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EMPOWERING IVD

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NOTICE of CHANGE dated 16/02/2023

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HEV ELITe MGB® Kit»
Ref. RTS130ING

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

- *Troubleshooting section: added the possibility of discriminating possible mutants by the presence of a defined peak of the Melting Temperature with a value different from that of the Positive Control*
- *New data of Inclusivity*

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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HEV ELITe MGB® Kit

reagents for RNA reverse transcription and cDNA Real Time amplification

REF RTS130ING CE IVD -20°C

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INTENDED USE

The **«HEV ELITe MGB® Kit»** product is part of a quantitative nucleic acids reverse transcription and amplification assay for the **detection and quantification of the RNA** of Hepatitis E virus **(HEV)** in RNA samples extracted from clinical samples.

The assay is able to detect the RNA of HEV belonging to 1, 2, 3, 3a and 4 genotypes.

The assay has to be carried out in association with **ELITe InGenius®** system starting from plasma collected in EDTA and stool supernatant.

The product is intended for use in the diagnosis of hepatitis caused by HEV, in conjunction with the patient's clinical data and other laboratory test results.

ASSAY PRINCIPLE

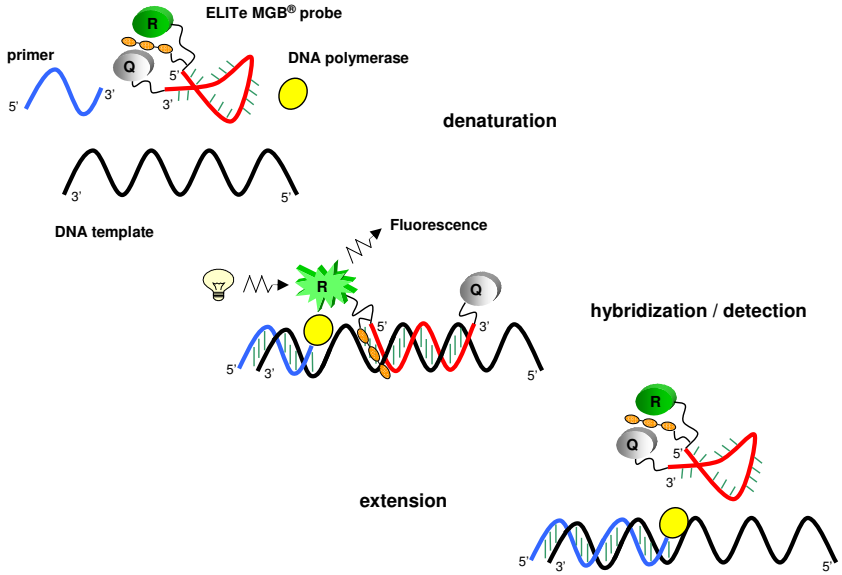
The assay consists of a reverse transcription and a real-time amplification reaction (one-step method).

Starting from RNA extracted from sample to be tested, in the PCR cassette a reaction of reverse transcription and amplification specific for the ORF-2 region of HEV and for a region of the genomic RNA of MS2 phage (exogenous Internal Control of extraction and inhibition) is carried out.

The HEV specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridized with the specific product of the HEV amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with 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 HEV RNA in the sample.

The assay has been validated in association with **ELITe InGenius**, automated integrated system for extraction, amplification, detection of nucleic acids and result interpretation.

In the following picture is synthetically showed the mechanism of activation and fluorescence emission of ELITe MGB® technology probe. Note that the probe is not hydrolysed during the amplification cycle so as it can be utilized for the dissociation curve analysis and melting temperature calculation.



PRODUCT DESCRIPTION

The « **HEV ELITe MGB® Kit»** product provides the following components:

- **HEV 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, without insert). Each tube contains **600 µL** of solution, sufficient for **24 tests** (processing at least 5 samples per session) in association with **ELITe InGenius**.

Primers and the probe for HEV (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non-fluorescent molecule) are specific for the ORF-2 region of HEV. The HEV signal is detected by Channel 1 of the **ELITe InGenius** system**.**

Primers and the probe for Internal Control (stabilized by MGB® group, labelled by AP525 fluorophore and quenched by a non-fluorescent molecule) are specific for a region of the **phage MS2** genomic RNA. The Internal Control (IC) signal is detected by Channel 2 of the **ELITe InGenius** system.

The reaction mixture provides also the buffer, magnesium chloride, the nucleotide triphosphates, AP593 fluorophore, that can be used as passive reference for fluorescence normalisation, 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** in association with **ELITe InGenius**.

The mixture provides the enzyme for reverse transcription.

The product is sufficient for **96 tests in association with «ELITe InGenius»**, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
HEV PCR Mix	mixture of reagents for reverse transcription and real time amplification **WHITE cap**	**4 x 600 µL**	**-**
RT EnzymeMix	Reverse transcriptase **cap with BLACK insert**	**2 x 20 µL**	**-**

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Sarstedt 2.0 mL 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, the internal control template, the amplification positive control, the known quantity DNA standards and the consumables are **not** included in this product.

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For automatic nucleic acid extraction, Real Time amplification and result interpretation of samples to be analyzed, the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) and the following specific Assay protocols (ELITechGroup S.p.A.), are required:

- parameters for calibrators amplification **«HEV ELITe_STD»,**
- parameters for positive control amplification **«HEV ELITe_PC»**,
- parameters for negative control amplification **«HEV ELITe_NC»**,
- parameters for plasma samples to be analyzed **«HEV ELITe_PL_200_100»,**
- parameters for stool samples to be analyzed **«HEV ELITe_ST_200_100»**.

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

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

As template of extraction and inhibition internal control, the generic product **«CPE - Internal Control»** (ELITechGroup S.p.A., ref. CTRCPE), is required. This is a stabilised solution containing two plasmid DNAs and the genomic RNA of MS2 phage.

If detection of HEV RNA is required for qualitative analysis, use the specifc product **«HEV - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR130ING). The product provides a stabilised solution of plasmid DNA.

If detection and quantification of HEV RNA is required for quantitative analysis, use the specifc product **«HEV ELITe Standard»** (ELITechGroup S.p.A., ref. STD130ING). The product provides four stabilised dilutions of plasmid DNA at known concentration to obtain the standard curve.

A conversion factor allows to express the results of the quantitative analysis in International Units of HEV of the "1st World Health Organization International Standard for Hepatitis E Virus RNA Nucleic Acid Amplification Techniques (NAT)-Based Assays" (PEI, Germany, code 6329/10).

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121 °C before disposal.

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

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

Warnings and precautions for molecular biology

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

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

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

The reagents must be handled under a laminar airflow hood. The reagents required for reverse transcription and amplification must be prepared in such a way that they can be used in a single session. 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 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

HEV PCR Mix

The **HEV PCR Mix** must be stored at -20 °C in the dark.

The **HEV PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

RT EnzymeMix

The **RT EnzymeMix** must be stored at -20 °C.

The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than 10 minutes. Can be frozen and thawed for no more than **ten times.**

SAMPLES AND CONTROLS

This product must be used with the following clinical samples:

Plasma collected in EDTA

Samples of plasma, intended for RNA extraction, must be collected in EDTA, transported and stored at room temperature (+18 / +30 °C) for a maximum of twenty four hours 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 thirty days or at -70 °C for longer period.

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: when the RNA extraction from plasma collected in EDTA is carried out with the **ELITe InGenius** system and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the Assay protocol **HEV ELITe_PL_200_100**. This protocol processes 200 µL of sample, adds the **CPE**-(Internal Control) at 10 µL / extraction and elutes the nucleic acids in 100 µL.

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Purified nucleic acids can be stored at +2 / +8 °C for 16 hours or at -20 °C for one month.

When the primary tube is used, the volume of the sample varies according to the type of tube loaded, refer to the instruction for use of the extraction kit for more information on how to set up and perform the extraction procedure.

Stool

Sample of stool, intended for RNA extraction, must be collected and identified according to laboratory guidelines, transported and stored at +2 / +8 °C for a maximum of twenty four hours. Otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer period.

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.

Before extraction, the following pre-treatment is recommended:

- transfer about 3 mL stool in the 50 mL conical tube (corresponding to fill the conical bottom),
- add 5 mL of molecular biology grade water,
- vortex until the sample is homogeneous,
- transfer 100 µL of water-treated stool sample to 900 µL of molecular biology grade water into the 1.5 mL tube,
- vortex until the sample is homogeneous,
- centrifuge at 13,000 RCF for 1 minute,
- carefully transfer 200 µL of the stool supernatant into a “Sonication tube” provided with **«ELITe InGenius SP 200 Consumable Set»**, being careful not to disturb the pelleted fecal material.

Note: when the RNA extraction from human stool samples is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.3 (or later equivalent versions), use the Assay protocol **HEV ELITe_ST_200_100**. This protocol processes 200 µL of sample, adds the **CPE** (Internal Control) at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Purified nucleic acids can be stored at +2 / +8 °C for 16 hours or at -20 °C for one month.

Interfering substances

Quantities of human genomic DNA and/or RNA higher than 1 µg extracted from the sample could inhibit the reverse transcription reaction and the real-time amplification.

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

Amplification calibrators and 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 **HEV ELITe Standard** (not provided with this kit), in association with the protocol **HEV ELITe_STD**,
- as amplification Positive Control, use the **HEV - ELITe Positive Control** (not provided with this kit), in association with the protocol **HEV ELITe_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit), in association with the protocol **HEV 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 set in association with the amplification reagent lot.
The amplification Control results, approved and stored in the database, will expire after **15 days**. At expiration date it is necessary to re-run the Positive and Negative Controls in association with the amplification reagent lot.
Furthermore, the 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 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 «**HEV ELITe MGB® Kit**» with the system **ELITe InGenius** 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 (**HEV Q-PCR Standard**) were run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not Calibrators approved or valid, run them as described in the following paragraphs,
- verify that the amplification Controls (Controls, **HEV Positive Control, HEV Negative Control**) were run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification Controls approved or valid, run them as described in the following paragraphs,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB® kits, **ELITe InGenius** instrument and the cited matrix.

The Assay Protocols available for sample testing with the product **HEV ELITe MGB® Kit** are described in the table below.

Assay protocol for HEV ELITe MGB® Kit

Name	Matrix	Report	Characteristics
HEV ELITe_PL_200_100	Plasma	Positive / copies/mL / IU/mL / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume:10 µL
HEV ELITe_ST_200_100	Stool supernatant	Positive / copies/mL / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume:10 µL

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

Setup of the session

The product **HEV ELITe MGB® Kit** can be used with the **ELITe InGenius** system in order to perform:

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

All the parameters needed for the session are included in the Assay protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

Note: the **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible to load the work session information. Refer to the instrument user's manual for more details.

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

1. If needed, thaw at room temperature (+18 / 25 °C) the test tubes containing the samples to be analysed. Mix by vortexing for 10 seconds, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep in ice.
2. Thaw for 30 minutes at room temperature (+18 / 25 °C) the **HEV PCR Mix** (WHITE cap) test tubes needed for the session, remembering that the content of each test tube is enough for **24 tests**. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in ice.
3. Take the **RT EnzymeMix** (cap with BLACK insert) tubes necessary for the session remembering that the content of each tube is sufficient to set up **48 tests**. Gently shake the tubes, centrifuge for 5 seconds to bring the contents to the bottom and keep in ice.

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

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

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

Sample Number (N)	HEV PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 12	(N + 2) x 20 µL	(N + 2) x 0.3 µL

6. Prepare the **complete reaction mixture** by adding into the dedicated 2 mL tube the calculated volumes of the two components.
7. 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 when kept on board in the refrigerated block. The complete reaction mixture **cannot** be stored.

The main steps for the setup of the four types of run are described here below.

A. Integrated run

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

1. Thaw the CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds to bring the content to the bottom and keep in ice.
2. Select "Perform Run" from the "Home" screen.
3. Ensure that the "Extraction Input Volume" is 200 µL and the Extracted Elute Volume is 100 µL.
4. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
5. Select the Assay protocol to be used in the "Assay" column (e.g. HEV ELITe_PL_200_100).
6. Ensure that the "Protocol" displayed is: "Extract + PCR".
7. Select the sample loading position in the "Sample Position" column:
   - if a primary tube is used, select "Primary Tube",
   - if a secondary tube is used, select "Sonication Tube".

   Click "Next" to continue the setup.
8. Load the **complete reaction mixture** and the CPE on the "Inventory Block" selected by following the GUI instruction. Click "Next" button to continue the setup.
9. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" button to continue the setup.

10. Load the “PCR Cassettes”, the “ELITe InGenius SP 200” extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Extracted Sample in the “Elution tube” must be removed from the instrument, capped, 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 reaction products and the consumables must be removed from the instrument and disposed of without 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.

B. Amplification run

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

1. Select “Perform Run” from the “Home” screen.
2. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
3. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
4. Select the Assay Protocol to be used in the “Assay” column (e.g. HEV ELITe_PL_200_100).
5. Select “PCR Only” in the “Protocol” column.
6. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load the “PCR Cassettes” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup.
10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

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

Note: At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without 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.

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C. Calibration run

To setup the Calibration run, carry out the following steps as per GUI:

1. Thaw **HEV Q-PCR Standard** tubes (Cal1: HEV Q-PCR Standards 10^2, Cal2: HEV Q-PCR Standards 10^3, Cal3: HEV Q-PCR Standards 10^4, Cal4: HEV Q-PCR Standards 10^5). 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 200 µL and the “Extracted Elute Volume” is 100 µL.
4. Select the Assay Protocol “HEV ELITe_STD” in the “Assay” column and fill in the lot number and expiry date of **HEV Q-PCR Standard.**
5. Click “Next” to continue the setup.
6. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
7. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load the “PCR Cassettes”, the **HEV Q-PCR Standard** tubes following the GUI instruction. Click “Next” to continue the setup.
9. Close the instrument door.
10. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

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

Note: At the end of the run the PCR Cassettes with the reaction products and consumables must be removed from the instrument and disposed of without 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.

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 **HEV Positive Control** tubes 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 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. In the Track of interest, select the Assay protocol to be used in the “Assay” column.
5. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
6. For the positive control, select the Assay Protocol “HEV ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of **HEV Positive Control**,
7. For the negative control, select the Assay Protocol “HEV ELITe_NC” and fill in the lot number and expiry date of the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load / check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.

11. Load the “PCR Cassettes”, the **HEV Positive Control** tube and the negative control tube following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

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

Note: At the end of the run the PCR Cassettes with the reaction products and consumables must be removed from the instrument and disposed of without 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.

Review and export of results

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

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

The **ELITe InGenius** system generates the results with the product **HEV ELITe MGB® Kit** through the following procedure:

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

A. Validation of Calibration curve

The fluorescence signals emitted by the probe for HEV (Channel 1 "HEV") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “HEV ELITe_STD”.

The Calibration curve, specific for the amplification reagent lot, is stored in the database (Calibration). It can be viewed and approved by personnel qualified as “Administrator” or “Analyst”, following the GUI instructions.

The Calibration curve, specific for the amplification reagent lot, will expire **after 60 days**.

Note: If the Calibration curve does not meet the acceptance criteria, the “Failed” message is shown on the “Calibration” screen and it is not possible to approve the curve. The Calibrator amplification reactions have to be repeated.

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 Positive Control and Negative Control results

The fluorescence signals emitted by the probe for HEV (Channel 1 "HEV") in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocols “HEV ELITe_PC” and “HEV ELITe_NC”.

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

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire **after 15 days**.

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The results of Positive Control and Negative Control amplification runs are used by the instrument software to calculate the setup 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 amplification Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction has to be repeated.

Note: If the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

C. Validation of Sample results

The fluorescence signals emitted by the probe for HEV (Channel 1 "HEV") and by the probe of Internal Control (Channel 2 “IC”) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocol HEV ELITe_PL_200_100 and HEV ELITe_ST_200_100.

Results are shown in the reports generated by the instrument ("Result Display").
The sample run can be approved when the three conditions reported in the table below are met.

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

For each sample, the assay result is automatically interpreted by the system as established by the ELITe InGenius software algorithm and the Assay protocol parameters.

The possible result messages are listed in the table below.

Result of sample run	**Interpretation**
HEV: RNA Detected, quantity equal to XXX copies / mL or IU / mL.	**HEV RNA was detected** in the sample within the measurement range of the assay, quantity as shown.
HEV: RNA Detected, quantity below LLoQ copies / mL or IU / mL.	**HEV RNA was detected** in the sample below the lower limit of quantification of the assay.
HEV: RNA Detected, quantity beyond ULoQ copies / mL or IU / mL.	**HEV RNA was detected** in the sample beyond the upper limit of quantification of the assay.
HEV: RNA Not Detected or below the LoD copies / mL or IU / mL.	**HEV RNA was not detected** in the sample. The sample is negative for HEV 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.

Note: The results obtained with stool supernatant have to be considered as semi-quantitative. These results are expressed only in copies / mL.

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

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode.

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

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".

D. Sample result reporting

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

The "Sample Report" shows the details of a work session sorted by selected sample (SID).

The "Track Report" shows the details of a work session by selected Track.

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

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: Limit of Detection (LoD)

The analytical sensitivity of this assay, as sensitivity of reverse transcription and amplification reaction, allows the detection of about 10 copies of RNA in 10 µL of sample added to the reaction.

The analytical sensitivity of this assay, as Limit of Detection (LoD), was defined in association Plasma EDTA samples and stool supernatant samples and ELITe InGenius system.

The LoD was calculated by testing a panel of negative Plasma EDTA spiked with HEV certified reference material (PEI) at known titre. Six levels of dilutions were prepared starting from a concentration higher than the expected LoD value. Each dilution level was processed in 12 replicates on ELITe InGenius system in "Extract + PCR" mode. The LoD was obtained by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call. The LoD value was verified through testing 20 replicates of Plasma collected in EDTA and 20 replicates of Stool supernatant spiked with HEV certified reference material at the claimed concentration.

The final results are reported in the following table.

Limit of Detection of HEV in plasma and stool supernatant with ELITe InGenius (IU / mL)		
LoD	95% confidence interval	
	lower bound	upper bound
288 IU / mL	183 IU / mL	768 IU / mL

The LoD as copies / mL for Plasma EDTA was calculated by applying the specific conversion factor reported at page 17. The analytical sensitivity as copies / mL is reported below.

Limit of Detection of HEV in plasma with ELITe InGenius (copies / mL)		
LoD	95% confidence interval	
	lower bound	upper bound
153 copies / mL	97 copies / mL	409 copies / mL

Linear measuring range

The analytical sensitivity of this assay, as linear measuring range, allows the quantification from about 10,000,000 to about 100 copies per mL. The linear measuring range was determined using a panel of artificial samples spiked with HEV certified reference material.

The linearity of this assay used in association to ELITe InGenius was verified using a panel of HEV RNA dilutions. The panel was prepared by diluting the "Quantitative Synthetic Hepatitis E virus RNA" (ATCC, code VR-3258SD) in a stabilizing buffer. The panel consisted of six dilution points (1 Log dilution steps) from 10^7 copies / mL to 10^2 copies / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure by ELITe InGenius in "Extract + PCR" mode and ELITechGroup S.p.A. products. The analysis of the obtained data, performed by polynomial regression, demonstrated that the assay shows a linear response for all the dilutions.

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The lower limit of the linear measuring range was set at the lowest concentration that gives 100% of positivity and quantitative results accurate and precise within ±0.5 Log.

The upper limit of the linear measuring range was set at the highest concentration that gives quantitative results accurate and precise within ±0.5 Log.

The linear measuring range as IU / mL for Plasma EDTA is calculated by applying the specific conversion factor reported at page 17.

The final results are reported in the following table.

Linear measuring range tested on HEV in plasma with ELITe InGenius	
Lower Limit	Upper Limit
288 IU / mL	18.800.000 IU / mL
153 copies / mL	10.000.000 copies / mL

Linear measuring range tested on HEV in stool supernatant with ELITe InGenius	
Lower Limit	Upper Limit
500 copies / mL	10.000.000 copies / mL

Inclusivity: Efficiency of detection and quantification efficiency on different genotypes / subtypes

The efficiency of detection for different genotypes was evaluated by comparison with sequences available in the EBI ENA nucleotide database.

The analysis of the regions chosen for the hybridization of the primers and of the fluorescent probes in the alignment of the sequences available in the database for the ORF-2 region of HEV of the 1, 2, 3, 3a and 4 genotypes showed their conservation and absence of significant mutations.

A recent sequence study in the EBI ENA nucleotide database identified two clusters of significant mutations (e.g. MW355395 and MH377727) in the probe's hybridization region. The effect of these mutations has been verified and the detection / quantification efficiency of these variants, although lower, falls within the variability of ± 0.5 Log. It is possible to identify the presence of these variants through the analysis of the Melting Temperature (Tm) by the operator (see "Problems and Solutions").

The inclusivity of the assay, as detection and quantification efficiency on different genotypes / subtypes, was evaluated using the "1st World Health Organization International Reference Panel for Hepatitis E Virus (HEV) Genotypes for Nucleic Acid Amplification Technique (NAT)-Based Assays" (Paul Enrlich Institut, code 8578/13).

Each sample of the panel was tested carrying out the whole analysis procedure in association with ELITe InGenius system in "Extract+PCR" mode.

The results are reported in the following table.

Sample	Genotype	Overall mean Log IU/mL	Std Dev.	Measured Log IU/mL
8567/13	1a	2.64	0.60	2.61
8568/13s	1a	4.25	0.43	4.37
8569/13	1e	3.25	0.51	3.13
8570/13	3b	4.20	0.18	4.45
8571/13	3c	3.40	0.22	3.46
8572/13	3e	3.50	0.22	3.66
8573/13	3f	3.84	0.41	3.61
8574/13s	3 (rabbit-like)	4.98	0.38	4.78
8575/13	4c	4.07	0.38	3.85
8576/13	4g	3.77	0.38	3.36
8577/13s	2a	5.42	0.49	4.96

All samples were correctly detected. Samples were quantified in the range defined by WHO Collaborative Study ± 1 SD, but the 8570/13, 8576/13 e 85/77/13s that, however, fall within ± 0.5 Log.

Potential interfering markers

The potential cross-reactivity with other unintended organisms of the assay was evaluated by *in silico* analysis of sequences available in the EBI ENA nucleotide database.

The regions chosen for the hybridization of the primers and the fluorescent probes were checked on the alignment of the sequences of other viruses and bacteria. The hybridization regions showed absence of significant homologies and indicated no potential interference.

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

Samples of genomic DNA or RNA from different potentially cross-reactive organisms (ATCC, NIBSC and Qnostics) were analyzed in three replicates in association with ELITe InGenius system in “PCR Only” mode.

The final results are reported in the following table.

Potential cross-reactive organisms		
Organism	**Strain**	**Outcome**
CMV	AD-169	No cross-reactivity
HSV-1	McIntyre	No cross-reactivity
HSV-2	G	No cross-reactivity
EV	Enterovirus 71	No cross-reactivity
EBV	B95-8	No cross-reactivity
BKV	1b-2	No cross-reactivity
JCV	1A	No cross-reactivity
WNV	NY99	No cross-reactivity

All the potential cross-reacting organisms were negative for the target when tested by HEV ELITe MGB® Kit.

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

Samples of genomic DNA or RNA from different potentially cross-reactive organisms (ATCC, NIBSC and Qnostics) were spiked with HEV certified reference material (ATCC) at low concentration (about 10 copies / reaction). The samples were analyzed in three replicates in association with ELITe InGenius system in “PCR Only” mode.

The final results are reported in the following table.

Potential interfering organisms		
Organism	**Strain**	**Outcome**
CMV	AD-169	No interference
HSV-1	McIntyre	No interference
HSV-2	G	No interference
EV	Enterovirus 71	No interference
EBV	B95-8	No interference
BKV	1b-2	No interference
JCV	1A	No interference
WNV	NY99	No interference

All the potential interfering organisms did not impact on the amplification of the target when tested by HEV ELITe MGB® Kit.

Potential interfering substances

The possible effect of interfering substances was evaluated by analyzing the panel "AcroMetrix® Inhibition Panel" (Life Technologies Inc.) containing endogenous substances, resulting from hemolysis, jaundice and lipemia, and exogenous substances, EDTA and Heparin anticoagulants.

The samples of the inhibition panel were spiked with HEV certified reference material (PEI) at a concentration equivalent to approximately 3X LoD. The samples were processed in three replicates on ELITe InGenius system in “Extract + PCR” mode.

The Ct values of the target and the Internal Control (reference and test samples) were used to calculate the percentage Coefficient of Variability (%CV) in order to evaluate the possible interference.

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The results are reported in the following table.

Potential interfering substances			
Sample	**Pos. / Rep.**	**%CV HEV Ct**	**%CV IC Ct**
EDTA Plasma	3 / 3	2.092	3.434
Haemolitic Blood low	3 / 3	1.134	0.842
Haemolitic Blood mid	3 / 3	1.209	0.590
Haemolitic Blood high	3 / 3	1.768	0.983
Heparinized Plasma	3 / 3	1.775	3.731
Lipemic Plasma	3 / 3	1.070	2.653
Icteric Plasma	3 / 3	1.313	0.797

All the samples resulted positive for the target of interest as expected. The percentage %CV of Ct values were lower than 4%. None of the tested substances at the tested concentrations were found to interfere with the target detection by HEV ELITe MGB® Kit.

Repeatability

The Repeatability of assay results in association with the ELITe InGenius system was tested by analyzing a panel of plasma EDTA samples. The panel included one negative sample and three samples spiked with HEV certified reference material (PEI) at concentration of 0.5X LoD (about 144 IU / mL), 1.5X LoD (about 431 IU / mL), and 3X LoD (about 861 IU / mL).

The Repeatability was obtained through the analysis of panel samples in three replicates, in two runs per day, with the same lot of product. Three lots of products were used in three different days on the same instrument by the same operator. Samples were processed 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	**Pos. / Neg.**	**HEV**			**Internal Control**		
		Mean Ct	**SD**	**% CV**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 6	NA	NA	NA	30.27	0.27	0.90
0.5X LoD	5 / 6	39.80	0.57	1.42			
1.5X LoD	6 / 6	38.77	0.89	2.28			
3X LoD	6 / 6	37.75	0.82	2.17			

Inter - Batch Repeatability							
Sample	**Pos. / Neg.**	**HEV**			**Internal Control**		
		Mean Ct	**SD**	**% CV**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 18	NA	NA	NA	29.95	0.37	1.23
0.5X LoD	16 / 18	39.62	0.71	1.80			
1.5X LoD	18 / 18	38.46	0.95	2.47			
3X LoD	18 / 18	37.55	0.75	2.01			

In the Repeatability test, the assay detected the HEV target as expected and showed low %CV of Ct values that did not exceed 3% for HEV and 2% for Internal Control.

Reproducibility

The Reproducibility of assay results in association with the ELITe InGenius system was tested by analyzing a panel of plasma EDTA samples. The panel included one negative sample and three samples spiked with HEV certified reference material (PEI) at concentration of 0.5X LoD (about 144 IU / mL), 1.5X LoD (about 431 IU / mL), and 3X LoD (about 861 IU / mL).

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

The Ct values of the 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 tables below.

Reproducibility							
Sample	Pos. / Neg.	HEV			Internal Control		
		Mean Ct	SD	% CV	Mean Ct	SD	% CV
Negative	0 / 18	NA	NA	NA	30.04	0.33	1.10
0.5X LoD	14 / 18	39.43	0.66	1.68			
1.5X LoD	18 / 18	38.24	0.60	1.58			
3X LoD	18 / 18	36.90	0.40	1.07			

In the Reproducibility test, the assay detected the HEV target as expected and showed low %CV of Ct values that did not exceed 2 % for HEV and for 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 calibrated reference material "1st World Health Organization International Standard for Hepatitis E Virus RNA Nucleic Acid Amplification Techniques (NAT)-Based Assays” (PEI, Germany, code 6329/10) in Plasma collected in EDTA tested negative for HEV RNA.

Each point of the panel was tested in 9 replicates with three different lots of product on three different instruments in three different days. Samples were processed on ELITe InGenius system in “Extract + PCR” mode.

A summary of results is shown in the table below.

Conversion factor to International Units with ELITe InGenius	
Plasma EDTA	Fc = 1.88 IU / copy

The Conversion factor was calculated only for Plasma samples. As the results with stool supernatant are just semi-quantitative, no Conversion factor was calculated for this matrix.

Diagnostic specificity: confirmation of negative samples

The Diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated by analyzing 30 plasma samples and 30 stool supernatant samples.

The samples were presumably negative and were tested by the assay in association with ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	N	positive	negative	invalid
HEV Negative Plasma EDTA	30	0	30	0
HEV Negative Stool supernatant	30	0	30	0

In the test, all the samples were confirmed valid and negative. In this test, the assay specificity was equal to 100 %.

Diagnostic sensitivity: confirmation of positive samples

The Diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing 30 plasma samples and 30 stool supernatant samples, spiked with HEV reference material.

The negative samples used in the diagnostic specificity test were spiked with HEV reference material (PEI) at three different concentrations (3x LoD, 10x LoD and 30x LoD). The spiked samples were tested by the assay in association with ELITe InGenius system in “Extract + PCR” mode.

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The results are summarized in the following table.

Samples	N	positive	negative	invalid
HEV spiked Plasma EDTA	30	30	0	0
HEV spiked Stool supernatant	30	29	1	0

In the test, 30 out of 30 plasma spiked samples were confirmed positive and 29 out of 30 stool supernatant spiked samples were confirmed positive. One sample spiked at 3X LoD gave a discrepant negative result.

In this test, the assay diagnostic sensitivity for plasma was equal to 100 % and the assay diagnostic sensitivity for stool supernatant was equal to 96.7 %.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File "HEV ELITe MGB Kit", FTP RTS130ING.

REFERENCES

J. J. Germer et al. (2017) *J Clin Microbiology* 55 (5): 1478 – 1487

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

PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: plasma collected in EDTA, Stool supernatant.

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

Do not use this product with samples containing too much faecal matrix: samples with high turbidity inhibit the amplification reaction of nucleic acids and can cause invalid results.

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

At the moment there are no data available concerning product performances with the following clinical samples: whole blood collected in EDTA.

The results obtained with this product depends 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 for nucleic acid extraction.

Owing to its high analytical sensitivity, the real-time amplification method used in this product is sensitive to cross-contaminations from positive clinical 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.

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 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.

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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 components.	Not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Prepare again the complete reaction mixture. Use a new aliquot of components.
Q-PCR Standards or Positive Control degradation.	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 components.	Prepare again the complete reaction mixture. Use a new aliquot of components.
Contamination of the Negative Control.	Use a new aliquot of molecular 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.

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 components.	Not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Prepare again the complete reaction mixture. Use a new aliquot of components.
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 primary sample in a “Extract + PCR” session.
Sample degradation.	Repeat the extraction with a new aliquot of sample.
Instrument error.	Contact ELITechGroup Technical Service.

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

Error 30103	
Possible Causes	**Solutions**
Sample with too high target concentration.	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.

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TH Error	
Possible Causes	**Solutions**
Sample with too high target concentration.	If significant amplification is observed in PCR plot with negative baseline: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a 1:10 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session.

SYMBOLS

Catalogue Number.


Upper limit of temperature.

Batch code.


Use by (last day of month).

in vitro diagnostic medical device.


Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.

Contains sufficient for "N" tests.


Attention, consult instructions for use.

Contents.


Keep away from sunlight.


Manufacturer.

NOTICE TO PURCHASER: LIMITED LICENSE

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

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