Rx Only

cobas® HBV

Quantitative nucleic acid test
for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® HBV	P/N: 09040820190
For use on the cobas® 5800 System	
cobas® HBV/HCV/HIV-1 Control Kit	P/N 09040773190
cobas® NHP Negative Control Kit	P/N 09051554190
For use on the cobas® 6800/8800 Systems	
cobas® HBV/HCV/HIV-1 Control Kit	P/N: 06997767190 or P/N: 09040773190
cobas® NHP Negative Control Kit	P/N: 07002220190 or P/N : 09051554190

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Reason: MoldSign Signature
Location: Moldova

Table of contents

Intended use

cobas® HBV for use on the **cobas®** 5800/6800/8800 Systems is an in vitro nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) DNA in human EDTA plasma or serum of HBV-infected individuals.

This test is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy. The test can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. The results from **cobas®** HBV must be interpreted within the context of all relevant clinical and laboratory findings.

Summary and explanation of the test

Background

HBV is one of several viruses known to cause viral hepatitis. Over 2 billion people throughout the world have been exposed to HBV and over 350 million are chronically infected carriers.[1] HBV is a major cause of liver disease in the United States (US), despite a decreasing incidence of acute infection associated with vaccination and universal needle use precautions.[2] The overall prevalence of HBV infection in the US has been estimated to be 0.3% to 0.5%, with 47% to 70% of cases attributed to people born outside the US.[2] However, targeted screening programs have shown prevalence rates in excess of 15% in certain high-risk immigrant populations.[3] Patients with chronic HBV infection are at high risk of long-term complications of infection, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[4-7] Serologic markers are commonly used as diagnostic and/or prognostic indicators of acute or chronic HBV infection.[8] The US Centers for Disease Control and Prevention expanded its recommendations for routine screening for high-risk individuals to now include screening in populations where HBV surface antigen (HBsAg) prevalence is greater than 2%, including people from endemic regions of the world (such as Asia and Africa), men who have sex with men, and injection drug users.[2]

The most common marker of HBV infection is the presence of HBsAg.[8] Although carriers may clear HBsAg and develop antibody to HBsAg, there still appears to be a risk of serious liver complications later in life.[9,10] HBe-antigen (HBeAg) is generally used as a secondary marker to indicate active HBV replication associated with progressive liver disease. Failure to clear HBeAg appears to increase the risk of end stage liver disease.[9,10] Variant strains of HBV precore mutants can lose the ability to produce HBeAg even when an active infection is present, limiting the use of this marker to monitor disease progression.[7]

Rationale for HBV testing

HBV DNA in EDTA plasma and serum can be quantitated by nucleic acid amplification technologies, such as PCR.[11-14] Several key guidelines recommend the use of real-time PCR methodology for HBV DNA quantitation primarily due to increased sensitivity and a broader linear range.[15,16]

Explanation of the test

cobas® HBV is a quantitative test performed on the **cobas®** 5800 System, **cobas®** 6800 System and **cobas®** 8800 System. **cobas®** HBV enables the detection and quantitation of HBV DNA in EDTA plasma or serum of infected patients for use in laboratories that support clinical trials as well as routine clinical practice in the management of patients with HBV. A single probe is used to detect and quantify, but not discriminate genotype A-H. The viral load is quantified against a non-HBV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample preparation. The DNA-QS

also functions to monitor for the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to HBV WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to HBV WHO International Standard.

Principles of the procedure

cobas® HBV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 5800 System is designed as one integrated instrument. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 5800 or **cobas**® 6800/8800 System software which assigns test results for all tests as target not detected, < LLoQ (lower limit of quantitation), > ULoQ (upper limit of quantitation) or HBV DNA detected, a value in the linear range LLoQ < x < ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples, external controls and added lambda DNA (DNA-QS) molecules are simultaneously extracted.

Viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of HBV. Selective amplification of DNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HBV genome. A thermostable DNA polymerase enzyme is used for amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[14,17,18] Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® HBV master mix contains detection probes which are specific for the HBV target sequences and the QS nucleic acid, respectively. The specific HBV and DNA-QS detection probes are each labeled with one of two unique fluorescent dyes which acts as a reporter. Each probe also has a second dye which acts as a quencher. The two reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified HBV target and the DNA-QS.[12,13] When not bound to the target sequence, the fluorescent signal of the intact probe is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified HBV target and the DNA-QS are possible.

Reagents and materials

cobas® HBV reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® HBV

(HBV)

Store at 2-8°C
192 test cassette (P/N 09040820190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 mL
DNA Quantitation Standard (DNA-QS)	Tris buffer, < 0.05% EDTA, < 0.001% non-HBV DNA construct containing non-HBV primer binding and a unique probe region (non-infectious DNA), 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxibenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
HBV Master Mix Reagent 2 (HBV MMX-R2)	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream HBV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HBV and the HBV Quantitation Standard, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 cobas® HBV/HCV/HIV-1 Control Kit

(HBV/HCV/HIV-1 CTL)

Store at 2–8°C

For use on the **cobas**® 5800 System (P/N 09040773190)

For use on the **cobas**® 6800/8800 Systems (P/N 06997767190 or P/N 09040773190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
HBV/HCV/HIV-1 Low Positive Control (HBV/HCV/HIV-1 L(+)C)	< 0.001% HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein armored, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 x 0.65 mL)	 **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H(+)C)	< 0.001% high titered synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	5.2 mL (8 x 0.65 mL)	 **WARNING** H317: May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273 Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2- methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance or mixture

Table 3 cobas® NHP Negative Control Kit

(NHP-NC)

Store at 2-8°C

For use on the **cobas**® 5800 System (P/N 09051554190)

For use on the **cobas**® 6800/8800 Systems (P/N 07002220190 or P/N 09051554190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing mist or vapours. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance

** Hazardous substance or mixture

cobas® omni reagents for sample preparation

Table 4 cobas® omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas® omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas® omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas® omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411 Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071 Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391 Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® HBV test kit. See listing of additional materials required (Table 8 and Table 9).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance or mixture

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5, Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 5800 or **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® HBV	2–8°C
cobas® HBV/HCV/HIV-1 Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas® **omni** Lysis Reagent	2–8°C
cobas® **omni** MGP Reagent	2–8°C
cobas® **omni** Specimen Diluent	2–8°C
cobas® **omni** Wash Reagent	15–30°C

Reagent handling requirements for the cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The **s**ystem allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® HBV	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® NHP Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

* Single use reagent

** Time is measured from the first time that reagent is loaded onto the **cobas**® 5800 System

Reagent handling requirements for the cobas® 6800/8800 Systems

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 7 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® HBV	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed	Not applicable*	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 10 hours
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

* Single use reagent

** Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Additional materials required for the cobas® 5800 System

Table 8 Material and consumables for use on the **cobas**® 5800 System

Material	P/N
cobas® **omni** Processing Plate 24	08413975001
cobas® **omni** Amplification Plate 24	08499853001
cobas® **omni** Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1 mL	04639642001
Tip CORE TIPS with Filter, 300 µL	07345607001
cobas® **omni** Liquid Waste Container	07094388001
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190
Solid Waste Bag or Solid Waste Bag With Insert	07435967001 or 08030073001

Additional materials required for the cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **cobas**® 6800/8800 Systems

Material	P/N
cobas® **omni** Processing Plate	05534917001
cobas® **omni** Amplification Plate	05534941001
cobas® **omni** Pipette Tips	05534925001
cobas® **omni** Liquid Waste Container	07094388001
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag with Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® HBV analysis package for the **cobas**® 5800 System shall be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

The **cobas**® 6800/8800 software and **cobas**® HBV analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 10 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems User Assistance and/or User Guides for additional information.

Note: Contact your local Roche representative for a detailed order list for primary and secondary tubes, for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- **cobas**® HBV has not been evaluated for use as a screening test for the presence of HBV in blood or blood products or as a diagnostic test to confirm the presence of HBV infection.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[19,20] Only personnel proficient in handling infectious materials and the use of **cobas**® HBV and **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water or follow appropriate site procedures.
- **cobas**® HBV/HCV/HIV-1 Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® **omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® HBV test kit, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.

- Do not allow **cobas**® **omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® HBV kits and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 or **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 5800 System or **cobas**® 6800/8800 Systems User Assistance and/or User Guides to properly clean and decontaminate the surface of instrument(s).

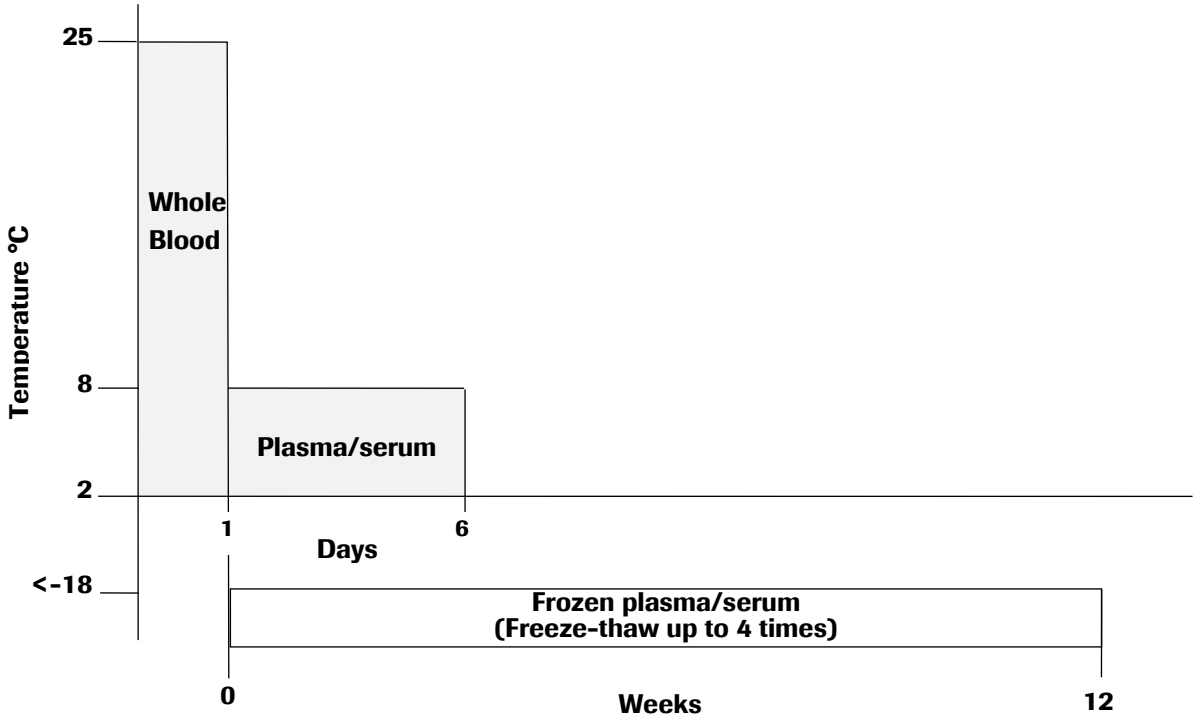
Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.
Store all samples at specified temperatures.
Sample stability is affected by elevated temperatures.
If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.

Samples

- Whole blood should be collected in SST™ Serum Separation Tubes, BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions. Refer to Figure 1.
- Whole blood collected in SST™ Serum Separation Tubes, BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2°C to 25°C prior to plasma/serum preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation plasma/serum samples may be stored in secondary tubes for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C.
- For long-term storage up to 6 months, temperatures at ≤ -60°C are recommended.
- Plasma/serum samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.

Figure 1 Sample storage conditions



- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Instructions for use

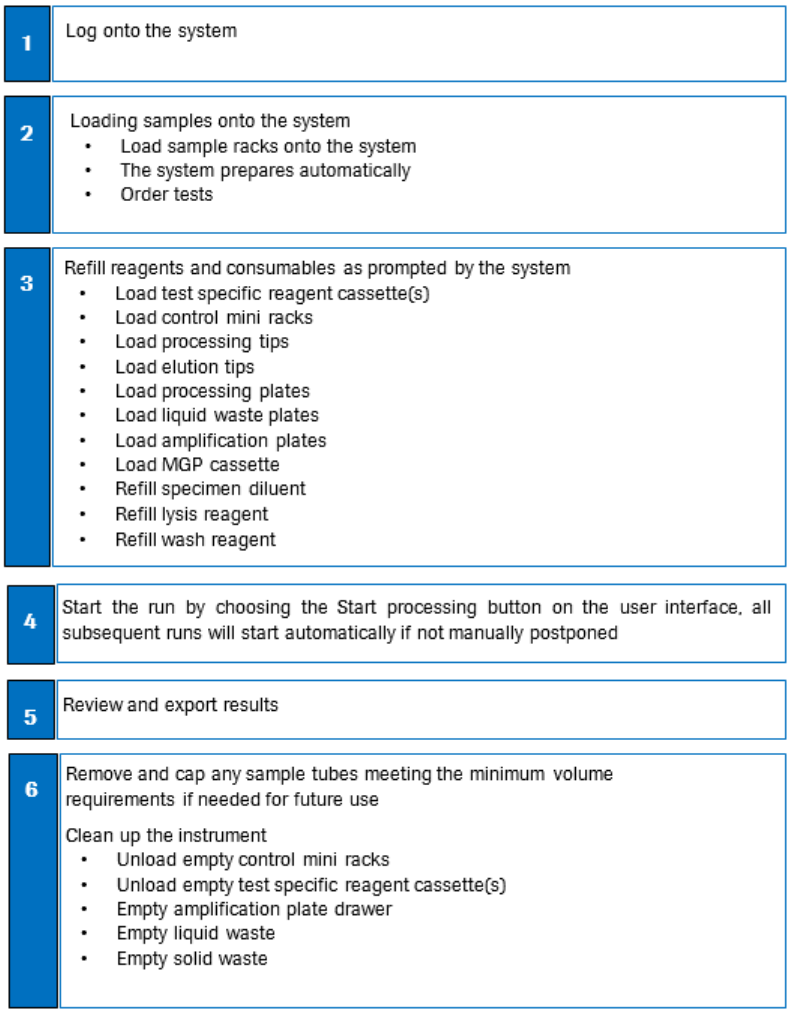
Procedural notes

- Do not use **cobas**® HBV test reagents, **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® NHP Negative Control Kit, or **cobas**® **omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems User Assistance and/or User Guides for proper maintenance of instruments.

Running cobas® HBV on the cobas® 5800 System

cobas® HBV can be run with two minimum required sample volumes of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). The test procedure is described in detail in the **cobas**® 5800 System User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

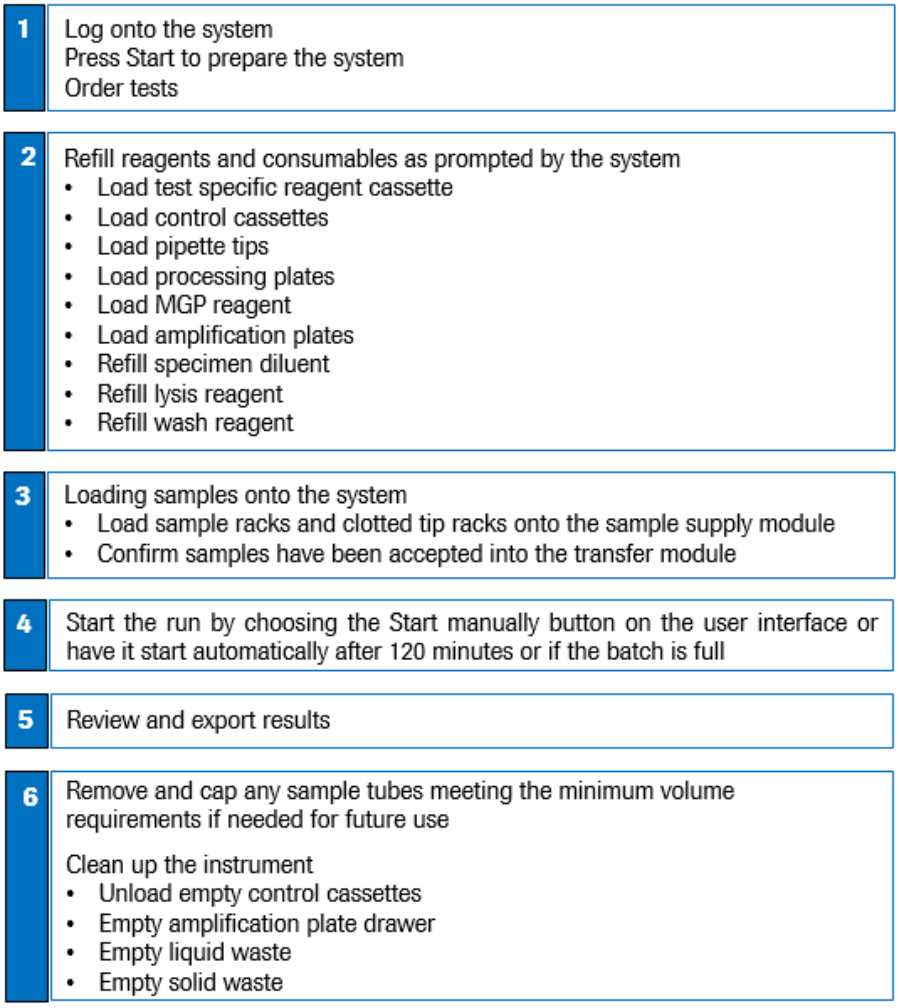
Figure 2 **cobas**® HBV test procedure on the **cobas**® 5800 System



Running cobas® HBV on the cobas® 6800/8800 Systems

cobas® HBV can be run with two minimum required sample volumes of 350 µL (for the 200 µL sample workflow) and 650 µL (for the 500 µL sample workflow). The test procedure is described in detail in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide. Figure 3 below summarizes the procedure.

Figure 3 **cobas**® HBV test procedure on the **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800 System and **cobas**® 6800/8800 Systems automatically determine the HBV DNA concentration for the samples and controls. The HBV DNA concentration is expressed in International Units per milliliter (IU/mL).

Quality control and validity of results on the cobas® 5800 System

- One negative control [(–) C] and two positive controls, a low positive control [HBV L (+) C] and a high positive control [HBV H (+) C] are processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 software and/or report, check for flags and their associated results to ensure the result validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: HBV L (+) C, HBV H (+) C. The negative control result is displayed as (–) C and the low and high positive controls are displayed as HxV L (+) C and HxV H (+) C.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Quality control results on the cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with 'Invalid' in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view.
- If one of the positive controls is invalid, repeat testing of all positive controls and all associated samples. If the negative control is invalid, repeat testing of all controls and all associated samples.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One negative control [(–) C] and two positive controls, a low positive control [HBV L (+) C] and a high positive control [HBV H (+) C] is processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: HBV L (+) C, HBV H (+) C. The negative control result is displayed as (–) C and the low and high positive controls are displayed as HxV L (+) C and HxV H (+) C.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

Control flags on the cobas® 6800/8800 Systems

Table 11 Control flags for negative and positive controls

Negative Control	**Flag**	**Result**	**Interpretation**
(–) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Flag**	**Result**	**Interpretation**
HxV L (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
HxV H (+) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

If the batch is invalid, repeat testing of the entire batch including samples and controls.

HxV L (+) C stands for **cobas**® HBV/HCV/HIV-1 low positive control and HxV H (+) C stands for **cobas**® HBV/HCV/HIV-1 high positive control in the **cobas**® 6800/8800 software.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 5800 and **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.

Table 12 Target results for individual target result interpretation

Results	Interpretation
Target Not Detected	HBV DNA not detected. Report results as "HBV not detected."
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "HBV detected, less than (Titer Min)." Titer min = 10 IU/mL (500 µL) Titer min = 25 IU/mL (200 µL)
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as "(Titer) of HBV detected".
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "HBV detected, greater than (Titer Max)." Titer max = 1.00E+09 IU/mL (500 µL and 200 µL)

[a] Sample result > Titer Max refers to HBV positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with HBV-negative EDTA plasma or serum, depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.
For a valid control batch, check each individual sample for flags in the **cobas**® 5800 software and/or report. The result interpretation should be as follows:

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
  - Q05D: Result validation failure because of an invalid positive control
  - Q06D: Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 12 above.
- If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Interpretation of results on the cobas® 6800/8800 Systems

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as show in Table 12 above.

Procedural limitations

- **cobas**® HBV has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® NHP Negative Control Kit, **cobas**® **omni** MGP Reagent, **cobas**® **omni** Lysis Reagent, **cobas**® **omni** Specimen Diluent, and **cobas**® **omni** Wash Reagent for use on the **cobas**® 5800/6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma and serum. Testing of other sample types may result in inaccurate results.
- Quantitation of HBV DNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® HBV, may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- **cobas**® HBV is not intended for use as a screening test for the presence of HBV in blood or blood products or as a diagnostic test to confirm the presence of HBV infection.

Non-clinical performance evaluation

Key performance characteristics performed on the cobas® 6800/8800 Systems

Limit of Detection (LoD)

WHO International Standard

The limit of detection of **cobas**® HBV was determined by analysis of serial dilutions of the WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Amplification Technology Assays (2nd WHO International Standard) genotype A obtained from NIBSC, in HBV-negative human EDTA plasma and serum using sample processing volumes of 500 µL and 200 µL. Panels of eight concentration levels plus a negative were tested for 500 µL sample processing volume and nine concentration levels for 200 µL sample process volume over three lots **cobas**® HBV test reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum from both sample processing volumes are shown in Table 13 to Table 16, respectively. The study demonstrates that **cobas**® HBV detected HBV DNA at a concentration of 3 IU/mL with a hit rate of ≥ 95% for the 500 µL sample processing volume and at a concentration of 17.5 IU/mL with a hit rate of ≥ 95% for the 200 µL sample processing volume in EDTA plasma. For serum the study demonstrates that **cobas**® HBV detected HBV DNA at a concentration of 3 IU/mL with a hit rate of ≥ 95% for the 500 µL sample processing volume and at a concentration of 15 IU/mL with a hit rate of ≥ 95% for the 200 µL sample processing volume.

Table 13 Limit of detection in EDTA plasma (500 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
20.0	189	189	100.00
10.0	189	189	100.00
8.0	189	189	100.00
6.0	189	189	100.00
5.0	189	188	99.47
4.0	189	185	97.88
3.0	189	183	96.83
2.0	189	166	87.83
LoD by PROBIT at 95% hit rate	2.7 IU/mL 95% confidence range: 2.4 – 3.1 IU/mL		

Table 14 Limit of detection in serum (500 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
20.0	189	189	100.00
10.0	189	189	100.00
8.0	189	189	100.00
6.0	189	189	100.00
5.0	189	188	99.47
4.0	189	186	98.41
3.0	189	187	98.94
2.0	189	172	91.01
LoD by PROBIT at 95% hit rate	2.4 IU/mL 95% confidence range: 2.0 – 2.7 IU/mL		

Table 15 Limit of detection in EDTA plasma (200 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
50.0	189	189	100.00
30.0	189	189	100.00
25.0	189	188	99.47
20.0	189	189	100.00
17.5	189	182	96.30
15.0	189	179	94.71
12.5	189	170	89.95
10.0	189	142	75.13
5.0	189	87	46.03
LoD by PROBIT at 95% hit rate	15.5 IU/mL 95% confidence range: 14.4 – 16.9 IU/mL		

Table 16 Limit of detection in serum (200 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
50.0	189	189	100.00
30.0	189	189	100.00
25.0	189	189	100.00
20.0	189	187	98.94
17.5	189	189	100.00
15.0	189	184	97.35
12.5	189	174	92.06
10.0	189	170	89.95
5.0	189	107	56.61
LoD by PROBIT at 95% hit rate	12.5 IU/mL 95% confidence range: 11.6 – 13.8 IU/mL		

Linear range

Linearity study of **cobas**® HBV was performed with a dilution series consisting of 15 panel members spanning the intended linear range for the predominant genotype (GT A). High titer panel members were prepared from a high titer HBV plasmid DNA stock whereas the lower titer panel members were prepared from a clinical sample. The linearity panel was designed to have an approximate 2 $\log_{10}$ titer overlap between the two material sources. The expected linear range of **cobas**® HBV is from LLoQ (10 IU/mL in 500 µL sample process volume and 25 IU/mL in 200 µL sample process volume) to ULoQ (1.00E+09 IU/mL). The linearity panel was designed to range from one concentration below LLoQ (e.g. 7.5 IU/mL) to one concentration level above ULoQ (e.g. 2.0E+09 IU/mL) and to include medical decision points. Moreover, the linearity panel was designed to partly support steps of 1.0 $\log_{10}$ throughout the linear range. For each panel member the nominal concentration in IU/mL and the source of the HBV DNA were given.

With 500 µL process volume, **cobas**® HBV is linear for EDTA plasma and serum from 10 IU/mL to 1.00E+09 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.2 $\log_{10}$. Across the linear range, the accuracy of the test was within ± 0.24 $\log_{10}$.

With 200 µL process volume, **cobas**® HBV is linear for EDTA plasma and serum from 25 IU/mL to 1.00E+09 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.2 $\log_{10}$. Across the linear range, the accuracy of the test was within ± 0.24 $\log_{10}$.

See Figure 4 to Figure 7 for representative results.

Figure 4 Linear range determination in EDTA plasma (500 µL)

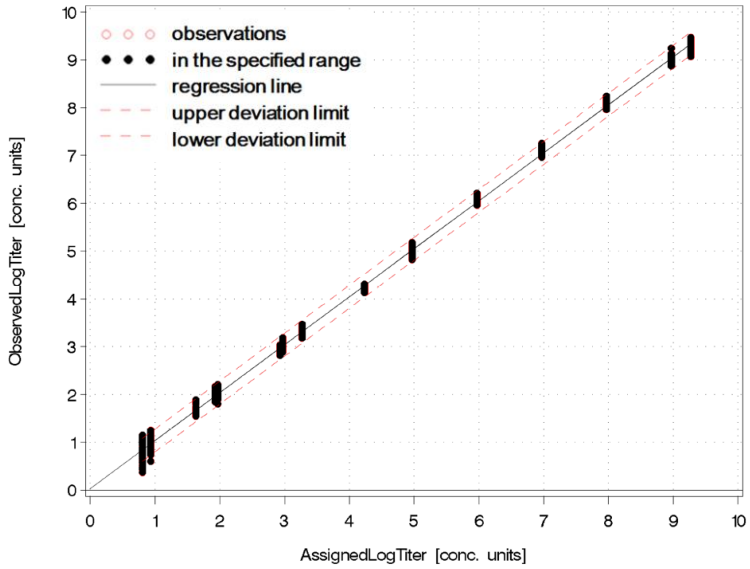


Figure 5 Linear range determination in EDTA plasma (200 µL)

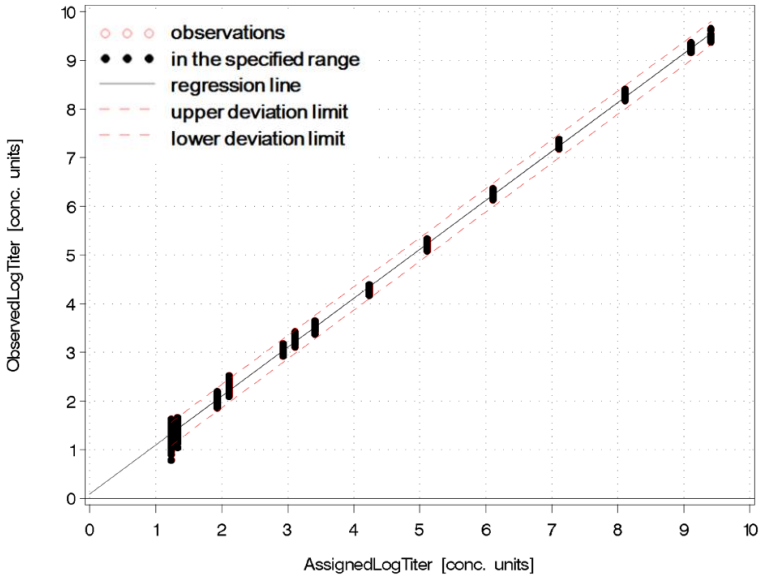


Figure 6 Linear range determination in serum (500 µL)

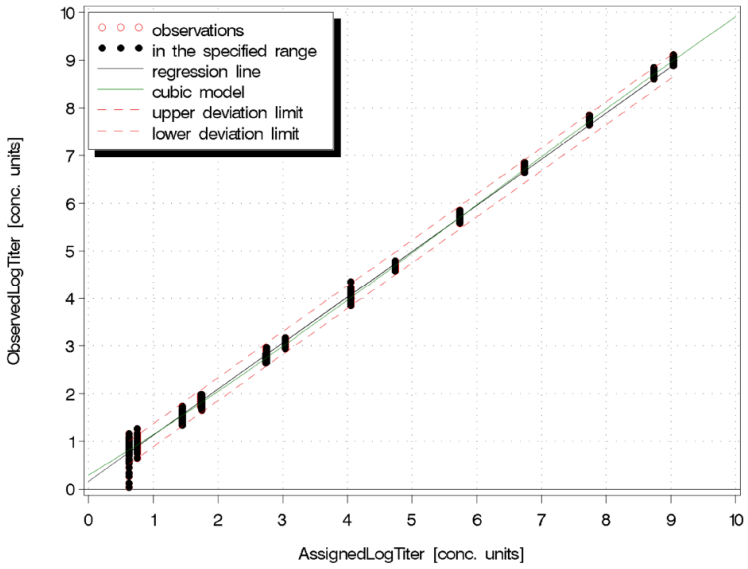
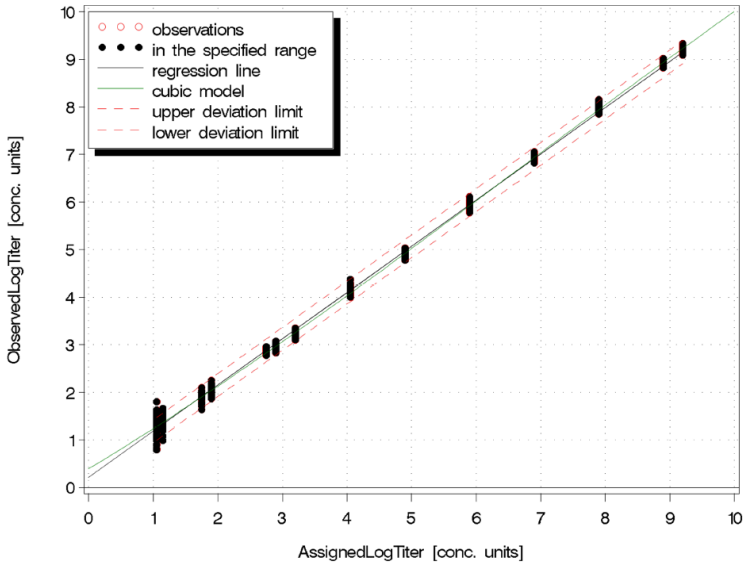


Figure 7 Linear range determination in serum (200 µL)



Precision – within laboratory

Precision of **cobas**® HBV was determined by analysis of serial dilutions of clinical HBV (Genotype A) samples (CS) or of HBV plasmid DNA in HBV negative EDTA plasma or in serum. Ten to 12 dilution levels were tested in 48 replicates for each level and process volume across three lots of **cobas**® HBV test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas**® HBV test procedure on a fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 17 through Table 20.

cobas® HBV showed high precision for three lots of reagents tested across a concentration range of 5.00E+01 IU/mL to 1.0E+09 IU/mL with 500 µL sample processing volume and 1.00E+02 IU/mL to 1.0E+08 IU/mL (EDTA plasma) and 1.0E+09 IU/mL (serum) with 200 µL sample processing volume.

Table 17 Within-laboratory precision of **cobas®** HBV (EDTA plasma samples – processing volume of 500 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All lots
			SD	SD	SD	Pooled SD
1.00E+09	9.32E+08	plasmid DNA	0.04	0.07	0.09	0.07
1.00E+08	9.32E+07	plasmid DNA	0.04	0.08	0.05	0.06
1.00E+07	9.32E+06	plasmid DNA	0.06	0.05	0.04	0.05
1.00E+06	9.32E+05	plasmid DNA	0.06	0.07	0.04	0.06
1.00E+05	9.32E+04	plasmid DNA	0.06	0.06	0.07	0.06
2.00E+04	1.71E+04	clinical specimen	0.05	0.03	0.03	0.04
2.00E+03	1.86E+03	plasmid DNA	0.05	0.04	0.07	0.05
1.00E+03	8.54E+02	clinical specimen	0.04	0.05	0.04	0.04
1.00E+03	9.32E+02	plasmid DNA	0.06	0.06	0.05	0.06
1.00E+02	8.54E+01	clinical specimen	0.07	0.08	0.07	0.07
1.00E+02	9.32E+01	plasmid DNA	0.10	0.08	0.09	0.09
5.00E+01	4.27E+01	clinical specimen	0.09	0.04	0.08	0.08

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 18 Within-laboratory precision of **cobas®** HBV (serum samples – processing volume of 500 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Serum			
			Lot 1	Lot 2	Lot 3	All lots
			SD	SD	SD	Pooled SD
1.00E+09	5.47E+08	plasmid DNA	0.05	0.06	0.03	0.05
1.00E+08	5.47E+07	plasmid DNA	0.03	0.04	0.03	0.04
1.00E+07	5.47E+06	plasmid DNA	0.05	0.05	0.03	0.05
1.00E+06	5.47E+05	plasmid DNA	0.04	0.06	0.06	0.05
1.00E+05	5.47E+04	plasmid DNA	0.04	0.03	0.03	0.04
2.00E+04	1.12E+04	clinical specimen	0.10	0.07	0.08	0.08
2.00E+03	1.09E+03	plasmid DNA	0.05	0.05	0.03	0.05
1.00E+03	5.62E+02	clinical specimen	0.03	0.14	0.03	0.09
1.00E+03	5.47E+02	plasmid DNA	0.04	0.05	0.04	0.04
1.00E+02	5.62E+01	clinical specimen	0.09	0.06	0.07	0.07
1.00E+02	5.47E+01	plasmid DNA	0.05	0.07	0.04	0.06
5.00E+01	2.81E+01	clinical specimen	0.07	0.06	0.10	0.08

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 19 Within-laboratory precision of **cobas®** HBV (EDTA plasma samples – processing volume of 200 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	EDTA plasma			
			Lot 1	Lot 2	Lot 3	All lots
			SD	SD	SD	Pooled SD
1.00E+08	1.28E+08	plasmid DNA	0.04	0.05	0.03	0.04
1.00E+07	1.28E+07	plasmid DNA	0.06	0.04	0.02	0.04
1.00E+06	1.28E+06	plasmid DNA	0.03	0.04	0.04	0.03
1.00E+05	1.28E+05	plasmid DNA	0.02	0.06	0.05	0.05
2.00E+04	1.71E+04	clinical specimen	0.03	0.05	0.03	0.04
2.00E+03	2.57E+03	plasmid DNA	0.05	0.06	0.05	0.05
1.00E+03	8.54E+02	clinical specimen	0.07	0.05	0.03	0.05
1.00E+03	1.28E+03	plasmid DNA	0.06	0.07	0.03	0.05
1.00E+02	8.54E+01	clinical specimen	0.09	0.09	0.07	0.09
1.00E+02	1.28E+02	plasmid DNA	0.06	0.09	0.11	0.09

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots

Table 20 Within-laboratory precision of **cobas®** HBV (serum samples – processing volume of 200 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Serum			
			Lot 1	Lot 2	Lot 3	All lots
			SD	SD	SD	Pooled SD
1.00E+09	7.92E+08	plasmid DNA	0.04	0.03	0.03	0.04
1.00E+08	7.92E+07	plasmid DNA	0.07	0.05	0.06	0.06
1.00E+07	7.92E+06	plasmid DNA	0.04	0.03	0.04	0.04
1.00E+06	7.92E+05	plasmid DNA	0.03	0.05	0.04	0.04
1.00E+05	7.92E+04	plasmid DNA	0.06	0.07	0.03	0.06
2.00E+04	1.12E+04	clinical specimen	0.16	0.08	0.03	0.11
2.00E+03	1.58E+03	plasmid DNA	0.05	0.04	0.05	0.05
1.00E+03	5.62E+02	clinical specimen	0.07	0.04	0.04	0.05
1.00E+03	7.92E+02	plasmid DNA	0.07	0.05	0.06	0.06
1.00E+02	5.62E+01	clinical specimen	0.09	0.10	0.07	0.09
1.00E+02	7.92E+01	plasmid DNA	0.08	0.09	0.09	0.08

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Genotype determination and verification

The performance of **cobas**® HBV on HBV genotypes was evaluated by:

- Determination of the limit of detection for genotypes B through H and the predominant precore mutant with EDTA-plasma and serum for 500 µL processing volume
- Verification of the limit of detection for genotypes B through H and the predominant precore mutant with EDTA-plasma and serum for 200 µL processing volume
- Verification of the linearity for genotypes B through H and the predominant precore mutant

Limit of detection for genotypes B through H and the predominant precore mutant

The limit of detection of **cobas**® HBV was determined by analysis of serial dilutions for seven different genotypes (B, C, D, E, F, G, H) and the predominant precore mutant (G1896A; C1858T) in HBV-negative human EDTA plasma and serum using sample processing volumes of 500 µL. Panels of eight concentration levels plus a negative were tested using three lots of **cobas**® HBV test reagents, over multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum for 500 µL processing volume are shown in Table 21 and Table 22, respectively. The study demonstrates that **cobas**® HBV detected all HBV genotypes tested with a similar LoD as HBV genotype A.

Table 21 HBV DNA genotype limit of detection in EDTA plasma (500 µL)

Genotype	95% LoD by PROBIT	95% Confidence Interval
GT B	3.45 IU/mL	2.95 IU/mL - 4.32 IU/mL
GT C	4.13 IU/mL	3.32 IU/mL - 5.82 IU/mL
GT D	4.52 IU/mL	3.59 IU/mL - 6.49 IU/mL
GT E	3.21 IU/mL	2.76 IU/mL - 3.98 IU/mL
GT F	1.87 IU/mL	1.66 IU/mL - 2.24 IU/mL
GT G	2.49 IU/mL	2.17 IU/mL - 3.02 IU/mL
GT H	6.55 IU/mL	5.33 IU/mL - 8.77 IU/mL
precore mutant	2.38 IU/mL	2.08 IU/mL - 2.90 IU/mL

Table 22 HBV DNA genotype limit of detection in serum (500 µL)

Genotype	95% LoD by PROBIT	95% Confidence Interval
GT B	3.30 IU/mL	2.76 IU/mL - 4.30 IU/mL
GT C	3.34 IU/mL	2.83 IU/mL - 4.23 IU/mL
GT D	2.59 IU/mL	2.17 IU/mL - 3.42 IU/mL
GT E	2.67 IU/mL	2.25 IU/mL- 3.49 IU/mL
GT F	1.98 IU/mL	1.72 IU/mL - 2.45 IU/mL
GT G	2.07 IU/mL	1.75 IU/mL - 2.66 IU/mL
GT H	3.48 IU/mL	2.89 IU/mL - 4.60 IU/mL
precore mutant	1.65 IU/mL	1.43 IU/mL - 2.03 IU/mL

Verification of limit of detection for genotypes B through H and the predominant precore mutant

HBV DNA clinical specimens from all genotypes (B, C, D, E, F, G, H) and the predominant precore mutant (G1896A; C1858T) were diluted to three different concentration levels in EDTA plasma and serum. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® HBV reagents. The results from EDTA plasma and serum using 200 µL are shown in Table 23 and Table 24. These results verify that **cobas**® HBV detected HBV DNA for the seven different genotypes and the predominant precore mutant at concentrations of 12.50 IU/mL with a hit rate of ≥ 93.65% with an upper one-sided 95% confidence interval of 97.80%.

Table 23 HBV DNA genotype verification of limit of detection in EDTA plasma (200 µL)

Genotype	6.25 IU/mL			12.50 IU/mL			18.75 IU/mL		
	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)
B	63	51	80.95 (88.63)	63	63	100.00 (100.00)	63	63	100.00 (100.00)
C	63	45	71.43 (80.65)	63	62	98.41 (99.92)	62	62	100.00 (100.00)
D	61	49	80.33 (88.63)	63	63	100.00 (100.00)	62	61	98.39 (99.92)
E	63	51	80.95 (88.63)	63	63	100.00 (100.00)	63	63	100.00 (100.00)
F	63	54	85.71 (92.34)	63	63	100.00 (100.00)	63	63	100.00 (100.00)
G	63	46	73.02 (82.02)	63	63	100.00 (100.00)	63	63	100.00 (100.00)
H	63	33	52.38 (63.26)	63	59	93.65 (97.80)	63	59	93.65 (97.80)
Precore mutant	63	54	85.71 (92.34)	63	62	98.41 (99.92)	63	63	100.00 (100.00)

* Upper one-sided 95% confidence interval

Table 24 HBV DNA genotype verification of limit of detection in serum (200 µL)

Genotype	6.25 IU/mL			12.50 IU/mL			18.75 IU/mL		
	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)	Number of valid replicates	Number of positives	Hit rate in % (95% CI*)
B	63	51	80.95 (88.63)	63	62	98.41 (99.92)	63	63	100.00 (100.00)
C	63	54	85.71 (92.34)	63	62	98.41 (99.92)	63	63	100.00 (100.00)
D	63	53	84.13 (91.13)	63	62	98.41 (99.92)	63	63	100.00 (100.00)
E	63	54	85.71 (92.34)	62	62	100.00 (100.00)	63	63	100.00 (100.00)
F	63	59	93.65 (97.80)	63	63	100.00 (100.00)	62	62	100.00 (100.00)
G	63	59	93.65 (97.80)	62	62	100.00 (100.00)	63	63	100.00 (100.00)
H	63	47	74.60 (83.37)	63	61	96.83 (99.43)	63	62	98.41 (99.92)
Precore mutant	63	60	95.24 (98.66)	63	62	98.41 (99.92)	63	63	100.00 (100.00)

* Upper one-sided 95% confidence interval

Linearity for genotypes B through H and the predominant precore mutant

The dilution series used in the verification of genotypes linearity study of **cobas**® HBV consists of 10 panel members spanning the intended linear range. High titer panel members were prepared from a high titer plasmid DNA stock whereas the lower titer panel members were made from a high titer clinical sample. The linearity panel was designed to have an approximate 2 $\log_{10}$ titer overlap between the two material sources. The linear range of **cobas**® HBV spanned from below the LLoQ (10 IU/mL for a sample processing volume of 500 µL; 25 IU/mL for a sample processing volume of 200 µL) to the ULoQ (1.00E+09 IU/mL) and included at least one medical decision point. Twenty-one replicates were tested across three lots of **cobas**® HBV reagent for each level in EDTA plasma and serum.

The linearity within the linear range of **cobas**® HBV was verified for all seven genotypes (B, C, D, E, F, G, H) and predominant precore mutant (G1896A; C1858T). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.2 $\log_{10}$.

Specificity

The specificity of **cobas**® HBV was determined by analyzing HBV negative EDTA plasma and serum samples from individual donors. Three hundred individual EDTA plasma and 300 individual serum samples (600 total results) were tested with two lots of **cobas**® HBV reagents. All samples tested negative for HBV DNA. In the test panel the specificity of **cobas**® HBV was 100% (with a one-sided 95% confidence interval of 99.5%).

Analytical specificity

The analytical specificity of **cobas**® HBV was evaluated by diluting a panel of microorganisms with HBV DNA positive and HBV DNA negative EDTA plasma. The microorganisms were added to negative human EDTA plasma and tested with and without HBV DNA. None of the non-HBV pathogens interfered with test performance. Negative results were obtained with **cobas**® HBV for all microorganism samples without HBV target and positive results were obtained on all of the microorganism samples with HBV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HBV samples containing potentially cross-reacting organisms was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 25 Microorganisms tested for cross-reactivity

Viruses		**Bacteria**	**Yeast**
Adenovirus type 5	West Nile Virus	Propionibacterium acnes	Candida albicans
Cytomegalovirus	St. Louis encephalitis Virus	Staphylococcus aureus	-
Hepatitis A Virus	Dengue virus types 1, 2, 3, and 4	-	-
Hepatitis C Virus	FSME virus (strain HYPR)	-	-
Hepatitis D Virus	Yellow Fever Virus	-	-
Human Immunodeficiency Virus-1	Human Papillomavirus	-	-
Human T-Cell Lymphotropic Virus types 1 and 2	Varicella-Zoster Virus	-	-
Human Herpes Virus Type-6	Influenza A	-	-
Herpes Simplex Virus Type-1 and 2	Zika Virus	-	-

Analytical specificity – interfering substances

Elevated levels of triglycerides (34.5 g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) in samples have been tested in the presence and absence of HBV DNA. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® HBV.

Moreover, the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody were tested.

In addition, drug compounds listed in Table 26 were tested at 3 times the C_{max} in presence and absence of HBV DNA.

All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with **cobas**® HBV for all samples without HBV target and positive results were obtained on all of the samples with HBV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HBV samples containing potentially interfering substances was within ± 0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 26 Drug compounds tested for interference with the quantitation of HBV DNA by **cobas**® HBV

Class of drug	Generic drug name	
Immune modulator	Peginterferon α-2a	Peginterferon α-2b
	Ribavirin	
HIV entry inhibitor	Maraviroc	
HIV integrase inhibitor	Elvitegravir/Cobicistat	Raltegravir
Non-nucleoside HIV Reverse transcriptase inhibitor	Efavirenz	Nevirapine
	Etravirine	Rilpivirine
HIV protease inhibitor	Atazanavir	Lopinavir
	Tipranavir	Nelfinavir
	Darunavir	Ritonavir
	Fosamprenavir	Saquinavir
HCV protease inhibitor	Boceprevir	Telaprevir
	Simeprevir	
Reverse transcriptase or DNA polymerase inhibitors	Abacavir	Tenofovir
	Emtricitabine	Adefovir dipivoxil
	Entecavir	Telbivudine
	Foscarnet	Zidovudine
	Cidofovir	Aciclovir
	Lamivudine	Valganciclovir
	Ganciclovir	Sofosbuvir
Compounds for treatment of opportunistic infections	Azithromycin	Pyrazinamide
	Clarithromycin	Rifabutin
	Ethambutol	Rifampicin
	Fluconazole	Sulfamethoxazole
	Isoniazid	Trimethoprim

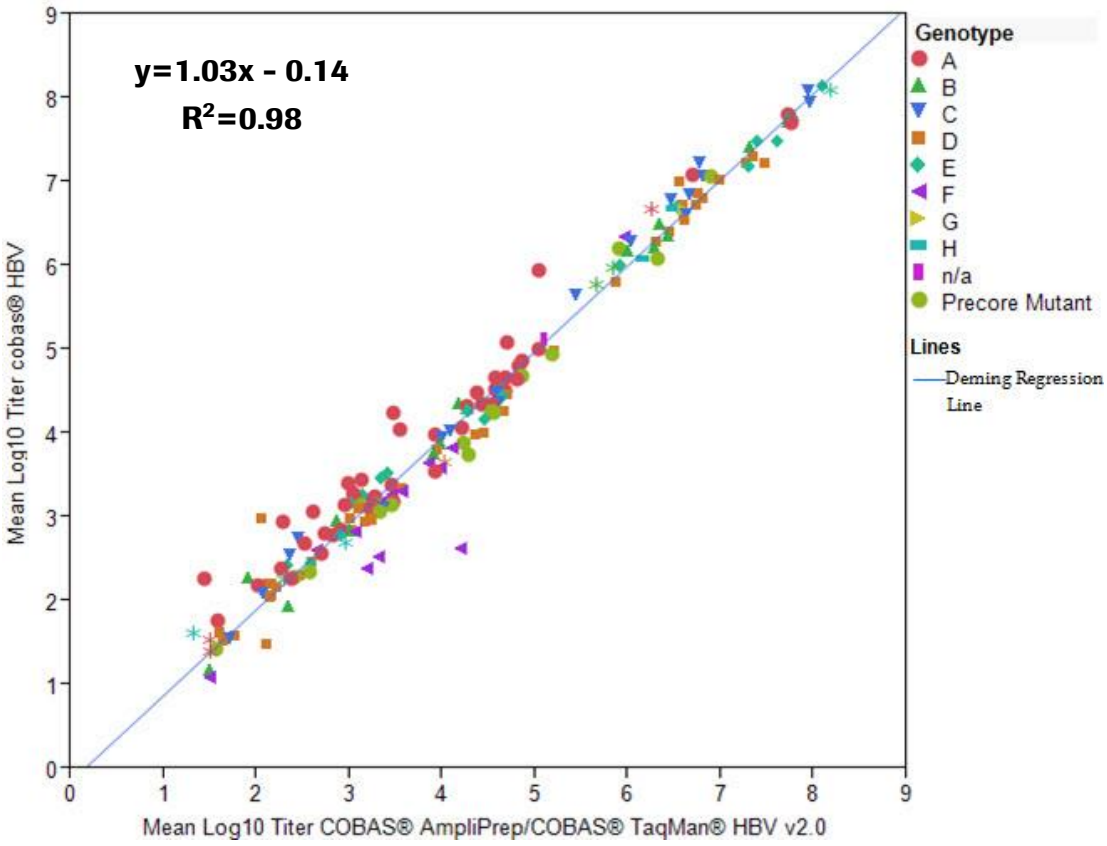
Method correlation

Performance evaluation of cobas® HBV compared to the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0

The performance of **cobas**® HBV and the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 (TaqMan® HBV Test, v2.0) were compared by analysis of EDTA plasma and serum samples from HBV-infected patients. A total of 103 EDTA plasma and 85 serum samples across all HBV genotypes, analyzed in duplicate, were valid and within the quantitation range of both tests. Deming regression analysis was performed. The mean titer deviation of the samples tested with the two tests was -0.03 $\log_{10}$.

The Deming regression results are shown in Figure 8.

Figure 8 Regression analysis of **cobas**® HBV vs TaqMan® HBV Test, v2.0, EDTA plasma and serum samples

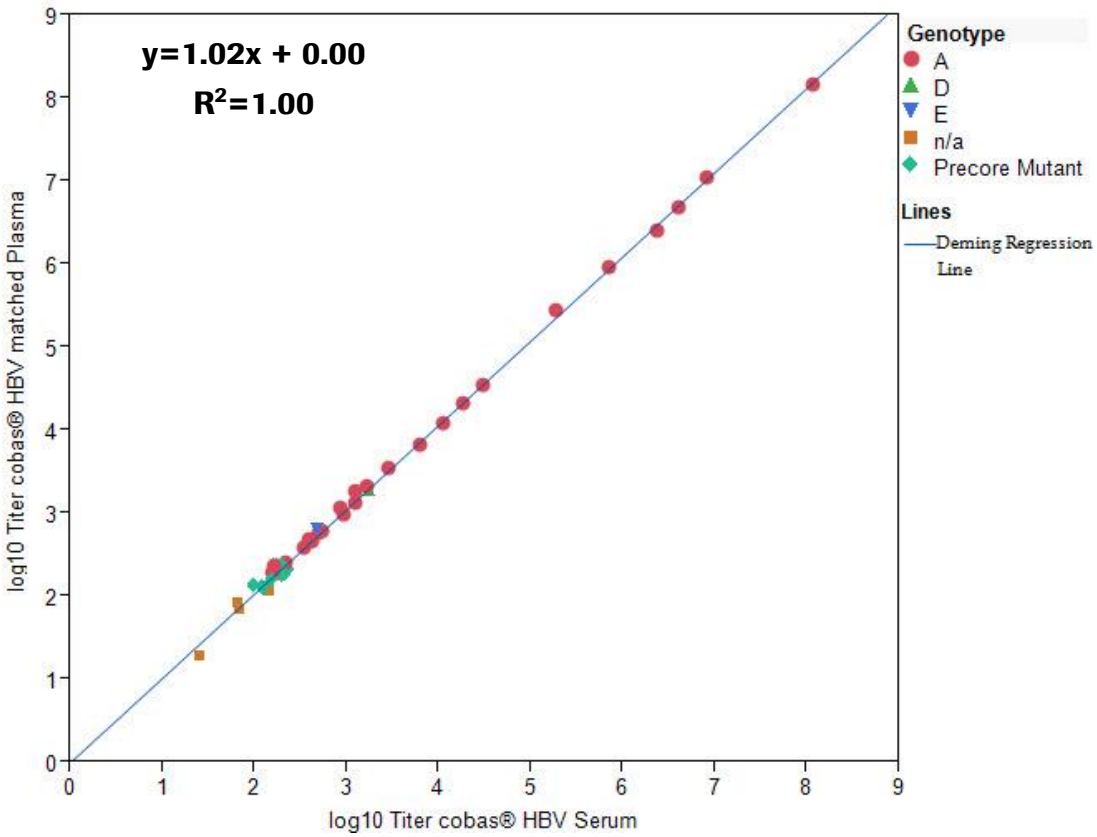


Matrix equivalency – EDTA plasma versus serum

Fifty paired EDTA plasma and serum samples were analyzed for matrix equivalency. The HBV positive samples covered genotype A, genotype D, genotype E and precore mutant. Samples had titers across the entire linear range.

Matrix equivalency was shown in the tested samples with a mean titer deviation of 0.05 $\log_{10}$ (Figure 9).

Figure 9 Matrix equivalency performance between EDTA plasma and serum



Whole system failure

The whole system failure rate for **cobas**® HBV was determined by testing 100 replicates of EDTA plasma and 100 replicates for serum spiked with HBV for a total of 200 replicates. These samples were tested at a target concentration of approximately 3 x LoD. The study was performed using the **cobas**® 6800 System.

The results of this study determined that all replicates were reactive for each target, resulting in a whole system failure rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.62% for the upper bound for each matrix [0%: 3.62%].

Cross contamination

The cross-contamination rate for **cobas**® HBV was determined by testing 240 replicates of a normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma sample and 225 replicates of a high titer HBV sample at 1.00E+09 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were non-reactive, resulting in a cross-contamination rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 1.53% for the upper bound [0%: 1.53%].

System equivalency / system comparison

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 Systems was demonstrated via performance studies.

The results presented in the Instructions for Use support equivalent performance for all systems.

Additional information

Key test features

Sample type	EDTA plasma, serum		
Minimum amount of sample required	650 µL or 350 µL*		
Sample process volume	500 µL or 200 µL		
Analytical sensitivity		500 µL	200 µL
	EDTA plasma	2.7 IU/mL	15.5 IU/mL
	Serum	2.4 IU/mL	12.5 IU/mL
Linear range	500 µL: 10 IU/mL – 1.0E+09 IU/mL		
	200 µL: 25 IU/mL – 1.0E+09 IU/mL		
Specificity	100% (one-sided 95% confidence interval: 99.5%)		
Genotypes detected	HBV Genotype A-H, and predominant precore mutant		

* Dead volume of 0.150 mL is identified for the **cobas**® **omni** Secondary Tubes. Other tubes compatible with **cobas**® 5800/6800/8800 Systems (consult User Assistance and/or User Guides) may have different dead volume and require more or less minimum volume.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 27 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical Support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer

Table 28 Manufacturer

Manufactured in the United States

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Made in USA

Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

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IVD

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Document revision

Document Revision Information	
Doc Rev. 1.0 01/2023	First publishing. Updated **Trademarks and patents** section, including the link. Updated to current economic operators. Updated hazard information. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.
Doc Rev. 2.0 04/2023	The **cobas**® HBV test claim extension to run as well on the **cobas**® 5800 System was shown and with that the respective information added to the whole document. Added information to the **Explanation of the test** section. Updated **References** section. Updated **cobas**® branding. Please contact your local Roche Representative if you have any questions.