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EMPOWERING IVD

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HBV ELITe MGB® Kit

reagents for DNA Real Time amplification

REF RTK602ING

CE 0344 IVD

-20°C

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INTENDED USE

The **HBV ELITe MGB® Kit** is a nucleic acid amplification assay for the **detection and the quantification** of Hepatitis B Virus (**HBV**) DNA extracted from clinical specimens.

The assay is able to detect HBV belonging to genotypes A, B, C, D, E, F, G, H, I and RF.

The assay is validated in association with **ELITe InGenius®** and "**ELITe BeGenius®**" using human plasma (collected in EDTA or ACD) and serum.

The product is intended for use as an aid in managing of HBV-infected individuals undergoing antiviral therapy. The results must be interpreted in combination with all relevant clinical observations and laboratory results.

This product is not intended for use as a screening test for the presence of HBV in blood or blood products or as a diagnostic test to confirm the presence of HBV infection.

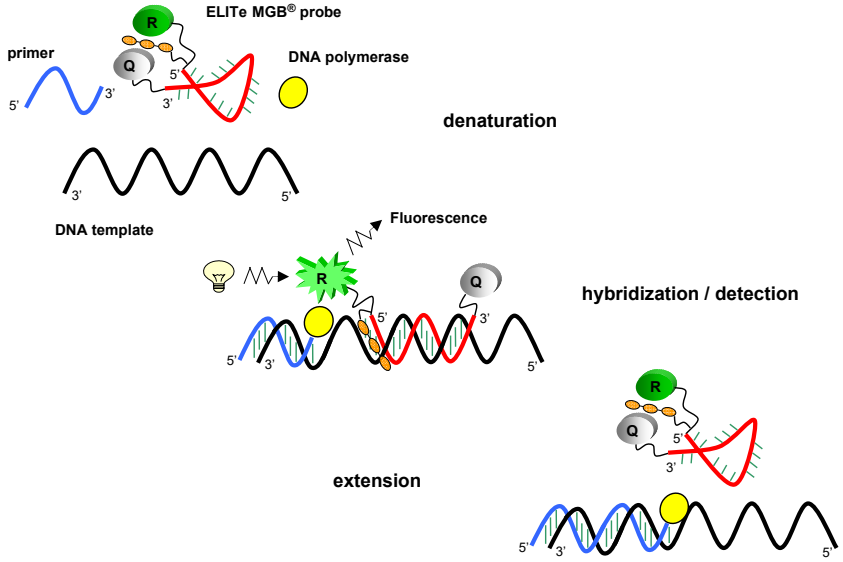
ASSAY PRINCIPLE

The assay is a quantitative Real-Time PCR validated on **ELITe InGenius** and **ELITe BeGenius**, automated and integrated systems for extraction, amplification and detection of nucleic acids and results interpretation.

HBV DNA is isolated from serum or plasma (collected in EDTA or ACD) specimens then amplified in a Real-Time PCR with **HBV PCR Mix**. Assay reagents contain primers and probes targeting the HBV polymerase gene (P gene). The HBV probe utilizes ELITe MGB technology and is labeled with FAM fluorophore. In addition, primers and probes specific to a heterologous Internal Control target are included in the assay reagents. The Internal Control probe also utilizes ELITe MGB technology and is labeled with AquaPhluor® 525 (AP525) dye. The exogenous Internal Control, IC2, is added to the lysis buffer and monitors for extraction and PCR efficiency.

The HBV and Internal Control specific probes are activated when hybridize with the related PCR products. ELITe InGenius monitors fluorescence increase and calculates the Ct and quantity based on a stored calibration curve.

The ELITe MGB technology is depicted in the illustration below. The fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe - amplicon duplex as the quencher is spatially separated from the fluorophore. Note the fluorophore is not cleaved during PCR.



PRODUCT DESCRIPTION

The **HBV ELITe MGB Kit** contains the following components:

- **HBV ELITe MGB Mix**

The **HBV ELITe MGB Mix** contains the subcomponent **HBV PCR Mix**, an optimized and stabilized PCR mixture aliquoted into **eight ready-to-use tubes**. Each tube contains **280 µL** and is sufficient for **12 tests** on the **ELITe InGenius** and **ELITe BeGenius** if processing at least 2 samples per session.

Primers and probe for HBV are specific for the polymerase gene (P gene) of **HBV**. The HBV probe is stabilized by MGB®, quenched by the Eclipse Dark Quencher®, and labeled with the FAM fluorophore for detection in Channel 1 of the **ELITe InGenius** and **ELITe BeGenius**.

Primers and probe for the exogenous Internal Control are specific for **IC2** artificial sequence. The IC2 probe is stabilized by MGB, quenched by the Eclipse Dark Quencher, and labeled with the AP525 fluorophore for detection in Channel 2 of the **ELITe InGenius** and **ELITe BeGenius**.

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

The **HBV ELITe MGB Mix** contains sufficient reagents for **96 tests on the ELITe InGenius** and **ELITe BeGenius**, with 20 µL used per reaction.

- **HBV ELITe Standard**

The **HBV ELITe Standard** contains the subcomponents **HBV Q-PCR Standards**, four stabilized solutions of plasmid DNA with the HBV polymerase gene region at **known titer** aliquoted into **ready-to-use tubes**. Each tube contains **160 µL** of solution, sufficient for **2 sessions**. The **HBV Q-PCR Standards** must be used with the **HBV PCR Mix** on the **ELITe InGenius** and **ELITe BeGenius** to construct the calibration curve of the system (product batch and instrument) for HBV quantification.

The plasmid DNA concentration was determined by UV spectroscopy as copies/mL, which was correlated to the "4th WHO International Standard for HBV DNA for NAT" (NIBSC, UK, code 10/266) by a conversion factor allowing HBV quantification in International Unit/mL (IU/mL).

The plasmid DNA concentration was also correlated to the "5th WHO International Standard for HBV DNA for NAT" (NIBSC, UK, code 22/120) as per Conversion factor to International Units, as indicated in Performance characteristics section.

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

- **HBV - ELITe Positive Control**

The **HBV – ELITe Positive Control** contains the subcomponent **HBV Positive Control**, a stabilized solution of plasmid DNA with the HBV polymerase gene region aliquoted into **two ready-to-use tubes**. Each tube contains **160 µL** of solution, sufficient for **4 sessions**. The **HBV Positive Control** must be used with the **HBV PCR Mix** on the **ELITe InGenius** and **ELITe BeGenius** to construct control plots for the verification of the system (product batch and instrument).

The **HBV – ELITe Positive Control** contains sufficient material for **8 sessions on ELITe InGenius** and **ELITe BeGenius**, with 20 µL used per reaction.

- **HBV Internal Control**

The **HBV Internal Control** contains the subcomponent **HBV CPE** (exogenous Internal Control), a stabilized solution of plasmid DNA containing the artificial IC2 sequence aliquoted into **eight ready-to-use tubes**. Each tube contains **160 µL** of solution, sufficient for **12 samples** if processing at least 2 samples per session. The **HBV CPE** is added to extraction reagents, purified along with sample nucleic acids, and subsequently combined with **HBV PCR Mix** for Real-Time PCR to validate the results of HBV negative samples.

The **HBV Internal Control** contains sufficient material for **96 tests on ELITe InGenius** and **ELITe BeGenius,** with 10 µL used per extraction.

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MATERIALS PROVIDED IN THE PRODUCT

Component	Sub-Component	Description	Quantity	Classification of hazards
HBV ELITe MGB Mix Ref. RTS602ING	HBV PCR Mix Ref. RTS602ING	Mixture of reagents for Real-Time PCR with **WHITE cap**	**8 x 280 µL**	-
HBV ELITe Standard Ref. STD602ING	HBV Q-PCR Standard 10^5 Ref. STD602ING-5	plasmid solution in tube with **RED cap**	**1 x 160 µL**	-
	HBV Q-PCR Standard 10^4 Ref. STD602ING-4	plasmid solution in tube with **BLUE cap**	**1 x 160 µL**	
	HBV Q-PCR Standard 10^3 Ref. STD602ING-3	plasmid solution in tube with **GREEN cap**	**1 x 160 µL**	
	HBV Q-PCR Standard 10^2 Ref. STD602ING-2	plasmid solution in tube with **YELLOW cap**	**1 x 160 µL**	
HBV - ELITe Positive Control Ref. CTR602ING	HBV Positive Control Ref. CTR602ING	Plasmid solution in tube with **BLACK** cap	**2 x 160 µL**	-
HBV Internal Control Ref. CPE602ING	HBV CPE Ref. CPE602ING	Solution of Plasmid DNAs and genomic RNA of MS2 phage 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).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA and the consumables are **not** provided with this kit.

For nucleic acid extraction and sample analysis with the **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) the following are required:

- **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200) extraction cartridges
- **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS)
- **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000)
- **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR)
- **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S)
- Assay protocols (ELITechGroup S.p.A.)
  - Calibration Standards **HBV ELITe_STD**,
  - Positive PCR Control **HBV ELITe_PC**,
  - Negative PCR Control **HBV ELITe_NC**,
  - One of the following for sample analysis: **HBV ELITe_PL_200_50**, **HBV ELITe_Se_200_50**.

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

- **ELITe InGenius® SP 200** (ELITechGroup S.p.A., ref. INT032SP200) extraction cartridges
- **ELITe InGenius® SP 200 Consumable Set** (ELITechGroup S.p.A, ref. INT032CS)
- **ELITe InGenius® Waste Box** (ELITechGroup S.p.A, ref. F2102-000)
- **ELITe InGenius® PCR Cassette** (ELITechGroup S.p.A, ref. INT035PCR)
- **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118)
- Assay protocols (ELITechGroup S.p.A.)
  - Calibrators Standards **HBV ELITe_Be_STD**,
  - Positive PCR Control **HBV ELITe_Be_PC**,
  - Negative PCR Control **HBV ELITe_Be_NC**,
  - One of the following for sample analysis: **HBV ELITe_Be_PL_200_50**, **HBV ELITe_Be_Se_200_50.**

Note: in case of need, the Calibrators and the Positive Control are also available as separate products: **HBV ELITe Standard,** ref. STD602ING, and **HBV - ELITe Positive Control,** ref. CTR602ING.

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. 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 infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be 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 regulations in force.
Carefully read all instructions provided before running the assay.
While running the assay, follow the product instructions provided.
Do not use the product after the indicated expiry date.
Use only 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 except where indicated.

Warnings and precautions for molecular biology

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

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

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

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

The extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination. The pipettes used to handle extraction products must be exclusively used for this purpose.

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

Warnings and precautions specific for the product components

- **HBV ELITe MGB Mix**
  The **HBV PCR Mix** must be stored at temperature below -20 °C and protected from light.
  The **HBV PCR Mix** must be used within 60 days from the first opening.
  The **HBV PCR Mix** can be frozen and thawed up to **seven times**: further freeze / thaw cycles may cause a loss of product performance.
  The **HBV PCR Mix** can be kept on board in the inventory area cool block for up **seven separete sessions of three hours each** (Extract + PCR mode, with intermediate freeze / thaw cycles) or for **three consecutive sessions of three hours each** (Extract + PCR mode).
- **HBV ELITe Standard**
  The **HBV Q-PCR Standard** must be stored at temperature below -20°C.
  The **HBV Q-PCR Standard** must be used within 60 days from the first opening.
  The **HBV Q-PCR Standard** can be frozen and thawed up to **two times**: further freeze / thaw cycles may cause a loss in titre.
  The **HBV Q-PCR Standard** can be kept on board in the extraction area for up to **two separate sessions of two hours each** (PCR Only mode).
- **HBV - ELITe Positive Control**
  The **HBV Positive Control** must be stored at temperature below -20 °C.
  The **HBV Positive Control** must be used within 60 days from the first opening.
  The **HBV Positive Control** can be frozen and thawed up to **four times**: further freeze / thaw cycles may cause a loss of product performance.
  The **HBV Positive Control** can be kept on board in the extraction area for up to **four separate sessions of three hours each** (Extract + PCR mode).
- **HBV Internal Control**
  The **HBV CPE** must be stored at temperature below -20 °C.
  The **HBV CPE** must be used within 60 days from the first opening.
  The **HBV CPE** can be frozen and thawed up to **twelve times**: further freeze / thaw cycles may cause a loss of product performance.
  The **HBV CPE** can be kept on board in the inventory area cool block for up to **six separate sessions of three hours each** (Extract + PCR mode).

ELITe InGenius

SAMPLES AND CONTROLS

Samples

This product is intended for use with the following clinical specimens:

Plasma collected in EDTA or ACD

Plasma specimens for nucleic acid extraction must be collected in EDTA or ACD, 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. Alternatively, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for six months.

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

Note: Nucleic acid extraction from EDTA plasma or ACD plasma is performed on **ELITe InGenius** with **ELITe InGenius Software** version 1.3 (or later) using the assay protocol **HBV ELITe_PL_200_50** to processes 200 µL of sample, adds 10 µL of **HBV CPE** (Internal Control) to each extraction and elutes the nucleic acids in 50 µL.

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

Serum

Serum specimens for nucleic acid 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. Alternatively, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for six months.

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

Note: Nucleic acid extraction from serum is performed on **ELITe InGenius** with **ELITe InGenius Software** version 1.3 (or later) using the assay protocol **HBV ELITe_Se_200_50** to processes 200 µL of sample, adds 10 µL of **HBV CPE** (Internal Control) to each extraction, and elutes the nucleic acid in 50 µL.

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.

Interfering substances

Available data concerning inhibition caused by drugs and other substances are reported in Potentially Interfering Substances in the Performance Characteristics section.

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

Calibration curve and Amplification controls

Before analysing any sample, it is necessary to generate the calibration curve and run the amplification controls for each lot of PCR reagent:

- for the calibration curve, use the four levels of the **HBV ELITe Standard** provided with this kit and **HBV ELITe_STD** Assay Protocol,
- for the Positive Control, use the **HBV - ELITe Positive Control** provided with this kit and **HBV ELITe_PC** Assay Protocol,
- for the Negative Control, use molecular biology grade water (not provided with this kit) and **HBV ELITe_NC** Assay Protocol.

Note: ELITe InGenius requires an approved and valid calibration curve and amplification controls for each lot of PCR reagent.
Calibration curves stored in the database expire after **60 days**, at which time it is necessary to re-run the **HBV Standard** with the appropriate PCR reagent lot.
Amplification control results stored in the database expire after **15 days**, at the which time it is necessary to re-run the Positive and Negative Controls with the appropriate PCR reagent lot.
Furthermore, the calibrators and amplification controls must be re-run when:

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

Quality controls

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

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PROCEDURE

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

- Verification of the system readiness,
- Session setup,
- 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 login in "**CLOSED**" mode,
- verify the Calibrators (**HBV Q-PCR Standards**) are approved and valid (Status) for the **HBV PCR Mix** lot to be used. If no valid Calibrators are available for the **HBV PCR Mix** lot, perform calibration as described below,
- verify the amplification Controls (**HBV Positive Control**, **HBV Negative Control**) are approved and valid (Status) for the **HBV PCR Mix** lot to be used. If no valid amplification Controls are available for the **HBV PCR Mix** lot, run the amplification 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 InGenius instrument with the indicated matrices.

The Assay Protocols available for sample testing with the product **HBV ELITe MGB Kit** are described in the table below:

Assay Protocol for HBV ELITe MGB Kit			
Name	**Matrix**	**Report unitage**	**Characteristics**
HBV ELITe_PL_200_50	Plasma samples	Positive / IU/mL / copies/mL / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HBV ELITe_Se_200_50	Serum samples	Positive / IU/mL / copies/mL / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Session Setup

The **HBV ELITe MGB Kit** can be used on **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 Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

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

The main steps for the setup of the three types of runs are described below.

A. Integrated run

To setup an integrated run with sample extraction and amplification, follow the steps below while referring to the GUI:

1. Thaw samples at room temperature (+18 / 25 °C) and handle according to laboratory guidelines and to the “Samples and Controls” section.
2. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25 °C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

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

3. Thaw the needed **HBV CPE** tubes at room temperature (~+25 °C) for 30 minutes. Each tube is sufficient for 12 extractions. Mix gently then spin down the contents for 5 seconds.
4. Select “Perform Run” from the “Home” screen.
5. Ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 50 µL.
6. For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.
7. Select the Assay Protocol to be used in the “Assay” column (e.g., HBV ELITe_PL_200_50).
8. Ensure that the “Protocol” displayed is: “Extract + PCR”.
9. Select the sample loading position as “Extraction Tube” in the “Sample Position” column. Click “Next” to continue.
10. Load **HBV CPE** and **HBV PCR Mix** on the designated “Inventory Block” referring to the Load List and enter the reagent lot number and expiry date. Click “Next” to continue.
11. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
12. Load the **PCR Cassettes**, the **ELITe InGenius SP 200** extraction cartridges, and all required consumables and samples to be extracted, following the GUI instruction. Click “Next” to continue.
13. Close the instrument door.
14. Press “Start” to start the run.

When the session is finished, the ELITe InGenius allows users to view, approve, and 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, labeled, and stored at -20 °C for one month. Avoid spilling the Extracted Sample.

Note: At the end of the run, the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

Note: The PCR Mix can be used for 7 separate sessions of 3 hours each or can be kept on board in the refrigerated block for up to 3 consecutive sessions of 3 hours each. Mix gently then spin down the contents for 5 seconds before starting the next session.

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B. Amplification run

To set up the amplification run starting from extracted nucleic acid, follow the steps below while referring to the GUI:

1. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25 °C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently then spin down the content for 5 seconds.

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

2. Select “Perform Run” from the “Home” screen.
3. Ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 50 µL, even if extraction is not being performed.
4. For each sample, assign the Track and enter the SID by typing or by scanning the sample barcode.
5. Select the Assay Protocol to be used in the “Assay” column (e.g., HBV ELITe_PL_200_50).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue.
8. Load **HBV PCR Mix** on the “Inventory Block” referring to the Load List and enter the reagent lot number and expiry date. Click “Next” to continue.
9. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
10. Load the **PCR Cassettes** and 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** allows users to view, approve, and 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 spilling the Extracted Sample.

Note: At the end of the run, the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

Note: The PCR Mix can be used for 7 separate sessions of 3 hours each or can be kept on board in the refrigerated block for up to 3 consecutive sessions of 3 hours each. Mix gently then spin down the contents for 5 seconds before starting the next session.

C. Calibration run

To set up the Calibration run for Q-PCR Standards, follow the steps below while referring to the GUI:

1. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25 °C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

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

2. Thaw the **HBV Q-PCR Standard** tubes (Cal1: HBV Q-PCR Standard 10^2, Cal2: HBV Q-PCR Standard 10^3, Cal3: HBV Q-PCR Standard 10^4, Cal4: HBV Q-PCR Standard 10^5) at room temperature (~+25 °C) for 30 minutes. Each tube is sufficient for 2 reactions. Mix gently then spin down the contents for 5 seconds.
3. Select “Perform Run” from the “Home” screen.
4. Ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 50 µL, even if extraction is not being performed.
5. For the **HBV Q-PCR Standard**, assign the Track, select the Assay Protocol “HBV ELITe_STD” in the “Assay” column and enter the reagent lot number and expiry date. Click “Next” to continue.
6. Load **HBV PCR Mix** on the “Inventory Block” referring to the Load List and enter the reagent lot number and expiry date. Click “Next” to continue.
7. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
8. Load the **PCR Cassettes** and the **HBV Q-PCR Standard** tubes following the GUI instruction. Click “Next” to continue.
9. Close the instrument door.
10. Press “Start” to start the run.

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

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

Note: At the end of the run, the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

Note: The Q-PCR Standards can be used for 2 separate sessions of 2 hours each.

Note: The PCR Mix can be used for 7 separate sessions of 3 hours each or can be kept on board in the refrigerated block for up to 3 separate sessions of 3 hours each. Mix gently then spin down the contents for 5 seconds before starting the next session.

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D. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, follow the steps below while referring to the GUI:

1. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25 °C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds.

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

2. Thaw **HBV Positive Control** tubes at room temperature (~+25 °C) for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently then spin down the contents for 5 seconds.
3. Prepare the HBV Negative Control by transferring at least 50 µL of molecular biology grade water to an “Elution tube”, provided with the **ELITe InGenius SP 200 Consumable Set**.
4. Select “Perform Run” from the “Home screen”.
5. Ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 50 µL, even if extraction is not being performed.
6. For the Positive Control, assign the Track, select the Assay Protocol “HBV ELITe_PC” in the “Assay” column and enter the reagent lot number and expiry date.
7. For the Negative Control, assign the Track, select the Assay Protocol “HBV ELITe_NC” in the “Assay” column and enter the molecular biology grade water lot number and expiry date. Click “Next” to continue.
8. Load **HBV PCR Mix** on the “Inventory Block” referring to the Load List and enter the reagent lot number and expiry date. Click “Next” to continue.
9. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
10. Load the **PCR Cassettes**, the **HBV 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** allows users to view, approve, and 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. The remaining Negative Control must be discarded.

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

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

Note: The PCR Mix can be used for 7 separate sessions of 3 hours each or can be kept on board in the refrigerated block for up to 3 consecutive sessions of 3 hours each. Mix gently then spin down the contents 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 results and the run information are shown. From this screen, results can be approved, and reports printed or saved ("Sample Report" or "Track Report"). Refer to the instrument manual for more details.

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

The **ELITe InGenius** generates results with the **HBV ELITe MGB Kit** through the following procedure:

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

A. Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the HBV probe (Channel "HBV") of the Calibrator reactions with the **HBV ELITe_STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

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

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

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

B. Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the HBV probe (Channel "HBV") of the Positive Control and Negative Control reactions with the **HBV ELITe_PC** and **HBV ELITe_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and used to validate the system (reagents lot and instrument).

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

The Positive Control and Negative Control results expire **after 15 days**.

Before analysing any sample, it is mandatory to verify that Positive Control and Negative Control results are approved and valid for the PCR reagent lot. The Status of Positive Control and Negative Control results for each lot of PCR reagent is shown in the "Controls" module. If the results of Positive Control and/or Negative Control are missing or expired, run the control(s) as described above.

The **ELITe InGenius software** processes the Positive Control and Negative Control results and generates Control Charts. Four approved Positive Control and Negative Control results are used to set up the initial Control Chart. For subsequent controls, the results are analysed by the software to ensure the system performances are within the acceptance criteria, shown in the Control Chart plots. Refer to the instrument manual for more details.

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

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

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C. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the HBV probe (Channel "HBV") and the Internal Control probe (Channel "IC") with the **HBV ELITe_PL_200_50** and **HBV ELITe_Se_200_50** Assay Protocol parameters. The resulting HBV Ct values are converted to concentration.

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

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

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

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

Samples reported as "DNA Detected, quantity below LLoQ" are not suitable for quantification. The concentration of HBV DNA 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.

Samples reported as "DNA Detected, quantity beyond ULoQ" are not suitable for quantification. The concentration of HBV DNA 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.

Samples reported as "HBV DNA Not Detected or below LoD" are suitable for analysis but HBV DNA was not detected. In this case, the sample may be either negative for HBV DNA or the HBV DNA is present at a concentration below the limit of detection of the assay (see "Performance characteristics").

HBV DNA positive samples at a concentration below the LoD, if detected, are reported as "HBV: DNA Detected, quantity below LLoQ" (see "Performance characteristics").

Samples reported as "Invalid - Retest Sample" are not suitable for result interpretation. In this case, the Internal Control DNA was not efficiently detected, which could be due to problems in the PCR or extraction step (degradation or loss of DNA during the extraction or inhibitors in the eluate), which may cause incorrect results.
If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in "PCR Only" mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using "Extract + PCR" mode.

Note: The results obtained with this assay must be interpreted along with all other clinical data and other laboratory test for the patient.

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

D. Sample result reporting

The sample results are stored in the database and reports can be exported as “Sample Report” and “Track Report”.
The “Sample Report” shows the results details by selected sample (SID).
The “Track Report” shows the results details by selected Track.
The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

ELITe BeGenius

SAMPLES AND CONTROLS

Samples

This product is intended for use with the following clinical specimens:

Plasma collected in EDTA or ACD

Plasma specimens for nucleic acid extraction, must be collected in EDTA or ACD, 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.

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 DNA 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 **HBV ELITe_Be_PL_200_50**. This protocol processes 200 µL of sample, adds 10 µL per extraction of the **HBV 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 specimens for nucleic acid 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 six 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 DNA 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 **HBV ELITe_Be_Se_200_50**. This protocol processes 200 µL of sample, adds 10 µL per extraction of the **HBV CPE** (Internal Control) and elutes the nucleic acids in 50 µL.

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

Other samples

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

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.

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

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 **HBV 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 InGenius and login in “**CLOSED**” mode,
  verify the Calibrators (**HBV Q-PCR Standard**) are approved and valid (Status) for the **HBV ELITe MGB Kit** lot to be used. If no valid Calibrators are available for the **HBV ELITe MGB Kit** lot, perform calibration as described below,
- verify that the amplification Controls (**HBV Positive Control**, **HBV Negative Control**) are approved and valid (Status) for the **HBV ELITe MGB Kit** lot to be used. If no valid amplification Controls are available for the **HBV ELITe MGB Kit** lot, run the Controls as described below,
- choose the type of run, follow the instructions on the Graphical User Interface (GUI) for the session setup and use 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 indicated matrices.

The Assay Protocols available for sample testing with the product **HBV ELITe MGB Kit** are described in the table below:

Assay Protocol for HBV ELITe MGB Kit			
Name	**Matrix**	**Report units**	**Characteristics**
HBV ELITe_Be_PL_200_50	Plasma samples	Positive / IU/mL / copies/mL / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
HBV ELITe_Be_Se_200_50	Serum samples	Positive / IU/mL / copies/mL / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Session Setup

The **HBV ELITe MGB Kit** can be used on **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 required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

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

The main steps for the setup of the three types of runs are described below.

A. Integrated run (Extract + PCR)

To set up an integrated run with sample extraction and amplification, follow the steps below while referring to the GUI:

1. Thaw samples at room temperature (~+25°C) and handle according to laboratory guidelines and to the "Samples and Controls" section.
2. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently, then spin down the content for 5 seconds.

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

3. Thaw the needed **HBV CPE** tubes at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 12 extractions. Mix gently,then spin down the content for 5 seconds.
4. Select "Perform Run" from the "Home screen".
5. Remove the racks from the "Cooler Unit" and place them on the preparation table.
6. Select the "run mode": "Extract + PCR".
7. Load the specimens into Racks 5 and 4 (always starting from Rack 5), using adaptors for appropriate fit if necessary.
8. Insert the Rack into the "Cooler Unit". Click "Next" to continue.

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

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9. Ensure that the "Extraction Input Volume" is 200 µL and the "Extraction Elution Volume" is 100 µL.
10. Select the assay protocol to be used in the "Assay" column (i.e. HBV ELITe_Be_PL_200_50). Click "Next" to continue.
11. If Rack 4 is used, repeat step 7 to 9.
12. Load the Elution tubes into the Racks 3 and 2 (start always from Rack 3).

Note: Elution tubes can be labelled to improve traceability.

13. Insert the Rack into the "Cooler Unit". Click "Next" to continue.
14. If Rack 2 is used, repeat step 12.
15. Load **CPE** and **HBV-PCR Mix** into the Rack 1.
16. Insert the Rack 1 into the "Cooler Unit". Click "Next" to continue.
17. Verify the tips in the Tip Racks in the "Inventory Area" and replace Tip Rack(s) if necessary. Click "Next" to continue.
18. Load the Basket with **PCR Cassette** in the "Inventory Area" by following the GUI instruction. Click "Next" to continue.
19. Load the Basket with the **ELITe InGenius SP 200** extraction cartridges and the required extraction consumables by following the GUI instruction. Click "Next" to continue.
20. Close the instrument door.
21. Press "Start" to start the run.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, and 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

Note: At the end of the run the **PCR Cassettes** and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction product.

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. 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 DNA, follow the steps below while referring to the GUI:

1. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently, and spin down the contents for 5 seconds.

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

2. Select "Perform Run" on 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.
7. Ensure that the "Extraction Input Volume" is 200 µL and the "Extraction Elution Volume" is 100 µL, even if extraction is not being performed.
8. Select the assay protocol to be used in the "Assay" column (e.g. HBV ELITe_Be_PL_200_50). Click "Next" to continue.
9. If Rack 2 is used, repeat steps 7 to 9.
10. Load **HBV PCR Mix** into Rack 1.

11. Insert the rack into the “Cooler Unit”. Click “Next” to continue.
12. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
13. Load the basket with **PCR Cassette** in the “Inventory Area” by following the GUI instruction. Click “Next” to continue.
14. Close the instrument door.
15. Press “Start” to start the run.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, and 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. Avoid spilling of the Extracted Sample.

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run (PCR only)

To set up a Calibration run with the Q-PCR Standards, follow the steps below while referring to the GUI:

1. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for 12 reactions in optimized conditions (at least 2 tests per session). Mix gently, then spin down the content for 5 seconds.

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

2. Thaw the **HBV Q-PCR Standard** tubes (Cal1: HBV Q-PCR Standards 10^2, Cal2: HBV Q-PCR Standards 10^3, Cal3: HBV Q-PCR Standards 10^4, Cal4: HBV Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 2 reactions. Mix gently, then spin down the content for 5 seconds.
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 calibrator standards into Racks 3.
7. Insert the rack into the “Cooler Unit”. Click “Next” to continue.
8. Select the assay protocol to be used in the “Assay” column (HBV ELITe_Be_STD). Click “Next” button to continue.
9. Load **HBV PCR Mix** into Rack 2.
10. Insert Rack 2 into the “Cooler Unit”. Click “Next” to continue.
11. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
12. Load the basket with **PCR Cassette** in the “Inventory Area” by following the GUI instruction. Click “Next” to continue.
13. Close the instrument door.
14. Press “Start” to start the run.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, and 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.

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Note: At the end of the run, the PCR Cassettes and consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

D. Amplification run for Positive Control and Negative Control (PCR only)

To setup the amplification run for Positive Control and Negative Control, follow the steps below while referring to the GUI:

1. Thaw the needed **HBV PCR Mix** tubes at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently, then spin down the content for 5 seconds.

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

2. Thaw **HBV Positive Control** tubes at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently, then spin down the content for 5 seconds.
3. Transfer ≥50 µL of the molecular biology grade water (as Negative Control) in an Elution tube, provided with the **ELITe InGenius SP Consumable Set**.
4. Select “Perform Run” from the “Home screen”.
5. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
6. Select the run mode: “PCR Only”.
7. Load the Positive Control and Negative Control tubes into Racks 3.
8. Insert the rack into the “Cooler Unit”. Click “Next” to continue.
9. Select the assay protocol to be used in the “Assay” column (HBV ELITe_Be_PC and HBV ELITe_Be_NC). Click “Next” button to continue.
10. Load the **HBV PCR Mix** into Rack 2.
11. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
12. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
13. Load the Basket with **PCR Cassette** in the “Inventory Area” by following the GUI instruction. Click “Next” to continue.
14. Close the instrument door.
15. Press “Start” to start the run.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, and 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.

Note: At the end of the run, the PCR Cassettes and other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.
Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. 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 results and the run information are shown. From this screen, results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

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

The **ELITe BeGenius** generates the results using the **HBV 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 BeGenius** chapters for the details.

PERFORMANCE CHARACTERISTICS
ELITe InGenius and ELITe BeGenius

Limit of Detection (LoD)

The HBV ELITe MGB® Kit Limit of Detection (LoD) of was determined with Plasma samples on ELITe InGenius.

The LoD was determined by testing a panel of HBV negative ACD Plasma spiked with HBV certified reference material (4th WHO International Standard, NIBSC) at known titres. Six dilution levels of were prepared starting from 18 IU/mL to 1 IU/mL. Each dilution level was processed in 24 replicates on ELITe InGenius in "Extract + PCR" mode. Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Limit of Detection (IU/mL) for ACD Plasma samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
HBV	9	6	18

The LoD as copies/mL for ACD Plasma was calculated by applying the specific Conversion factor reported on page 23. The analytical sensitivity as copies/mL is reported below.

Limit of Detection (copies/mL) for ACD Plasma samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
HBV	38	27	73

The calculated LoD value was verified by testing 30 replicates of ACD Plasma, 30 replicates of EDTA Plasma and 30 replicates of Serum samples spiked with HBV certified reference material (4th WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 27 out of 30 replicates give a positive result.

The results are reported in the following table.

Limit of Detection for Plasma and Serum samples and ELITe InGenius					
Sample	Titer	Target	N	Positive	Negative
ACD Plasma	9 IU/mL	HBV	30	30	0
EDTA Plasma	9 IU/mL	HBV	30	28	2
Serum	9 IU/mL	HBV	30	29	1

The LoD value for HBV target was confirmed at 9 IU/mL for ACD Plasma, EDTA Plasma and Serum.

The calculated LoD in association with **ELITe BeGenius** was verified by testing 30 replicates of ACD Plasma, 30 replicates of EDTA Plasma and 30 replicates of Serum samples spiked by HBV certified reference material (4th WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 27 out of 30 replicates give a positive result.

The results are reported in the following table.

Limit of Detection for Plasma and Serum samples and ELITe BeGenius					
Sample	Titer	Target	N	Positive	Negative
ACD Plasma	9 IU/mL	HBV	30	28	2
EDTA Plasma	9 IU/mL	HBV	30	28	2
Serum	9 IU/mL	HBV	30	30	0

The LoD value for HBV target was confirmed at 9 IU/mL for ACD Plasma, EDTA Plasma and Serum.

Matrix equivalence: EDTA Plasma versus ACD Plasma and Serum

The Matrix equivalence of the HBV ELITe MGB Kit was verified using samples of ACD Plasma, EDTA Plasma, and Serum on ELITe InGenius.

A test was performed on 30 samples of EDTA Plasma and 30 samples of ACD Plasma from the same 30 individual donors (paired samples), which tested negative for HBV by a CE IVD marked immunoassay. The samples were tested on ELITe InGenius in "Extract + PCR" mode. The Negative Percent Agreement (NPA) was evaluated. The Coefficient of Variation (%CV) of Internal Control Ct values was calculated in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

Sample	N	Positive	Negative	NPA	IC Ct %CV	Whole IC Ct %CV
EDTA Plasma	30	0	30	100%	0.86	0.98
ACD Plasma	30	0	30		1.01	

A test was performed on 30 paired samples of EDTA Plasma and Serum, which tested negative for HBV by a CE IVD marked immunoassay. The samples were tested on ELITe InGenius in "Extract + PCR" mode. The Negative Percent Agreement was evaluated. The Coefficient of Variation (%CV) of Internal Control Ct values was calculated in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

Sample	N	Positive	Negative	NPA	IC Ct %CV	Whole IC Ct %CV
EDTA Plasma	30	0	30	97%	0.90	0.86
Serum	30	1	29		0.82	

The positive Serum sample showed a very low titre (lower than 9 IU/mL) which is consistent with a negative result by the immunologic CE IVD assay used to certify the negativity of the sample.

A test was performed on 30 paired samples of EDTA Plasma and ACD Plasma, which tested negative for HBV by a CE IVD marked immunoassay, then spiked with certified reference material (4th WHO HBV International Standard, NIBSC) at a concentration of 3 x LoD (about 27 IU/mL). The samples were tested on ELITe InGenius in "Extract + PCR" mode. The Positive Percent Agreement (PPA) was evaluated. The Coefficient of Variation (%CV) of HBV target Ct values was calculated in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

Sample	N	Positive	Negative	PPA	HBV Ct %CV	Whole HBV Ct %CV	ΔQty (Log IU/mL)
EDTA Plasma	30	30	0	100%	1.75	1.81	0.0458
ACD Plasma	30	30	0		1.88		

A test was Performed on 30 paired samples of EDTA Plasma and Serum, which tested negative for HBV by a CE IVD marked immunoassay, then spiked with certified reference material (4th WHO HBV International Standard, NIBSC) at a concentration of 3 x LoD (about 27 IU/mL). The samples were tested on ELITe InGenius in "Extract + PCR" mode. The Positive Percent Agreement was evaluated. The Coefficient of Variation (%CV) of HBV target Ct values was calculated in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

Sample	N	Positive	Negative	PPA	HBV Ct %CV	Whole HBV Ct %CV	ΔQty (Log IU/mL)
EDTA Plasma	30	30	0	100%	1.59	1.49	0.0910
Serum	30	30	0		1.29		

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

Additional Matrices equivalence testing was performed in the Linear Measuring Range study reported on page 23.

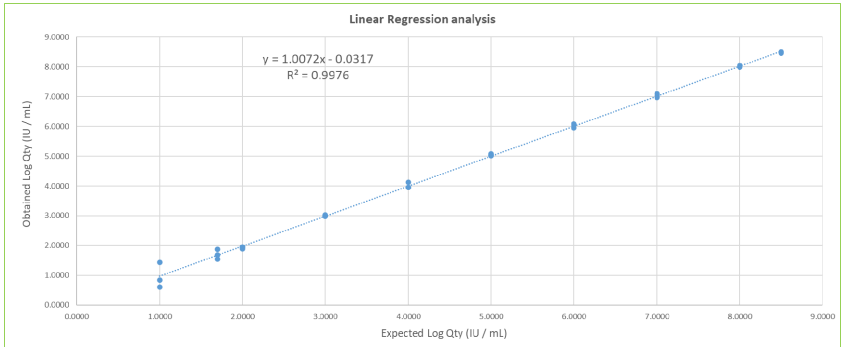
Linear measuring range

The Linear measuring range of HBV ELITe MGB Kit was determined with Plasma samples on ELITe InGenius.

The Linear measuring range was determined using a panel of dilutions of HBV reference material (ZeptoMetrix) in negative EDTA Plasma samples. The panel consisted of ten dilution points from about $3.2x10^8$ IU/mL to 10 IU/mL. Each sample of the panel was tested in triplicate on ELITe InGenius in “Extract + PCR” mode.

The data were analysed by linear regression and polynomial analysis, and results demonstrated that the assay shows a linear response for all the dilutions with a Squared Correlation Coefficient (R2) equal to 0.998.

The results are reported in the following figure.



The Lower Limit of Quantification (LLoQ) was determined to be 9 IU/mL, the same value as LoD, where measured results were within ±0.5 Log IU/mL of the target concentration. Precision and Accuracy at the LLoQ were calculated with Standard Deviation = 0.2813 Log IU/mL and Bias=0.2767 Log IU / mL.

The Upper Limit of Quantification (ULoQ) was determined to be 317,750,000 IU/mL, where measured results were within ±0.5 Log IU/mL of the target concentration. Precision and Accuracy at the ULoQ were calculated with Standard Deviation = 0.0275 Log IU/mL and Bias = 0.0175 Log IU/mL.

The linear measuring range as copies/mL for EDTA Plasma is calculated by applying the specific conversion factor reported on page 31.

The final results are summarized in the following table.

Linear measuring range for EDTA Plasma samples and ELITe InGenius	
Lower Limit	Upper Limit
9 IU/mL	317,750,000 IU/mL
38 copies/mL	1,323,958,333 copies/mL

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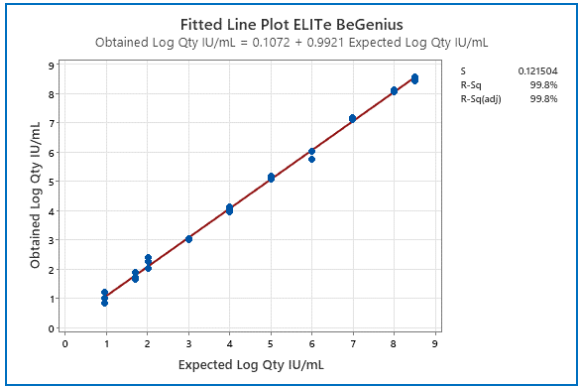
The Linear measuring range of HBV ELITe MGB® Kit was verified in association with Plasma samples and **ELITe BeGenius** system using a panel of dilutions of HBV reference material (ZeptoMetrix) in negative EDTA Plasma samples. The panel consisted of ten dilution points from about $3.2x10^8$ IU/mL to 9 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 linear regression analysis, demonstrated that the assay shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.998.

The results are reported in the following table.



The Lower Limit of Quantification (LLoQ) was confirmed to be 9 IU/mL, the same value as LoD, where measured results were within ±0.5 Log IU/mL of the target concentration. Precision and Accuracy at LLoQ were calculated with Standard Deviation = 0.1939 Log IU/mL Bias = 0.0709 Log IU / mL.

The Upper Limit of Quantification (ULoQ) was confirmed to be 317,750,000 IU/mL, where measured results were within ±0.5 Log IU/mL of the target concentration. Precision and Accuracy at the ULoQ were calculated with Standard Deviation = 0.0579 Log IU/mL and Bias = 0.0131 Log IU/mL.

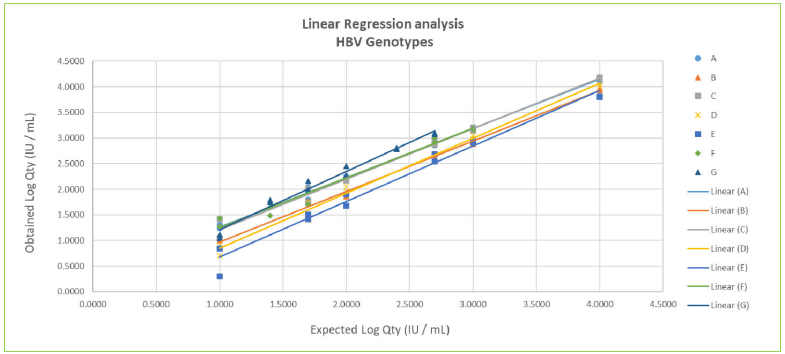
The linear measuring range as copies/mL for EDTA Plasma is calculated by applying the specific conversion factor reported on page 31.

The final results are summarized in the following table.

Linear measuring range for EDTA Plasma samples and ELITe BeGenius	
Lower Limit	Upper Limit
9 IU / mL	317,750,000 IU / mL
38 copies / mL	1,323,958,333 copies / mL

The Linear Measuring Range was verified by analysis of negative EDTA Plasma spiked with HBV reference material (1st WHO International Reference Panel for HBV Genotypes, PEI) for the main HBV genotypes (A, B, C, D, E, F, G). Each HBV genotype was tested in a panel of 6 dilution levels. Each dilution level was tested in duplicate on ELITe InGenius in “Extract + PCR” mode.

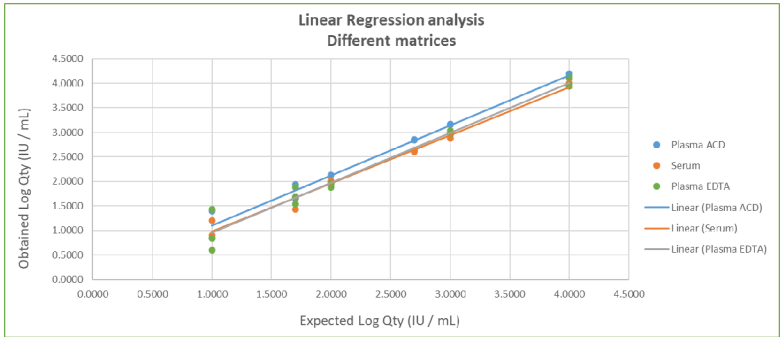
The results are reported in the following figure.



The linearity of the assay was confirmed for the main HBV genotypes (A, B, C, D, E, F, G) giving quantitative results within ±0.5 Log IU/mL and an R2 from 0.979 to 0.996.

The Linear Measuring Range was verified by analysis of negative ACD Plasma and negative Serum spiked with HBV reference material (4th WHO International Standard, NIBSC). Each matrix was tested in a panel of 6 dilution levels. Each dilution level was tested in duplicate on ELITe InGenius in "Extract + PCR" mode. Corresponding results of testing with EDTA Plasma were reported as reference.

The results are reported in the following figure.



The linearity of the assay was confirmed for ACD Plasma and Serum giving quantitative results within ±0.5 Log IU/mL and an R2 of 0.982 and 0.988, respectively.

Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

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

The analysis of the regions specific for the hybridization of primers and probe in the Polymerase gene (P gene) showed sequence conservation and absence of significant mutations in the HBV genotypes A, B, C, D, E, F, G, H, I and RF for all available sequences in the nucleotide database. So, an efficient detection and quantification for the different HBV genotypes is expected.

The Inclusivity of the assay, assessed by detection and quantification efficiency on different genotypes, was verified by testing the 1st WHO International Reference Panel for HBV Genotypes (PEI) including the main HBV genotypes (A, B, C, D, E, F, G).

Each sample of the panel was prepared at a concentration of 3 x LoD (about 27 IU/mL) in negative ACD Plasma samples and tested on ELITe InGenius in "Extract + PCR" mode.

The results are reported in the following table.

1st WHO International Reference Panel for HBV Genotypes (PEI)				
Sample ID	**Genotype**	**Pos. / Rep.**	**Mean HBV Ct**	**Mean HBV IU/mL**
HBV 1/A	A	3 / 3	37.71	25
HBV 2/A		3 / 3	37.76	25
HBV 3/A		3 / 3	38.20	17
HBV 4/B	B	3 / 3	38.15	17
HBV 5/B		3 / 3	38.56	17
HBV 6/B		3 / 3	38.23	18
HBV 7/B		3 / 3	37.75	24
HBV 8/C	C	3 / 3	38.01	20
HBV 9/C		3 / 3	38.12	18
HBV 10/D	D	3 / 3	37.84	22
HBV 11/D		3 / 3	37.97	20
HBV 12/D		3 / 3	38.29	16
HBV 13/E	E	3 / 3	38.02	19
HBV 14/F	F	3 / 3	37.81	22
HBV 15/G	G	3 / 3	37.08	37

The Inclusivity of the assay was also verified by testing the "AccuSet™ HBV DNA Genotype Performance Panel" (SeraCare) including the HBV genotypes A, B, C, D, E, F, G, H.

Each sample of the panel was prepared at a concentration of 3 x LoD (about 27 IU/mL) in negative ACD plasma samples and tested on ELITe InGenius in "Extract + PCR" mode.

The results are reported in the following table.

"AccuSet™ HBV DNA Genotype Performance Panel" (SeraCare)				
Sample ID	**Genotype**	**Pos. / Rep.**	**Mean HBV Ct**	**Mean HBV IU/mL**
A	A	3 / 3	38.01	19
B	B	3 / 3	37.99	20
C	C	3 / 3	38.14	18
D	D	3 / 3	38.16	18
E	E	3 / 3	38.15	17
F	F	3 / 3	37.92	21
H	H	3 / 3	37.80	22

All samples were correctly detected and quantified within ±0.5 Log IU/mL (9 – 85 IU/mL) by the HBV ELITe MGB Kit on ELITe InGenius.

Potentially interfering markers: cross-reactivity

The Potential cross-reactivity of the assay with other unintended organisms was evaluated by *in silico* analysis of sequences available in nucleotide databases.

The primer and probe sequences were assessed for homology with the sequences of other organisms available in nucleotide databases. The results showed no significant homology and therefore, no cross- reactivity is expected.

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 triplicate on ELITe InGenius in "PCR Only" mode. The genomic DNA or RNA of each organism was also 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	HBV Pos. / Rep.	Outcome
Adenovirus 2	0 / 3	No cross-reactivity
CMV	0 / 3	No cross-reactivity
EBV	0 / 3	No cross-reactivity
HHV6	0 / 3	No cross-reactivity
VZV	0 / 3	No cross-reactivity
HSV1	0 / 3	No cross-reactivity
HSV2	0 / 3	No cross-reactivity
HTLVI	0 / 3	No cross-reactivity
HTLVII	0 / 3	No cross-reactivity
Parvovirus B19	0 / 3	No cross-reactivity
Echovirus 4	0 / 3	No cross-reactivity
Dengue Virus Type 3	0 / 3	No cross-reactivity
WNV	0 / 3	No cross-reactivity
Influenza A virus (H1N1)	0 / 3	No cross-reactivity
Influenza B virus (Florida)	0 / 3	No cross-reactivity
RSV A2	0 / 3	No cross-reactivity
HAV	0 / 3	No cross-reactivity
HCV	0 / 3	No cross-reactivity
HEV	0 / 3	No cross-reactivity
HIV-1	0 / 3	No cross-reactivity
HIV-2	0 / 3	No cross-reactivity
Candida albicans	0 / 3	No cross-reactivity
Staphylococcus aureus	0 / 3	No cross-reactivity

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

Potentially interfering markers: Interference

The Absence of interference caused by other organisms' presence in clinical Plasma samples was verified by testing a panel of certified reference materials.

Samples of genomic DNA or RNA from potentially interfering markers (ATCC, NIBSC, ZeptoMetrix) at high concentration (at least 10^5 copies/reaction) were spiked with HBV genomic DNA (NIBSC) at low concentration (about 10 copies/reaction). The samples were analyzed in triplicate on ELITe InGenius in "PCR Only" mode. Each sample was also added with 80,000 Internal Control copies per reaction in order to mimic the extracted clinical sample.

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The results are reported in the following table.

Sample	HBV Pos. / Rep.	Outcome
Adenovirus 2	3 / 3	No interference
CMV	3 / 3	No interference
EBV	3 / 3	No interference
HHV6	3 / 3	No interference
VZV	3 / 3	No interference
HSV1	3 / 3	No interference
HSV2	3 / 3	No interference
HTLVI	3 / 3	No interference
HTLVII	3 / 3	No interference
Parvovirus B19	3 / 3	No interference
Echovirus 4	3 / 3	No interference
Dengue Virus Type 3	3 / 3	No interference
WNV	3 / 3	No interference
Influenza A virus (H1N1)	3 / 3	No interference
Influenza B virus (Florida)	3 / 3	No interference
RSV A2	3 / 3	No interference
HAV	3 / 3	No interference
HCV	3 / 3	No interference
HEV	3 / 3	No interference
HIV-1	3 / 3	No interference
HIV-2	3 / 3	No interference
Staphylococcus aureus	3 / 3	No interference
Candida albicans	3 / 3	No interference

All potentially interfering organisms tested showed no inhibition of the HBV target amplification using the HBV ELITe MGB Kit.

Potentially interfering substances

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

The inhibition panel samples were spiked with HBV certified reference material (4th WHO HBV International Standard, NIBSC) at a concentration of 3 x LoD (about 27 IU/mL).

In addition, 6 other potentially interfering substances, drugs, were tested at relevant concentration: Ganciclovir, Azithromycin, Glecaprevir, Entecavir, Tenofovir, Lamivudine. The substances were each added to negative ACD plasma spiked with HBV certified reference material (4th WHO HBV International Standard, NIBSC) at a concentration of 3 x LoD (about 27 IU/mL).

The samples were processed in triplicate on ELITe InGenius in "Extract + PCR" mode. The Ct values (reference and test samples) of the HBV target and Internal Control were used to calculate the Coefficient of Variation (%CV) in order to evaluate possible interference.

The results are reported in the following table.

Sample	Pos. / Rep.	%CV HBV Ct	%CV IC Ct
EDTA	3 / 3	1.78	0.87
Heparin	1 / 3	N.A.	7.11
Haemolitic Blood high	3 / 3	1.11	0.90
Lipemic Plasma	3 / 3	1.30	0.72
Icteric Plasma	3 / 3	1.13	1.41
Ganciclovir	3 / 3	0.96	0.76
Azithromycin	3 / 3	0.88	0.85
Glecaprevir	3 / 3	1.04	0.57
Entecavir	3 / 3	1.07	0.88
Tenofovir	3 / 3	0.88	0.76
Lamivudine	3 / 3	1.13	0.78

The test showed that EDTA, Hemoglobin, Triglycerides, Bilirubin, Ganciclovir, Azithromycin, Glecaprevir, Entecavir, Tenofovir and Lamivudine do not interfere with the HBV or Internal Control amplification. The HBV and IC Ct value %CV were lower than 2%.

Heparin was confirmed to be capable of inhibiting the amplification of HBV; however, due to the Internal Control Ct cut-off (IC Ct < 31), the sample results were "not valid" instead of "false negative".

Absence of cross-contamination

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

Five sets of samples, alternating six positive samples with six negative samples, were tested on ELITe InGenius in "Extract + PCR" mode.

The results are reported in the following table.

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

None of the tested HBV negative samples gave false positive results. In this test cross-contamination was neither detected within sessions nor between sessions.

Whole system failure rate

The Whole system failure rate was verified, in association with **ELITe InGenius,** by analysing a panel of samples spiked for HBV DNA at low titre and determining the frequency of "false negative" results.

100 individual samples of EDTA Plasma, 30 individual samples of ACD Plasma and 30 individual samples of Serum (all of which tested negative for HBV DNA) were spiked with certified reference material (4th WHO HBV International Standard, NIBSC) at a concentration of 3 x LoD (about 27 IU/mL). The samples were tested on ELITe InGenius in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	Negative	Positive	Mean HBV IU/mL
Spiked EDTA Plasma	100	0	100	26
Spiked ACD Plasma	30	0	30	27
Spiked Serum	30	0	30	23

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

The Whole system failure rate was verified in association with **ELITe BeGenius** by analysing a panel of samples spiked for HBV DNA at low titre and determining the frequency of "false negative" results.

100 individual samples of EDTA plasma, tested negative for HBV DNA, were spiked with certified reference material (4th WHO HBV International Standard, NIBSC) at a concentration of 3 x LoD (about 27 IU/mL). The samples were tested on ELITe BeGenius in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	Negative	Positive	Mean HBV IU/mL
Spiked EDTA Plasma	100	0	100	15

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

Repeatability

The Repeatability of results obtained with the HBV ELITe MGB Kit on **ELITe InGenius** was assessed by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HBV certified reference material (4th WHO HBV International Standard, NIBSC) at concentrations of 3 x LoD (about 27 IU/mL) and of 10 x LoD (about 90 IU/mL).

The Repeatability was determined by analysing of panel members in four replicates, in two runs per day, one lot of product per day, on two different days. Testing was performed with a total of three lots of product, all using the same instrument, by the same operator. Samples were processed in randomized positions on ELITe InGenius in "Extract + PCR" mode.

The Ct values of the HBV target and 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	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	28.89	0.23	0.79
3 x LoD	8 / 8	38.07	0.38	1.00				
10 x LoD	8 / 8	36.26	0.25	0.69				

Inter – Session Repeatability								
Sample	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	28.79	0.28	0.97
3 x LoD	16 / 16	38.01	0.41	1.08				
10 x LoD	16 / 16	36.18	0.28	0.77				

In the Repeatability test, the assay detected the HBV target as expected and showed Ct values with %CV below 1.1% for HBV and 1% for Internal Control.

The Repeatability of results obtained by the product HBV ELITe MGB Kit on **ELITe BeGenius** system was assessed by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked by HBV certified reference material (4th WHO HBV International Standard, NIBSC) at concentration of 3 x LoD (about 27 IU / mL) and of 10 x LoD (about 90 IU / mL).

The Repeatability was obtained through the analysis of panel samples in eight replicates, in one run 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 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 Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability								
Sample	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	30.06	0.37	1.24
3 x LoD	8 / 8	38.64	0.46	1.19				
10 x LoD	8 / 8	36.83	0.34	0.93				

Inter – Session Repeatability								
Sample	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	30.04	0.54	1.80
3 x LoD	16 / 16	38.93	0.86	2.22				
10 x LoD	16 / 16	36.87	0.35	0.94				

In the Repeatability test, the assay detected the HBV target as expected and showed low %CV of Ct values that did not exceed 2.2% for HBV and 1.8% for Internal Control.

Reproducibility

The Reproducibility of results obtained by the HBV ELITe MGB Kit on **ELITe InGenius** was assessed by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HBV certified reference material (4th WHO HBV International Standard, NIBSC) at concentrations of 3 x LoD (about 27 IU/mL) and of 10 x LoD (about 90 IU/mL).

The Reproducibility was determined by analysing panel members in four replicates, in one run per day, in two days per site. Three different lots of product were used at three different sites with three different instruments by three different operators. Samples were processed in randomized positions on ELITe InGenius 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	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 24	N.A.	N.A.	N.A.	72 / 72	28.73	0.45	1.58
3 x LoD	24 / 24	37.60	0.68	1.80				
10 x LoD	24 / 24	35.63	0.35	0.98				

Inter – Batch Reproducibility								
Sample	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 48	N.A.	N.A.	N.A.	144 / 144	28.67	0.41	1.44
3 x LoD	48 / 48	38.19	0.44	1.16				
10 x LoD	48 / 48	36.25	0.38	1.06				

In the Reproducibility test, the assay detected the HBV target as expected and showed Ct values with %CV below 1.8% for HBV and 1.6%for Internal Control.

The Reproducibility of results obtained by the product HBV ELITe MGB Kit on the **ELITe BeGenius** system was assessed by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HBV certified reference material (4th WHO HBV International Standard, NIBSC) at concentration of 3 x LoD (about 27 IU / mL) and of 10 x LoD (about 90 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	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 24	N.A.	N.A.	N.A.	72 / 72	30.67	0.86	2.80
3 x LoD	24 / 24	38.54	1.08	2.79				
10 x LoD	24 / 24	36.53	0.76	2.09				

Inter – Batch Reproducibility								
Sample	HBV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 48	N.A.	N.A.	N.A.	144 / 144	29.89	0.51	1.71
3 x LoD	48 / 48	38.19	0.85	2.24				
10 x LoD	48 / 48	36.38	0.57	1.57				

In the Reproducibility test, the assay detected the HBV target as expected and showed low %CV of Ct values that did not exceed 2.8% for HBV and 2.8%for Internal Control.

Conversion factor to International Units

The Conversion factor to report 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 "4th WHO HBV International Standard” (NIBSC) in EDTA Plasma which tested negative for HBV DNA.

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

The Conversion factor was determined by calculating the logarithmic concentration difference between the reference titre in IU/mL and the obtained results in copies/mL, resulting in 0.24 IU / copy.

A summary of results is shown in the table below.

Conversion factor to International Units, Fc = 0.24 IU / copy						
Sample			Result			Log difference (ref. - test)
IU/mL	Log IU/mL	N	Mean c./mL	Mean IU/mL	Mean Log IU/mL	
31,600	4.5000	27	133,240	31,748	4.4877	+0.0123
10,000	4.0000	27	41,965	9,999	3.9917	+0.0083
3,160	3.5000	27	14,275	3,401	3.5187	-0.0187
1,000	3.0000	27	4,337	1,033	3.0020	-0.0020

As the equivalence between EDTA Plasma, ACD Plasma, and Serum was demonstrated (pages 15 and 16), the Conversion factor can be applied to the three matrices.

The Conversion Factor of HBV ELITe MGB® Kit in association with EDTA Plasma was verified in association with **ELITe BeGenius** and **ELITe InGenius** using a panel of five dilutions (0.5 Log between dilutions) of the certified calibrated reference material "4th WHO HBV International Standard” (NIBSC) in EDTA Plasma which tested negative for HBV DNA. The panel consisted of five dilution points from about 4.5 Log IU/mL to 2.5 Log IU/mL. Each sample of the panel was tested in 4 replicates.

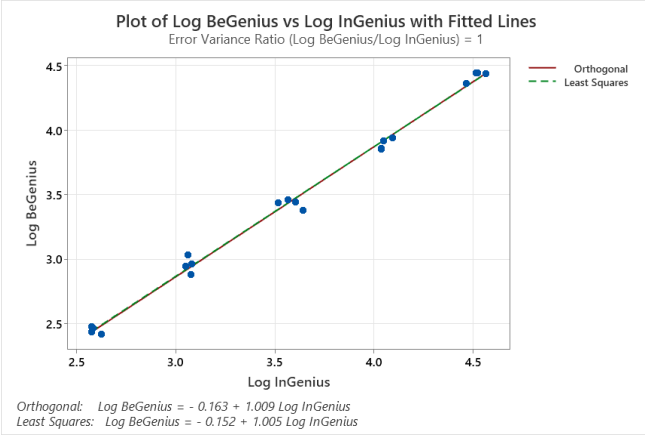
The target quantification precision, as Standard Deviation of Log IU/mL, was lower than 0.5 Log.

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

These results confirmed the Conversion factor calculated for Plasma 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 - 0.163 (95% CI: - 0.294; - 0.032) and a slope equal to 1.009 (95% CI: 0.973; 1.045). The linear regression analysis generated a R2 of 0.994.

Note: The Conversion Factors to International Standard (0.24 IU/copies), calculated with the 4th WHO International Standard for HBV DNA for NAT, can be applied also for the "5th WHO International Standard for HBV DNA for NAT" (NIBSC, UK, code 22/120)

Reproducibility with Reference Material

The Reproducibility of the assay results versus other methods in different laboratories was verified by testing the proficiency study panel “QCMD 2020 Hepatitis B Virus DNA EQA Programme” (QCMD).

Each panel member was tested on ELITe InGenius in “Extract + PCR” mode.

The results are reported in the following table, where the consensus values are derived from

commercial Real Time amplification systems.

Sample Code	Sample Content	Consensus Log IU/mL	SD Log IU / mL	Test Results Log IU / mL	Difference (cons. – test)
HBVDNA101S-01	HBV Type A	2.823	0.130	2.695	+0.128
HBVDNA101S-02	HBV Type D	2.673	0.148	2.625	+0.048
HBVDNA101S-03	HBV Type D	3.642	0.155	3.579	+0.063
HBVDNA101S-04	HBV Negative	N.A.	N.A.	N.A.	N.A.
HBVDNA101S-05	HBV Type A	1.869	0.229	1.688	+0.181
HBVDNA101S-06	HBV Type A	3.803	0.156	3.781	+0.022
HBVDNA101S-07	HBV Type A	2.848	0.176	2.696	+0.152
HBVDNA101S-08	HBV Type D	1.724	0.227	1.422	+0.302

In this test, the assay correctly detected all the panel members. Seven samples were detected within ± 1 Standard Deviation (SD) of the consensus values. The HBVDNA101S-08 sample (53 IU/mL) was underestimated, and the result is both within ± 2 SD and ± 0.5 Log IU / mL of the consensus value.

Diagnostic Sensitivity: method correlation

The Diagnostic Sensitivity of the assay, assessed by correlation analysis of different methods, was evaluated by analysing HBV DNA positive clinical samples from patients undergoing antiviral therapy in association with **ELITe InGenius**. 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.

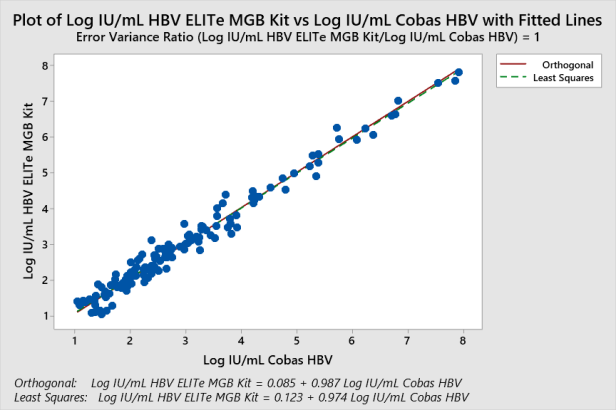
The samples' HBV DNA levels were within the measuring range of the HBV ELITe MBG Kit and of CE IVD marked molecular diagnostic reference methods ("cobas® HBV for use on the 4800 Systems" and "cobas® HBV for use on the 6800 Systems", Cobas HBV, Roche Diagnostics).

The correlation study was performed at three different sites on the following 131 samples of EDTA Plasma:

- site 1: 92 HBV DNA positive clinical samples of EDTA Plasma,
- site 2: 17 HBV DNA positive clinical samples of EDTA Plasma,
- site 3: 22 HBV DNA positive clinical samples of EDTA Plasma.

Each sample was subjected to the entire analysis procedure including extraction, amplification, detection, and result interpretation by the ELITechGroup S.p.A. products and by the reference methods. The results obtained with the HBV 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 summarized in the following figure.



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In this test, the orthogonal regression analysis generated a slope equal to 0.987, 95% CI [0.959, 1.015] and an intercept equal to 0.085, 95% CI [-0.009, 0.179]. The linear regression analysis generated an R2 of 0.974.

Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, assessed by Negative Percent Agreement of different methods, was evaluated in association with **ELITe InGenius** by analysing HBV DNA negative clinical samples tested by CE IVD marked molecular diagnostic reference methods (Roche Diagnostics).

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 three different sites on the following 127 samples of EDTA Plasma:

- site 1: 93 HBV DNA negative clinical samples of EDTA Plasma,
- site 2: 13 HBV DNA negative clinical samples of EDTA Plasma,
- site 3: 21 HBV DNA negative clinical samples of EDTA Plasma.

Each sample was subjected to the entire analysis procedure including extraction, amplification, detection, and result interpretation by the ELITechGroup S.p.A. products. The results obtained by the HBV ELITe MBG Kit were used to calculate the Negative Percent Agreement with the reference methods.

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

Samples	N	Positive	Negative	Invalid	Diagnostic Specificity
HBV DNA negative EDTA Plasma	127	3	124	0	97.6%

In this test, 124 samples were confirmed negative. Three samples gave a discordant positive result with titres lower than the LoD of the HBV ELITe MBG Kit and of the reference methods. These samples have very low titres which may randomly generate positive calls. The Diagnostic Specificity of the HBV ELITe MBG Kit was equal to 97.6%.

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 "HBV ELITe MGB Kit", FTP 602ING.

REFERENCES

S. Velkov et al. (2018) *Genes.* 9: 495.
D. N. Clark et al. (2017) *J. of Virology.* 91: e01785-16.
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 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.

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

This product is not intended for use as a screening test for the presence of HBV in blood or blood products or as a diagnostic test to confirm the presence of HBV infection.

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

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

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

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

This product requires the use of personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

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

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

A negative result obtained with this product indicates that the target DNA is not detected in the DNA extracted from the sample; however, it cannot be excluded that the target DNA has a lower 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, insertions or deletions within the region of the HBV DNA targeted by the product primers and probes may impair detection and quantification of target DNA.

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

As with any other diagnostic medical device, there is a residual risk of 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 Standards reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Q-PCR Standards and Positive Control. Check the volumes of PCR Mix, Q-PCR Standards and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area Cool Block). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
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 PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Contamination of the negative control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction area, of Racks or of Inventory Block.	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control, and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area). Do not leave the PCR Mix at room temperature for more than 30 minutes. Prepare a new aliquot of PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

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

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

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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 materials for "N" tests.
	Caution, 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 6972339, 7112684, 7319022, 7348146, 7381818, 7541454, 7582739, 7601851, 7671218, 7718374, 7723038, 7759126, 7767834, 7851606, 8008522, 8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1430147, 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

ELITe InGenius® and ELITe BeGenius® technology is covered by patents and pending applications.

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