. This document is available only in English.
Please refer to the complete document before use: www.elitechgroup.com

Intended use

The "HCV ELITe MGB® Kit" product is quantitative nucleic acids reverse transcription and amplification assay for the detection and the quantification of the RNA of Hepatitis C Virus (HCV) in RNA samples extracted from clinical specimens.
The assay is able to detect the RNA of HCV belonging to 1, 2, 3, 4, 5 and 6 genotypes.
The assay is validated in association with "ELITe InGenius®" and "ELITe BeGenius®" system starting from human plasma collected in EDTA or in ACD and serum samples.
The product is intended for use as an aid in the management of HCV-infected individuals undergoing antiviral therapy, together with patient's clinical data and other laboratory test results.
This 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.

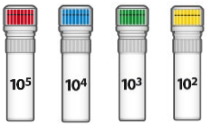
Amplified sequence

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

Validated matrix

› **Plasma EDTA** › **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
HCV PCR Mix 4 tubes of 600 µL 96 reactions per kit 5 freeze-thaw cycles	RT EnzymeMix 2 tubes of 20 µL 96 reactions per kit 10 freeze-thaw cycles	Ready-to-use Calibrators: 10^5, 10^4, 10^3, 10^2 1 set of 4 tubes of 160 µL 2 freeze-thaw cycles	Ready-to-use PC 2 tubes of 160 µL 8 reactions per kit 4 freeze-thaw cycles	Ready-to-use IC 8 tubes of 160 µL 96 extractions per kit 12 freeze-thaw cycles

Maximum shelf-life: **18 months**

Storage Temperature: **-20 °C**

Material required not provided in the kit

- ELITe InGenius instrument: INT030
- ELITe InGenius SP 1000 Extraction Cartridge: INT033SP1000
- ELITe InGenius PCR Cassette: INT035PCR
- ELITe InGenius SP200 Consumable Set: INT032CS
- ELITe InGenius Waste Box: F2102-000
- Filter Tips 300: TF-350-L-R-S
- 1000 µL Filter Tips Tecan : 30180118

ELITe InGenius protocol

› Sample volume	600 µL	› Unit of quantitative result	International Unit: IU/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
› HCV PCR Mix volume	20 µL		

Performance

Matrix	Limit of Detection	Method Correlation	Diagnostic Specificity
Plasma / Serum	**26 IU / mL** 11 copies / mL	**R^2 = 0.916** 128 *quantified samples*	**100%** 135 *confirmed samples* / 135 *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

Plasma samples collected in EDTA or ACD and Serum samples must be identified according to laboratory guidelines, transported and stored at room temperature (~+25 °C) for a maximum of three days or at +2 / +8 °C for a maximum of five days. Otherwise, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for six months. Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

ELITe InGenius Procedures

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

Before analysis

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

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

3. Thaw the **HCV PCR Mix** and the **HCV CPE** tubes.
Vortex gently.
Spin down 5 sec.
Keep the **RT EnzymeMix** in ice

4. Prepare the complete reaction mixture

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

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

Procedure 1 - Complete run: Extraction + PCR

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

Procedure 2 - PCR only

1 to 4: Follow the Complete Run procedure described above

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 acid

8. Close the door
Start the run

9. View, approve and store the results

Procedure 3 - Extraction only

1 to 4: Follow the Complete Run procedure described above

5. Select the method "Extraction Only" and set the sample position: Extraction Tube

6. Load the Internal Control in the inventory block

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

8. Close the door
Start the run

9. Archive the eluate sample

ELITe BeGenius Procedures

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

Before analysis

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

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

3. Thaw the **HCV PCR Mix** and the **HCV CPE** tubes.
Vortex gently.
Spin down 5 sec.
Keep the **RT EnzymeMix** in ice

4. Prepare the complete reaction mixture

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

Note: For more than 12 samples:

- prepare a first 2 mL tube with the complete reaction mixture for 12 samples;

- prepare a second 2 mL tube with the complete reaction mixture by calculating the volumes of the two
sub-components on the basis of the number of samples remaining (N-12).

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

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen

2. Verify the extraction volumes:
Input: "600 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_Be_PL_600_50 or HCV ELITe_Be_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

Procedure 2 - PCR only

1 to 4: Follow the Complete Run procedure described above

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 acid

8. Close the door
Start the run

9. View, approve and store the results

Procedure 3 - Extraction only

1 to 4: Follow the Complete Run procedure described above

5. Select the method "Extraction Only" and set the sample position:
Extraction Tube

6. Load the Internal Control in the inventory block

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

8. Close the door
Start the run

9. Archive the eluate sample