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EMPOWERING IVD

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NOTICE of CHANGE dated 05/03/2026

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HBV ELITe MGB® Kit»
Ref. RTK602ING

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

- *Update of the paragraph "Other product required".*
- *Update of the paragraph "Notice to the users".*
- *Update of the paragraph "Troubleshooting".*
- *Update of the paragraph "Procedure limitations"*
- *Update of the paragraph "Materials provided in the product".*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

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

PRODUCT REF.	Lot Number	Expiry date
RTK602ING	C1224-002	31/10/2026
RTK602ING	C0225-002	31/12/2026

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HBV ELITe MGB® Kit

reagents for DNA Real Time PCR

REF RTK602ING

CE 0123 IVD

-20°C

UDI 08033891487027

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INTENDED USE

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

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

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

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

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

ASSAY PRINCIPLE

The assay is a quantitative Real-Time PCR detecting HBV DNA isolated from specimens amplified using the assay reagent **HBV PCR Mix**, that contains primers and ELITe MGB® technology probes.

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

In the ELITe MGB probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore. Note the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

PRODUCT DESCRIPTION

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

- **HBV ELITe MGB Mix**

This component provides the sub-component **HBV PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probe for:

- **HBV** polymerase gene (P gene), detected in Channel **HBV**; the probe is stabilized by MGB®, quenched by Eclipse Dark Quencher®, and labeled by FAM dye.
- Internal Control (**IC**) specific for IC2 artificial sequence, detected in Channel **IC**; the probe is stabilized by MGB, quenched by Eclipse Dark Quencher, and labeled with AquaPhluor® AP525 dye.
- The **HBV PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates and hot-start DNA Polymerase. Each vial contains **280 µL** of solution and is sufficient for **12 tests**, if processing at least 2 samples per session.

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

- **HBV ELITe Standard**

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

The plasmid DNA concentration was determined by UV spectrophotometer 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 reagents for **2 sessions on ELITe InGenius** and **ELITe BeGenius**, with 20 µL used per reaction.

- **HBV – ELITe Positive Control**

This component contains the sub-component **HBV Positive Control**, a stabilized solution of plasmid DNA with the amplified HBV polymerase gene region at **known titre**. The **HBV Positive Control** must be used with **HBV PCR Mix** on **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 reagents for **8 sessions on ELITe InGenius** and **ELITe BeGenius (4 session each tube)**, with 20 µL used per reaction.

- **HBV Internal Control**

This component contains the sub-component **HBV CPE** (exogenous Internal Control), a stabilized solution of plasmid DNA containing the IC2 artificial sequence. The **HBV CPE** is added to extraction reagents, to validate the results of HBV negative samples.

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

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

MATERIALS PROVIDED IN THE PRODUCT

Component	Sub-Component	Description	Quantity	Classification of hazards
HBV ELITe MGB Mix ref. RTS602ING	HBV PCR Mix ref. RTS602ING	Mixture of reagents for Real-Time PCR with **NATURALcap**	**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 **NATURAL 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 centrifuge (~5,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (volume range: 0.5-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, ref. 72.694.005).
- 0.5 mL sterile screw capped tubes (Sarstedt, ref. 72.730.005)
- Molecular biology grade water.

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OTHER PRODUCTS REQUIRED

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

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA, ref. INT030) **ELITe InGenius Software** version 1.3.0.17 (or later) **HBV ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **HBV ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **HBV ELITe_STD,** Assay Protocol with parameters for Calibrators analysis **HBV ELITe_PL_200_50**, Assay Protocol with parameters for Plasma specimen analysis **HBV ELITe_Se_200_50**, Assay Protocol with parameters for Serum specimen analysis	**ELITe InGenius SP 200** (EG SpA, ref. INT032SP200) **ELITe InGenius** and **ELITe BeGenius** Reagents and Consumables (See ELITe InGenius and ELITe BeGenius Instruction for Use manuals)
ELITe BeGenius (EG SpA, ref. INT040) **ELITe BeGenius Software** version 2.1.0 (or later) **HBV ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **HBV ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **HBV ELITe_Be_STD,** Assay Protocol with parameters for Calibrators analysis **HBV ELITe_Be_PL_200_50**, Assay Protocol with parameters for Plasma specimen analysis **HBV ELITe_Be_Se_200_50**, Assay Protocol with parameters for Serum specimen analysis	

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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

Warnings and precautions for molecular biology

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

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

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

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

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

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

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Warnings and precautions specific for the components

Component (Sub-Component)	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
HBV ELITe MGB Mix (HBV PCR Mix)	-20 °C or below (protected from light)	60 days	up to seven	up to 7 separate sessions* of 3 hours each or up to 7 consecutive hours (2 sessions of 3 hours each and the time needed to start a third session)
HBV ELITe Standard (HBV Q-PCR Standard)	-20 °C or below	60 days	up to two	2 separate sessions of 2 hours each
HBV ELITe – Positive Control (HBV Positive Control)	-20 °C or below	60 days	up to four	4 separate sessions of 3 hours each
HBV ELITe Internal Control (HBV CPE)	-20 °C or below	60 days	up to six	6 separate sessions of 3 hours each

* with intermediate freeze / thaw cycles

SPECIMENS AND CONTROLS

Specimens

This product is intended for use on the **ELITe InGenius** and the **ELITe BeGenius** with the following clinical specimens identified and handled according to laboratory guidelines, and collected, transported, and stored under the following conditions.

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

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

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

Assay Protocols for HBV ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
Plasma ACD or EDTA	**ELITe InGenius**	**HBV ELITe_PL_200_50**	Positive / copies/mL / IU/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
	ELITe BeGenius	**HBV ELITe_Be_PL_200_50**	Positive / copies/mL / IU/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
Serum	**ELITe InGenius**	**HBV ELITe_Se_200_50**	Positive / copies/mL / IU/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
	ELITe BeGenius	**HBV ELITe_Be_Se_200_50**	Positive / copies/mL / IU/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

For all protocols, 200 µL of sample must be transferred into Extraction tube (for ELITe InGenius) or 2 mL tube (for ELITe BeGenius).

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

Purified nucleic acids can be left at room temperature for 16 hours and stored at -20 °C or below for no longer than one month.

Refer to "Potentially Interfering Substances" in the Performance Characteristics section to check data concerning interfering substances.

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

PCR calibrators and controls

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

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

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

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

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

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

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

Quality controls

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

ELITe InGenius PROCEDURE

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

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

STEP 1 -Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Calibration" menu on the Home page, verify the Calibrators (**HBV Q-PCR Standard**) 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 in the following sections,
- in the "Controls" menu on the Home page, verify the PCR Controls (**HBV Positive Control**, **HBV Negative Control**) are approved and valid (Status) for the **HBV PCR Mix** lot to be used. If no valid PCR Controls are available for the **HBV PCR Mix** lot, run the PCR Controls as described in the following sections.
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "Specimens and Controls").

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

STEP 2 - Session Setup

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

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

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

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

Before to setup a run:

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

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

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

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. For this assay, 200 µL of sample must be transferred in an Extraction tube previously labelled. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw** the **Elution tube** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 µL.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 µL.
4	For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.
5	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
6	Ensure the "Protocol" displayed is: "Extract + PCR".	Select "PCR Only" in the "Protocol" column.
7	Select the sample loading position as "Extraction Tube" in the "Sample Position" column. Ensure the "**Dilution factor**" is "**1**".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Ensure the "**Dilution factor**" is "**1**".
8	Click "Next" to continue.	Click "Next" to continue.
9	**Load CPE** and the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.
10	Click "Next" to continue.	Click "Next" to continue.
11	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
12	Click "Next" to continue.	Click "Next" to continue.
13	Load PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	Load PCR Cassette, Elution tubes with samples extracted.
14	Click "Next" to continue.	Click "Next" to continue.
15	Close the instrument door.	Close the instrument door.
16	Press "Start".	Press "Start".

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	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control tubes** at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 µL.	Ensure the "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 50 µL.
4	For the Q-PCR Standard, assign the "Track", **select** the **Assay Protocol** (see "Specimen and Controls") in the "Assay" column and enter the reagent lot number and expiry date.	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls"). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
5	Ensure "PCR Only" is selected in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
6	Ensure the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
7	**Load the PCR Mix** on the "Inventory Block" referring to the Load List and enter the PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter the PCR Mix lot number, expiry date and number of reactions for each tube.
8	Click "Next" to continue.	Click "Next" to continue.
9	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
10	Click "Next" to continue.	Click "Next" to continue.
11	Load the PCR Cassette and the Q-PCR Standard tubes.	Load PCR Cassette, Positive Control and Negative Control
12	Click "Next" to continue.	Click "Next" to continue.
13	Close the instrument door.	Close the instrument door.
14	Press "Start".	Press "Start".

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

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

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

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

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

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

STEP 3 - Review and approval of results

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

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

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

The **ELITe InGenius** generates results with the **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 target 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 target 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 verify the system (reagents lot and instrument).

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

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

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

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

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

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C. Validation of Sample results

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

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

1) Calibration Curve	**Status**
HBV Q-PCR Standard	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.
For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

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

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

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

Samples reported as "HBV:DNA Not detected or below "LoD" copies / mL or IU / mL" are suitable for analysis but HBV DNA was not detected. In this case, the sample may be either negative for HBV DNA or 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 Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as "HBV:DNA Detected, quantity below "LLoQ" copies / mL or IU / mL" (see "Performance Characteristics").

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

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

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

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

D. Sample result reporting

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

The "Sample Report" shows the results details by selected sample (SID).
The "Track Report" shows the results details by selected Track.
The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

ELITe BeGenius PROCEDURE

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

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

STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in "**CLOSED**" mode,
- in the "Calibrations" menu on the Home page, verify the Calibrators (**HBV Q-PCR Standard**) 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 in the following sections,
- in the "Controls" menu on the Home page, verify the PCR Controls (**HBV Positive Control**, **HBV Negative Control**) are approved and valid (Status) for the **HBV PCR Mix** lot to be used. If no valid PCR Controls are available for the **HBV PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "Specimens and Controls").

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

STEP 2 - Session Setup

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

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

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

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

Before to setup a run:

Thaw the needed **HBV PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient

for **12 tests** in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

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

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

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. For this assay, 200 µL of sample must be transferred in a 2 mL Sarstedt Tube previously labelled. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block	If needed, **thaw** the **Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run"** from the "Home" screen
3	Remove all the Racks from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2, and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table
4	Select the "Run mode": "Extract +PCR".	Select the "Run mode": "PCR Only".
5	**Load** the **samples** into the "Sample Rack". (**Note:** when secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack").	**Load the samples** into the "Elution Rack".
6	**Insert** the "**Sample Rack**" into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert "Sample ID" (SID) for each "Position" used.. (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually "Sample ID").	**Insert** the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3) If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 µL.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 50 µL.
9	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	When more than 12 samples are processed, repeat the procedure from point 6	When more than 12 samples are processed, repeat the procedure from point 6.
12	**Load** the "**Elution tubes"** into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable
13	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3). When more than 12 samples are processed, repeat using "Lane 2" (L2).	Not applicable
15	Click "Next" to continue.	Not applicable
16	**Load CPE** and the **PCR Mix** into the "Reagent/Elution Rack".	**Load** the **PCR Mix** into "Reagent/Elution Rack".
17	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix reagent and / or CPE enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions)
18	Click "Next" to continue.	Click "Next" to continue.
19	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.
20	Click "Next" to continue.	Click "Next" to continue.
21	**Load** the "**PCR Rack"** with "**PCR Cassette**" in the Inventory Area.	**Load** the "**PCR Rack"** with "**PCR Cassette**" in the Inventory Area.
22	Click "Next" to continue.	Click "Next" to continue.
23	**Load** "**Extraction Rack**" with the "ELITe InGenius SP 200" extraction cartridges and required extraction consumables.	Not applicable
24	Close the instrument door.	Close the instrument door.
25	Press "Start".	Press "Start".

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	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) for 30 minutes at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw the Positive Control tubes** at room temperature for 30 minutes. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block. **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Remove the "Racks" from "Lane 1, 2, and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2, and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode: PCR Only".	Select the "Run mode": "PCR Only".
5	**Load the Q-PCR** Standard tubes into the "Elution Rack".	**Load** the **Positive Control and Negative Control tubes** into the "Elution Rack".
6	**Insert** the **"Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	**Insert** the **"Elution Rack**" into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" (200 µL) and the "Extracted Elute Volume" (50 µL).	Ensure the "Extraction Input Volume" (200 µL) and the "Extracted Elute Volume" (50 µL).
9	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	**Load** the **PCR Mix** into "Reagent/Elution Rack".	**Load** the **PCR Mix** into "Reagent/Elution Rack".
12	**Insert** the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions)	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
13	Click "Next" to continue.	Click "Next" to continue.
14	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.
15	Click "Next" to continue.	Click "Next" to continue.
16	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.
17	Click "Next" to continue.	Click "Next" to continue.
18	Close the instrument door.	Close the instrument door.
19	Press "Start".	Press "Start".

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

Note: At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified, and stored at -20 ±10 °C for no longer than one month. Avoid the spilling of the Extracted Sample.

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

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

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

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

STEP 3 - Review and approval of results

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

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

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

The **ELITe BeGenius** generates the results with the **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.

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

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined on the ELITe InGenius instrument by testing a panel of HBV negative ACD Plasma spiked with certified reference material of HBV (4th WHO HBV International Standard, NIBSC). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.
The results are reported in the following tables.

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

The LoD as copies/mL for ACD Plasma was calculated by applying the specific Conversion factor (0.24 IU/copy). The analytical sensitivity as copies/mL is reported below.

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

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

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

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Matrix equivalence: EDTA Plasma versus ACD Plasma and Serum

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

For 30 samples tested negative for HBV by a CE IVD marked immunoassay, the Negative Percent Agreement (NPA) and the Coefficient of Variation (%CV) of Internal Control Ct values was evaluated.
The results are reported in the following tables.

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

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	

One out Serum sample showed a positive result with 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.

For 30 samples spiked with certified reference material (4th WHO HBV International Standard, NIBSC), the Positive Percent Agreement (PPA) and the Coefficient of Variation (%CV) of HBV Ct values was evaluated.
The results are reported in the following tables.

Sample	N	Positive	Negative	PPA	HBV Ct %CV	Whole HBV Ct %CV
EDTA Plasma	30	30	0	100%	1.75	1.81
ACD Plasma	30	30	0		1.88	

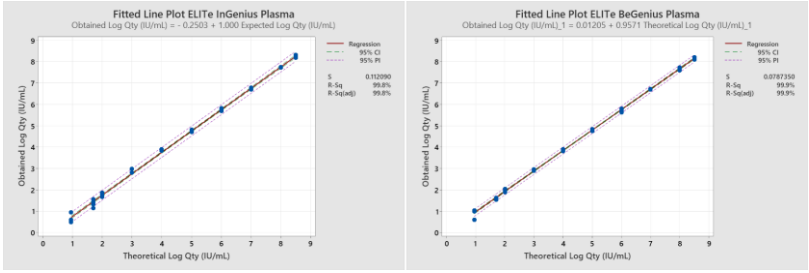
Sample	N	Positive	Negative	PPA	HBV Ct %CV	Whole HBV Ct %CV
EDTA Plasma	30	30	0	100%	1.59	1.49
Serum	30	30	0		1.29	

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 analyzed by HBV ELITe MGB Kit in association with ELITe InGenius.

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

Linear measuring range

The Linear measuring range of the assay was determined in association with ACD Plasma samples on ELITe InGenius and ELITe BeGenius using a panel of dilutions of HBV reference material (ZeptoMetrix) in negative ACD Plasma samples.
The results are reported in the following figures.



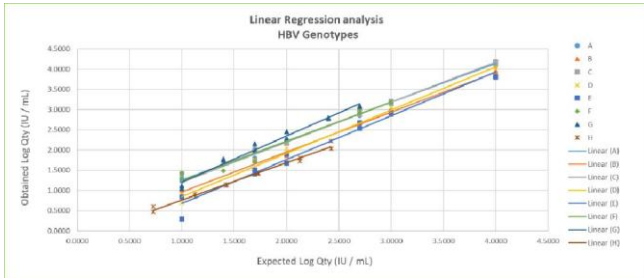
The linear measuring range as copies/mL for ACD Plasma is calculated by applying the specific conversion factor reported in the following section.

The final results are summarized in the following table.

Linear Measuring Range for HBV ELITe MGB Kit and ELITe InGenius and BeGenius	
Lower Limit	**Upper Limit**
9 IU / mL	317,750,000 IU / mL
38 copies / mL	1,323,958,333 copies / mL

For the main HBV genotypes (A, B, C, D, E, F, G), 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).

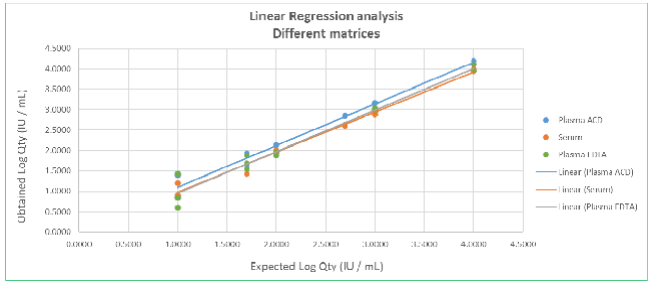
The results are reported in the following figure.



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.

For the three matrices, the Linear Measuring Range was verified by analysis of negative EDTA Plasma, negative ACD Plasma and negative Serum spiked with HBV reference material (4th WHO International Standard, NIBSC).

The results are reported in the following figure.



The linearity of the assay was verified for EDTA Plasma and ACD Plasma and Serum giving quantitative results within ±0.5 Log IU/mL and an R2 of 0.974, 0.982 and 0.988, respectively.

Standard Curve Uncertainty

The Uncertainty value of the Standard curve was calculated by combining the random errors (SD) of all level quantifications and multiplying for the Coverage factor k = 2 (Expanded Combined Uncertainty) and is equal to 0.2832 Log copies / reaction.

Standard curve levels	Theoretical Log c/rxn	SD	Expanded Combined Uncertainty
HBV Q - PCR Standard 10^5	5.0000	0.0652	0.2832
HBV Q - PCR Standard 10^4	4.0000	0.0641	
HBV Q - PCR Standard 10^3	3.0000	0.0489	
HBV Q - PCR Standard 10^2	2.0000	0.0964	

Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

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

The inclusivity of the assay was verified by testing two panels of HBV reference materials (PEI and SeraCare) at 3x LoD.

The results are reported in the following tables.

1st WHO International Reference Panel for HBV		
Sample ID	Pos. / Rep.	Outcome
HBV 1/A	3 / 3	HBV detected
HBV 2/A	3 / 3	HBV detected
HBV 3/A	3 / 3	HBV detected
HBV 4/B	3 / 3	HBV detected
HBV 5/B	3 / 3	HBV detected
HBV 6/B	3 / 3	HBV detected
HBV 7/B	3 / 3	HBV detected
HBV 8/C	3 / 3	HBV detected
HBV 9/C	3 / 3	HBV detected
HBV 10/D	3 / 3	HBV detected
HBV 11/D	3 / 3	HBV detected
HBV 12/D	3 / 3	HBV detected
HBV 13/E	3 / 3	HBV detected
HBV 14/F	3 / 3	HBV detected
HBV 15/G	3 / 3	HBV detected

AccuSet™ HBV DNA Genotype Performance Panel		
Sample ID	**Pos. / Rep.**	**Outcome**
A	3 / 3	HBV detected
B	3 / 3	HBV detected
C	3 / 3	HBV detected
D	3 / 3	HBV detected
E	3 / 3	HBV detected
F	3 / 3	HBV detected
H	3 / 3	HBV detected

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

Potential interfering markers: Cross-reactivity

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

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

The results are reported in the following table.

Sample ID	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

Sample ID	HBV Pos. / Rep.	Outcome
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 organisms tested showed no cross-reactivity for the HBV target amplification using the HBV ELITe MGB Kit.

Potential interfering markers: Inhibition

The potential inhibition of unintended organisms that may be found in clinical specimens was evaluated for the assay through the analysis of a panel of unintended organisms (ATCC, NIBSC, ZeptoMetrix) at high titre, spiked with HBV genomic DNA (NIBSC) at 3x LoD.

The results are reported in the following table.

Sample ID	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

Sample ID	HBV Pos. / Rep.	Outcome
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 detection and quantification using the HBV ELITe MGB Kit.

Potential interfering substances: Inhibition

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

The results are reported in the following table.

Sample	HBV Pos. / Rep.	Outcome
EDTA	3 / 3	No interference
Heparin	1 / 3	Interference
Haemolitic Blood high	3 / 3	No interference
Lipemic Plasma	3 / 3	No interference
Icteric Plasma	3 / 3	No interference
Ganciclovir	3 / 3	No interference
Azithromycin	3 / 3	No interference
Glecaprevir	3 / 3	No interference
Entecavir	3 / 3	No interference
Tenofovir	3 / 3	No interference
Lamivudine	3 / 3	No interference

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

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 called as “not valid” and not “false negative”.

Cross-contamination

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

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

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

Whole system failure rate

The Whole system failure rate for the assay was evaluated on ELITe InGenius and ELITe BeGenius by testing a panel of samples spiked by certified reference material (4th WHO HBV International Standard, NIBSC) at a concentration of 3 x LoD (about 27 IU/mL).

The results are summarized in the following tables.

ELITe InGenius - Whole system failure rate				
Samples	**N**	**Positive**	**Negative**	**Whole system failure rate**
Spiked EDTA Plasma	100	100	0	0%
Spiked ACD Plasma	30	30	0	0%
Spiked Serum	30	30	0	0%

ELITe BeGenius - Whole system failure rate				
Samples	**N**	**Positive**	**Negative**	**Whole system failure rate**
Spiked EDTA Plasma	100	100	0	0%

In this test with the HBV ELITe MGB Kit 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 Intra-Session and Inter-Session Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Plasma samples, including one negative sample and two samples spiked by HBV certified reference material (4th WHO HBV International Standard, NIBSC).

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

ELITe InGenius Intra-Session Repeatability (Day 1)								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**MeanCt**	**SD**	**%CV**
Negative	8	-	-	-	100%	29.10	0.23	0.79
3X LOD	8	38.90	0.50	1.29	100%			
10X LOD	8	36.50	0.16	0.44	100%			

ELITe BeGenius Intra-Session Repeatability (Day 1)								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	8	-	-	-	100%	30.06	0.37	1.24
3X LOD	8	38.64	0.46	1.19	100%			
10X LOD	8	36.83	0.34	0.93	100%			

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

ELITe InGenius Inter-Session Repeatability (Day 1 + Day 2)								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	16	-	-	-	100%	29.15	0.51	1.74
3X LOD	16	38.71	0.69	1.78	100%			
10X LOD	16	36.57	0.33	0.91	100%			

ELITe BeGenius Inter-Session Repeatability (Day 1 + Day 2)								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	16	-	-	-	100%	30.04	0.54	1.80
3X LOD	16	38.93	0.86	2.22	100%			
10X LOD	16	36.87	0.35	0.94	100%			

In the Repeatability test, the HBV ELITe MGB Kit correctly detected the target and showed a maximum variability of target Ct values as %CV equal to 2.22%.

Reproducibility

The Inter-Site, Inter-Instrument and Inter-Batch Reproducibility of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Plasma samples, including one negative sample and two samples spiked by HBV certified reference material (4th WHO HBV International Standard, NIBSC).

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

ELITe InGenius Inter-Site Reproducibility								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	24	Undet.	-	-	100%	28.73	0.45	1.58
3X LOD	24	37.60	0.68	1.80	100%			
10X LOD	24	35.63	0.35	0.98	100%			

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

ELITe InGenius Inter-Instrument Reproducibility								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	24	-	-	-	100%	29.08	0.31	1.05
3X LOD	24	37.69	0.69	1.83	100%			
10X LOD	24	36.04	0.55	1.53	100%			

ELITe BeGenius Inter-Instrument Reproducibility								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	24	-	-	-	100%	30.67	0.86	2.80
3X LOD	24	38.54	1.08	2.79	100%			
10X LOD	24	36.53	0.76	2.09	100%			

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

ELITe InGenius Inter-Batch Reproducibility								
Sample	**HBV**				**%Agreement**	**Internal Control**		
	N	**Mean Ct**	**SD**	**%CV**		**Mean Ct**	**SD**	**%CV**
Negative	48	-	-	-	100%	29.01	0.38	1.31
3X LOD	48	38.05	0.85	2.23	100%			
10X LOD	48	35.99	0.53	1.47	100%			

ELITe BeGenius Inter-Batch Reproducibility								
Sample	HBV				%Agreement	Internal Control		
	N	Mean Ct	SD	%CV		Mean Ct	SD	%CV
Negative	48	-	-	-	100%	29.89	0.51	1.71
3X LOD	48	38.19	0.85	2.24	100%			
10X LOD	48	36.38	0.57	1.57	100%			

In the Reproducibility test, the HBV ELITe MGB Kit correctly detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 2.79%.

Conversion factor to International Units

The Conversion factor to report the quantitative results in International Units/mL starting from copies/mL, was calculated using the certified calibrated reference material "4th WHO HBV International Standard" (NIBSC). The Conversion factor was determined as 0.24 IU/copy.

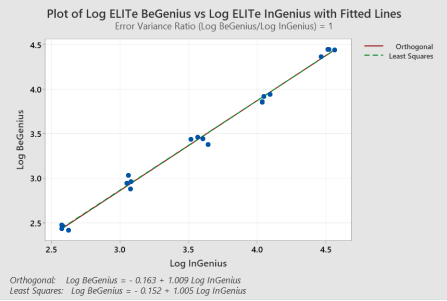
A summary of results is shown in the table below.

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 previously demonstrated, 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 instruments using the certified calibrated reference material "4th WHO HBV International Standard" (NIBSC). The results obtained were analysed by orthogonal and linear regression in order to calculate their correlation.

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 an R^2 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).

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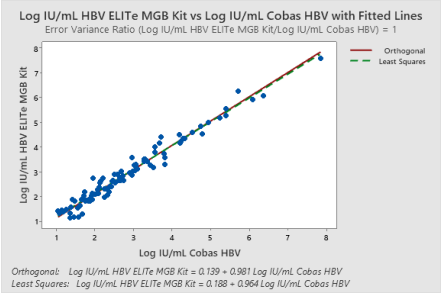
Diagnostic Sensitivity: method correlation

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

The correlation study was performed at one site on 93 HBV DNA positive clinical samples of plasma collected in EDTA using the "cobas® HBV for use on the 6800 System" as comparator.

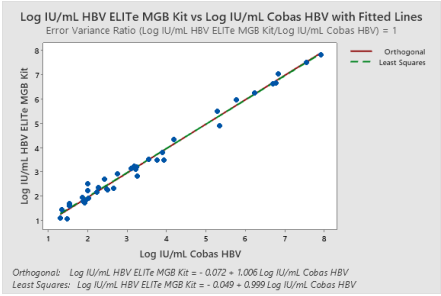
The results are summed up in the following figure.



In this test, the orthogonal regression analysis generated a slope equal to 0.981 (95% IC: 0.943; 1.020) and an intercept equal to 0.139 (95% CI: 0.018; 0.259). The linear regression analysis generated an R^2 of 0.964.

The correlation study was performed at other two sites on 38 HBV DNA positive clinical samples of plasma collected in EDTA using the "cobas® HBV for use on the 4800 System" as comparator.

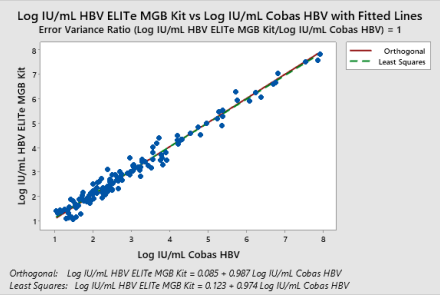
The results are summed up in the following figure.



In this test, the orthogonal regression analysis generated a slope equal to 1.006 (95% IC: 0.968; 1.043) and an intercept equal to -0.072 (95% CI: -0.219; 0.080). The linear regression analysis generated an R^2 of 0.987.

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

The results are summed up in the following figure.



In this test, the orthogonal regression analysis generated a slope equal to 0.987 (95% IC: 0.959; 1.015) and an intercept equal to 0.085 (95% CI: -0.009; 0.179). The linear regression analysis generated an R^2 of 0.974.

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

Diagnostic Specificity: confirmation of negative samples

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

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

HBV DNA negative EDTA Plasma samples	N	Positive	Negative	Diagnostic Specificity
Reference: cobas HBV for use on the 6800 System	93	3	90	96.8%
Reference: cobas HBV for use on the 4800 System	34	0	34	100%
Merged results	127	3	124	97.6%

Three samples gave discordant positive results with titres below the LoD of the HBV ELITe MBG Kit and of the reference methods. These samples may randomly generate positive calls.

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

Note: The complete data and results from the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File for the "HBV ELITe MGB® Kit", FTP602ING.

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.

https://www.mayocliniclabs.com (test ID HBVQN).

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PROCEDURE LIMITATIONS

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

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

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

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

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

The product is not intended for use as a diagnostic test to confirm the presence of 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 contamination from positive clinical samples, positive controls and PCR products. Cross-contamination cause false positive results. The product format is designed to limit cross-contamination. However, cross-contamination can only be avoided by good laboratory practices and following these instructions for use.

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

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

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

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques such as extraction, 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 titer than the product detection limit (see "Performance Characteristics"). In this case the result could be a false negative.

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

Although the region of the viral polymerase, selected as the target of this molecular assay, is the most conserved in the HBV genome, polymorphisms, insertions or deletions within this region are possible in nature. If these mutations affect the DNA region covered by the primers and probe of the assay, the accuracy of the detection and quantification of the target DNA could be variably altered.

As with any other gene sequence target diagnostic medical device, the quantitative results obtained with this product cannot be assessed absolutely on a single sample but on the kinetics of the values of multiple sequential biological samples and should be interpreted in combination with all relevant clinical observations and laboratory results. Specifically, the quantitative HBV-DNA assay is essential for monitoring therapy in HBV-positive patients, whether or not they have undergone antiviral therapy, but, as reported in the EASL 2025 Guidelines, for a correct evaluation of the efficacy of therapy and the stopping rules, it is necessary to combine the result of HBV-DNA kinetics with the quantitative assay of transaminases and HBsAg.

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

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

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TROUBLESHOOTING

Invalid Q-PCR Standard 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 or in the Cooler Unit). Do not use the PCR Mix for more than three consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 2 independent sessions (2 hours each in the Extraction Area or in the Cooler Unit). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

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

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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 or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
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.

Ct Calculation in the presence of an anomalous dissociation curve	
Possible causes	**Solutions**
Defined peak but Tm different from that of the other samples and that of the Standards or Positive Control: -high quantity of amplification reaction products	High quantity of amplification reaction products may interfere with the melting curve analysis: if the target Ct value is below 15, the sample can be considered positive.
Defined peak but Tm different from that of the other samples and that of the Standards or Positive Control: -low quantity of amplification reaction products	Low quantity of amplification reaction products at the end of the reaction may make the analysis of the melting curve unsuitable: if the Ct value of the target is below 35, the sample can be considered positive.
Defined peak but Tm different from that of the other samples and that of the Standards or Positive Control: -presence of one or more mutations on the target sequence detected by the probe	If the Ct value of the target is between 15 and 35, the sample can be considered positive, and the difference in Tm may be attributable to the genotypic variability of the virus. To confirm the presence of the target with a possible mutation, repeat the amplification reaction of the sample. The target in the sample can be sequenced to confirm the mutation.

No Ct calculation in the presence of an abnormal dissociation curve	
Possible Causes	**Solutions**
Defined peak, but Tm different from that of other samples and 6 °C or more lower than that of the standards or the positive control: - presence of one or more mutations on the target sequence detected by the probe	Repeat the amplification reaction of the sample to confirm the presence of the target with a possible mutation. The target in the sample must be sequenced to confirm the mutation.

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal	If significant amplification is observed in PCR plot select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: - repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a “PCR Only” session - repeat the extraction of the sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

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

NOTICE TO THE USERS

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

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

NOTICE TO PURCHASER: LIMITED LICENSE

IVD

SYMBOLS

CE 0123

Catalogue Number.

UDI

Upper limit of temperature.

Batch code.

Use by (last day of month).

in vitro diagnostic medical device.

CONT

Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.

Unique Device Identification

Contains sufficient for "N" tests.

Caution, consult instructions for use.

Contents.

Keep away from sunlight.

Manufacturer.

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

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

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

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.

HBV ELITe MGB® Kit used in association with Genius series®
RTK602ING

Caution, this document is a simplified version of the official instruction for use.
Please refer to the complete document before use: www.elitechgroup.com

Intended use

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

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

The product is intended for use as an aid in managing of HBV-infected individuals undergoing antiviral therapy.

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

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

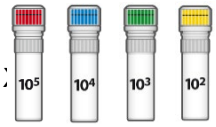
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	HBV polymerase gene (P gene)	FAM	HBV
Internal Control	IC2	AP525	IC

Validated matrix

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

Kit content

HBV ELITe MGB Mix	HBV ELITe Standard	HBV Internal Control	HBV - ELITe Positive Control
 X 8	 X 1	 X 8	 X 2
Ready-to-use PCR Mix 8 tubes of 280 µL 12 reactions per tube 96 reactions per kit 7 freeze-thaw cycles	Ready-to-use Calibrators: 4 levels 1 set of 4 tubes of 160 µL 2 reactions per tube 2 reactions per kit 2 freeze-thaw cycles	Ready-to-use IC 8 tubes of 160 µL 96 reactions per kit 6 freeze-thaw cycles	Ready-to-use Positive Control 2 tubes of 160 µL 4 reactions per tube 8 reactions per kit 4 freeze-thaw cycles

Maximum shelf-life: **24 months**

Storage Temperature: **-20 °C**

Other product required not provided in the kit

- ELITe InGenius instrument: INT030
- ELITe BeGenius instrument: INT040
- ELITe InGenius SP 200: INT032SP200.
  ELITe InGenius PCR Cassette: INT035PCR.
- ELITe InGenius SP200 Consumable Set: INT032CS
- ELITe InGenius Waste Box: F2102-000
- 300 µL Filter Tips Axigen: TF-350-L-R-S (ELITe InGenius only)
- 1000 µL Filter Tips Tecan : 30180118 (ELITe BeGenius only)

ELITe InGenius and ELITe BeGenius protocol

Sample volume	200 µL	Unit of quantitative result	International Unit: IU/mL
HBV CPE volume	10 µL	Conversion factor to IU	0.24 IU/copy
Total elution volume	50 µL	Frequency of controls	15 days
PCR elution input volume	20 µL	Frequency of calibration	60 days
HBV PCR Mix volume	20 µL		

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Diagnostic Sensitivity: Method Correlation	Diagnostic Specificity
Plasma / Serum	**9 IU / mL** 38 copies / mL	**R^2 = 0.974** 131 *quantified samples*	**97.6%** 124 *confirmed samples* / 127 *tested samples*

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

Sample preparation

This product is intended for use on the **ELITe InGenius®** and **ELITe BeGenius®** with the following clinical specimens identified according to laboratory guidelines, and collected, transported, and stored under the following conditions.

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

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

ELITe InGenius Procedures

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

Before analysis

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

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

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "50 µL"	**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: HBV ELITe_PL_200_50 or HBV ELITe_Se_200_50	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix and the Internal Control in the Inventory Block
7. Load: PCR Cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	**8.** Close the door Start the run	**9.** View, approve and store the results

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

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

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

ELITe BeGenius Procedures

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

Before analysis

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

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

1. Select "Perform Run" on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: "200 µL", Eluate: "50 µL"
4. Select the "Assay Protocol" of interest (HBV ELITe_Be_PL_200_50 or HBV ELITe_Be_Se_200_50) **Note**: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit.	**6.** Load the PCR-Mix and the CPE in the Reagent/Elution Rack and insert it in the Cooler Unit.
7. Load: "PCR Rack" with "PCR Cassette" and the "Extraction Rack" with the "ELITe InGenius SP 200" extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

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

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

1. Select "Perform Run" on the touch screen and then click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls/calibrators barcoded tubes in the Elution Rack and insert it in the Cooler Unit	**3.** Verify the extraction volumes: Input: "200 µL", Eluate: "50 µL"
4. Select the "Assay protocol" of interest (HBV ELITe_Be_PC and HBV ELITe_Be_NC or HBV ELITe_Be_STD)	**5.** Load the PCR-Mix in the Reagent/Elution Rack and insert it in the Cooler Unit	**6.** Load "PCR Rack" with "PCR Cassette"
7. **4.** Close the door. Start the run	**8.** . View, approve and store the results	