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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Intended use

cobas® HBV

cobas® HBV 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.

cobas® HBV/HCV/HIV-1 Control Kit

cobas® HBV/HCV/HIV-1 Control Kit is intended for use as a positive run/batch control on the **cobas**® 5800/6800/8800 systems with the **cobas**® HBV, **cobas**® HCV, and **cobas**® HIV-1 tests.

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, or **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 2nd WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to HBV 2nd WHO International Standard (NIBSC code 97/750).

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 softwares 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 the highly conserved pre-core and core 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

The materials provided for **cobas**® HBV can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 9 through Table 11.

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

Table 1 cobas® HBV

cobas® 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 EUH210: Safety data sheets available on request. EUH208: May produce an allergic reaction. Contains: Subtilisin, 9014-01-1	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

cobas® HBV/HCV/HIV-1 Control Kit

Store at 2–8°C

For use on the **cobas**® 5800 system, and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09040773190)

For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 06997767190 and 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

Table 3 cobas® NHP Negative Control Kit

cobas® **NHP Negative Control Kit**

Store at 2-8°C

For use on the **cobas**® 5800 system, and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09051554190)

For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 07002220190 and 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 dust/fume/gas/mist/vapours/spray. 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-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H - isothiazol-3- one [EC no. 220-239- 6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

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 + H332: Harmful if swallowed or if inhaled. H314: Causes serious skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face 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. 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

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Reagent storage 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**® 5800/6800/8800 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 cobas® 5800 system and cobas® 6800/8800 systems

Reagents loaded onto the **cobas**® 5800 system or **cobas**® 6800/8800 systems are stored at appropriate temperatures, their expiration is monitored and enforced by the system. The system allows reagents to be used only if all of the reagent handling conditions shown in Table 6, Table 7 and Table 8 are met. The system automatically prevents use of expired reagents. Remaining open-kit stability and number of kit uses information for assay specific reagents is accessible through the system user interface.

Table 6 Reagent expiry conditions monitored and enforced by the **cobas**® 5800 system

Reagent	Open-kit stability	Number of kit uses	On-board stability
cobas® HBV	90 days from first usage	40	36 days from loading
cobas® HBV/HCV/HIV-1 Control Kit	single use vial	8	36 days from loading
cobas® NHP Negative Control Kit	single use vial	16	36 days from loading

Table 7 Reagent expiry conditions monitored and enforced by the **cobas**® 6800/8800 systems

Reagent	Open-kit stability	Number of kit uses	On-board stability (outside on board refrigerator)
cobas® HBV	90 days from first usage	40	40 hours from loading
cobas® HBV/HCV/HIV-1 Control Kit	single use vial	8	8 hours from loading
cobas® NHP Negative Control Kit	single use vial	16	10 hours from loading

Table 8 shows the open-kit stability of the **cobas® omni** reagents. Prior to each run, the system verifies the open-kit stability and ensures sufficient fill volume. Therefore, these reagents have no number of kit uses or on-board stability assigned.

Table 8 cobas® omni reagent expiry conditions monitored and enforced by the **cobas®** 5800/6800/8800 systems

Reagent	Open-kit stability
cobas® omni Lysis Reagent	30 days from loading
cobas® omni MGP Reagent	30 days from first usage
cobas® omni Specimen Diluent	30 days from loading
cobas® omni Wash Reagent	30 days from loading

Additional materials required for cobas® 5800/6800/8800 systems

Table 9 Material for use on the **cobas**® 5800/6800/8800 systems

Material	P/N
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190

Table 10 Consumables for use on **cobas**® 5800 system*

Material
cobas® **omni** Processing Plate 24
cobas® **omni** Liquid Waste Plate 24
cobas® **omni** Amplification Plate 24
Tip CORE TIPS with Filter, 1mL
Tip CORE TIPS with Filter, 300µL
cobas® **omni** Liquid Waste Container
Solid Waste Bag or Solid Waste Bag With Insert
16-position tube S-carrier complete
5-position Rack Carrier

*For Part Numbers please refer to the **cobas**® 5800 system User Assistance

Table 11 Consumables for use on **cobas**® 6800/8800 systems*

Material
cobas® **omni** Processing Plate
cobas® **omni** Amplification Plate
cobas® **omni** Pipette Tips
cobas® **omni** Liquid Waste Container
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer

*For Part Numbers please refer to the **cobas**® 6800/8800 systems User Assistance

Instrumentation and software required

The **cobas**® 5800 software, the **cobas**® 6800/8800 systems software and **cobas**® HBV analysis package (ASAP) for the **cobas**® 5800/6800/8800 systems shall be installed.

For **cobas**® 5800 and **cobas**® 6800/8800 systems with software 2.0 or higher, the x800 Data Manager software and PC (or server) will be provided with the system.

For the **cobas**® 6800/8800 systems with software version 1.4, the Instrument Gateway (IG) server will be provided with the system.

Table 12 Instrumentation

Equipment	P/N
cobas® 5800 system	08707464001
cobas® 6800 system	05524245001 and 09575154001
cobas® 8800 system	05412722001 and 09575146001
Sample Supply Module for **cobas**® 6800/8800 systems	06301037001 and 09936882001

Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for additional information.

Note: Contact your local Roche representative for a detailed order list 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 system or **cobas**® 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 0.5% 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.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

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 or potassium hypochlorite 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 0.5% 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 to properly clean and decontaminate the surface of instrument(s).

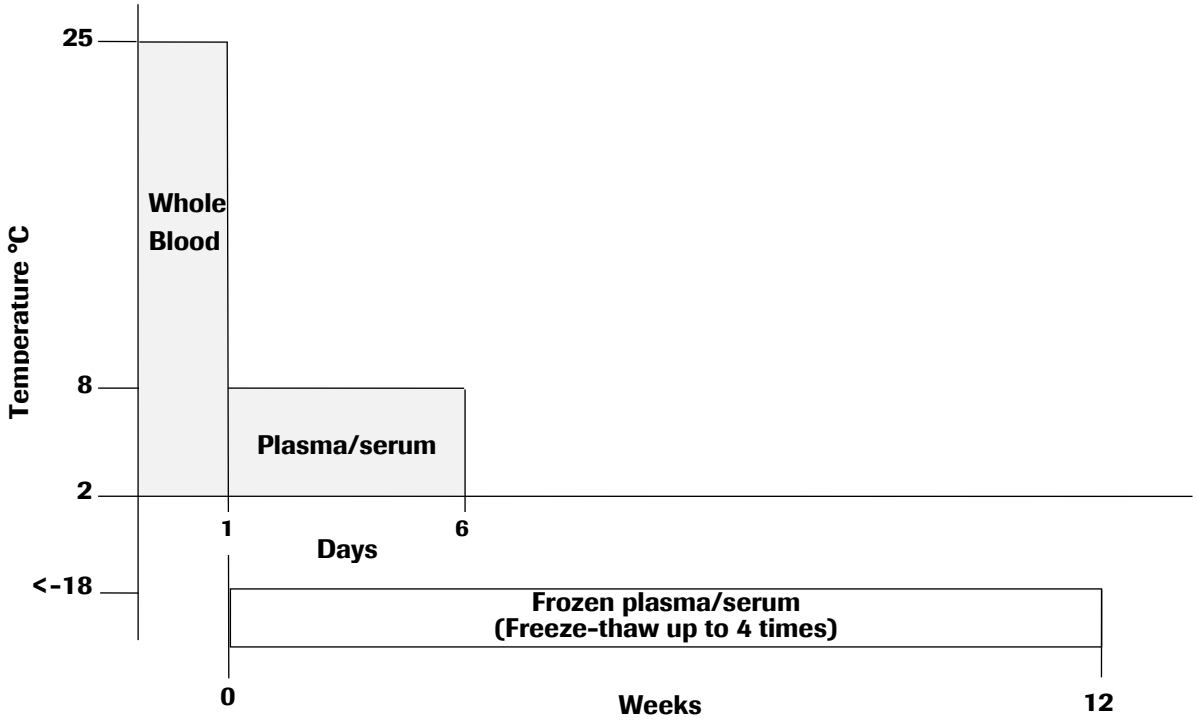
Sample collection, transport, and storage

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**® HBVreagents, **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.
- **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). Figure 2 and Figure 3 below summarize the test procedure.

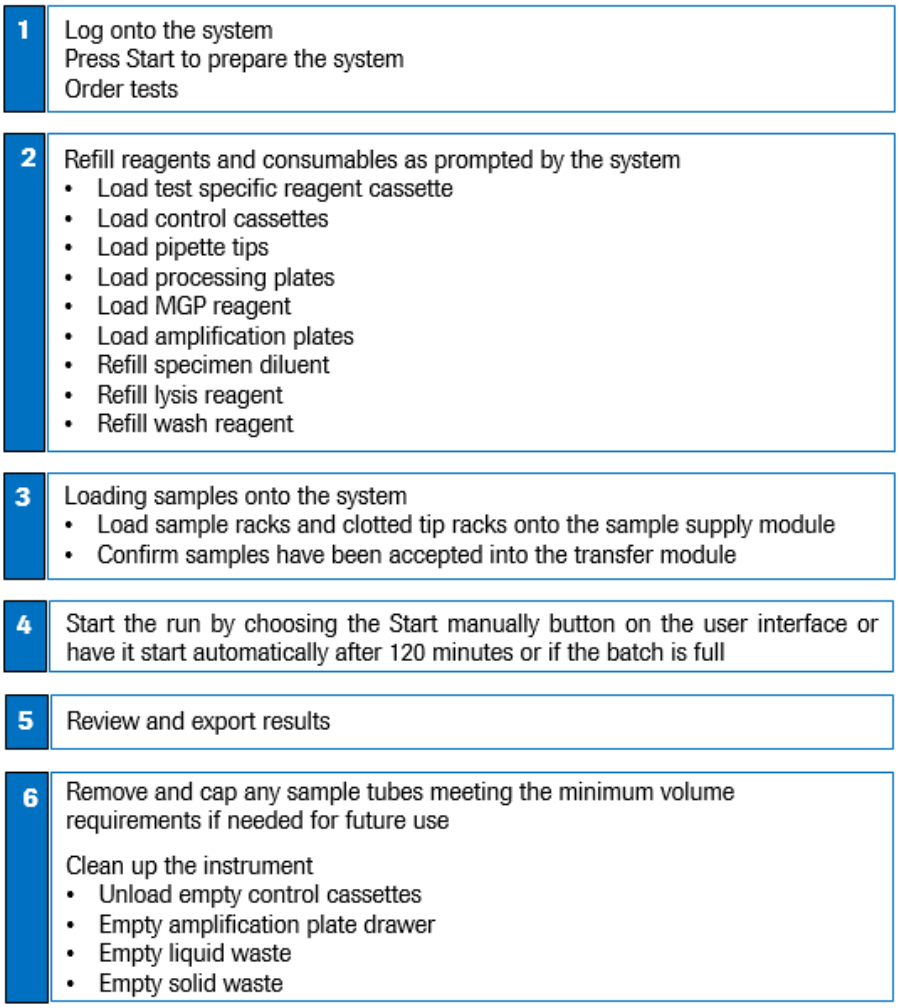
Running cobas® HBV on cobas® 5800/6800/8800 systems

- The operation of the instruments is described in detail in the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance.
- Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper maintenance of instruments.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of RD5 or MPA sample racks. Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper barcode specifications and additional information on loading sample tubes.

Figure 2 **cobas**® HBV test procedure on **cobas**® 5800 system

1	Log onto the system
2	Loading samples onto the system • Load sample racks onto the system • The system prepares automatically • Order tests
3	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette(s) • Load control mini racks • Load processing tips • Load elution tips • Load processing plates • Load liquid waste plates • Load amplification plates • Load MGP cassette • Refill specimen diluent • Refill lysis reagent • Refill wash reagent
4	Start the run by choosing the Start processing button on the user interface, all subsequent runs will start automatically if not manually postponed
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use Clean up the instrument • Unload empty control mini racks • Unload empty test specific reagent cassette(s) • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

Figure 3 **cobas**® HBV test procedure on **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 cobas® 5800 system and cobas® 6800/8800 systems with software version 2.0 or higher

- One **cobas**® NHP Negative Control [(–) C] and two **cobas**® HBV/HCV/HIV-1 Positive Controls, a low positive control [HxV L (+) C] and a high positive control [HxV 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 software and/or report, check for flags and their associated results to ensure the batch validity (refer to the x800 Data Manager User Assistance for a 'List of flag codes').
- The results of the controls are shown in the "Controls" app of the software.
- Controls are marked with 'Valid' in the column "Control result" if the respective target of the control is reported valid. Controls are marked with 'Invalid' in the column "Control result" if the respective target of the control is 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 controls is invalid, repeat testing of all controls and all associated samples is required.

Validation of results is performed automatically by the instrument software based on control results.

NOTE: The **cobas**® 5800 system and the **cobas**® 6800/8800 systems with software version 2.0 or higher 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 and validity of results on the cobas® 6800/8800 systems with software version 1.4

- One **cobas**® NHP Negative Control [(–) C] and two **cobas**® HBV/HCV/HIV-1 Positive Controls, a low positive control [HxV L (+) C] and a high positive control [HxV H (+) C] are processed with each batch.
- In the software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 systems User Assistance.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch is required.

Validation of results is performed automatically by the instrument software based on control results.

Control flags on cobas® 6800/8800 systems with software version 1.4

Table 13 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.

Interpretation of results for cobas® 5800/6800/8800 systems

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

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

Table 14 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 and cobas® 6800/8800 systems with software version 2.0 or higher

The results of the samples are shown in the "Results" app of the software.

For a valid control batch, check each individual sample for flags in the 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 14 above.
- If one or more sample targets are marked with 'Invalid' the 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 with software version 1.4

For a valid batch, check each individual sample for flags in the 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 14 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

System equivalency

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 systems was demonstrated via performance studies. The data presented in the Instructions for Use support equivalent performance for all systems.

Key performance characteristics

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, NIBSC code 97/750) genotype A obtained from National Institute for Biological Standards and Control (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 15 to Table 18, 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 15 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 16 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 17 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 18 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)

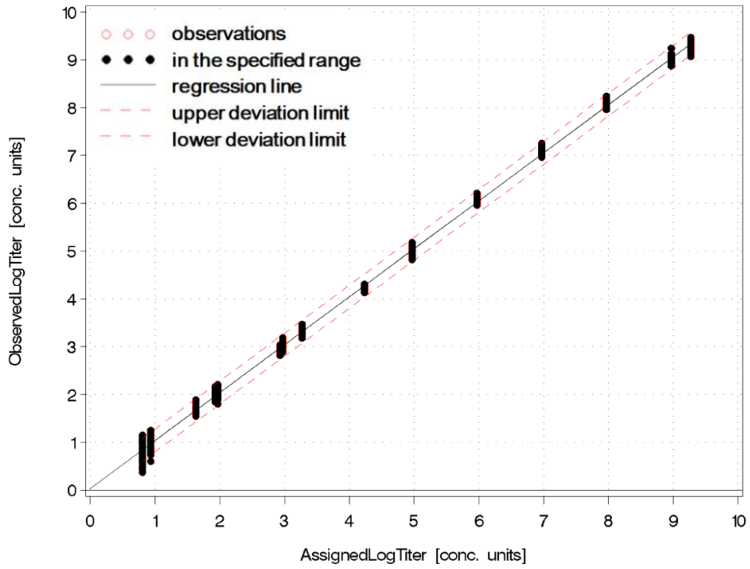


Figure 5 Linear range determination in EDTA plasma (200 µL)

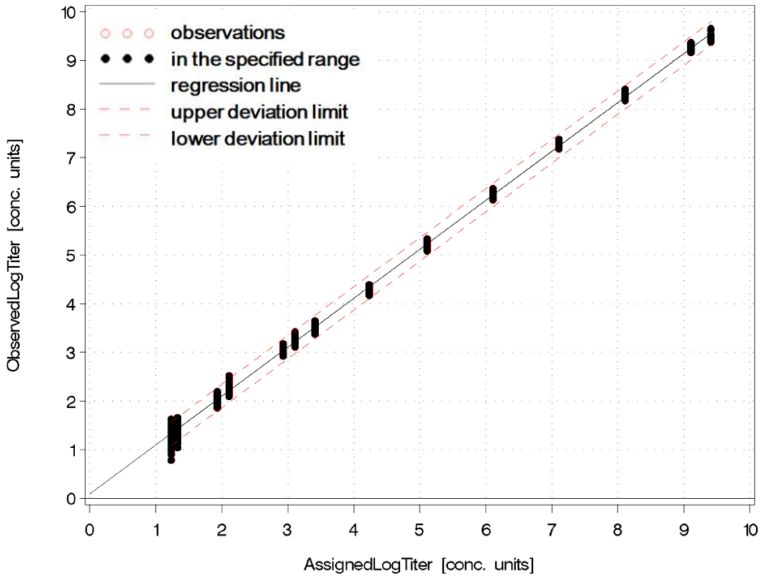


Figure 6 Linear range determination in serum (500 µL)

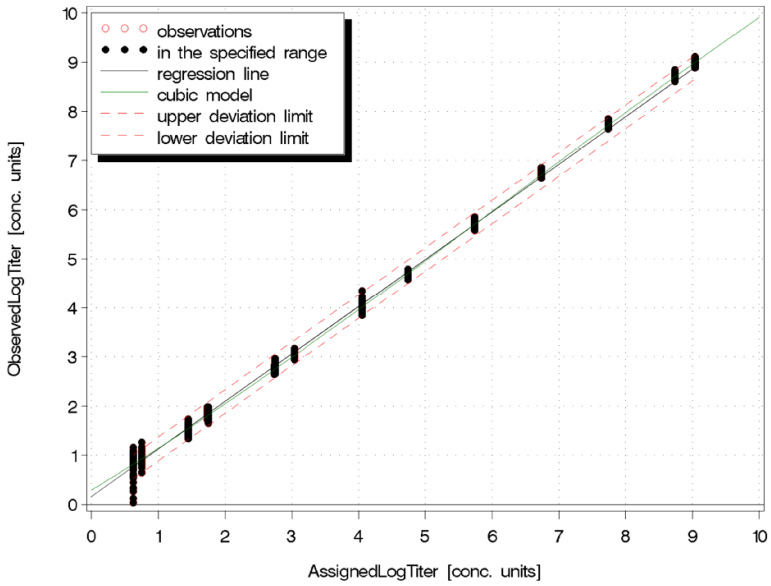
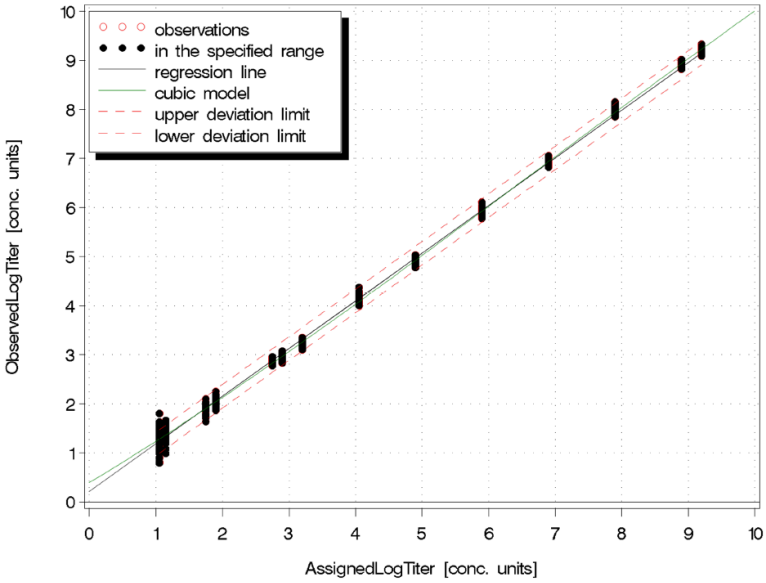


Figure 7 Linear range determination in serum (200 µL)



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 19 through Table 22.

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 19 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 20 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 21 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 22 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 23 and Table 24, respectively. The study demonstrates that **cobas**® HBV detected all HBV genotypes tested with a similar LoD as HBV genotype A.

Table 23 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 24 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 25 and Table 26. 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 25 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 (88.24)	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 26 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 (98.69)	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 27 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 28 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 28 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

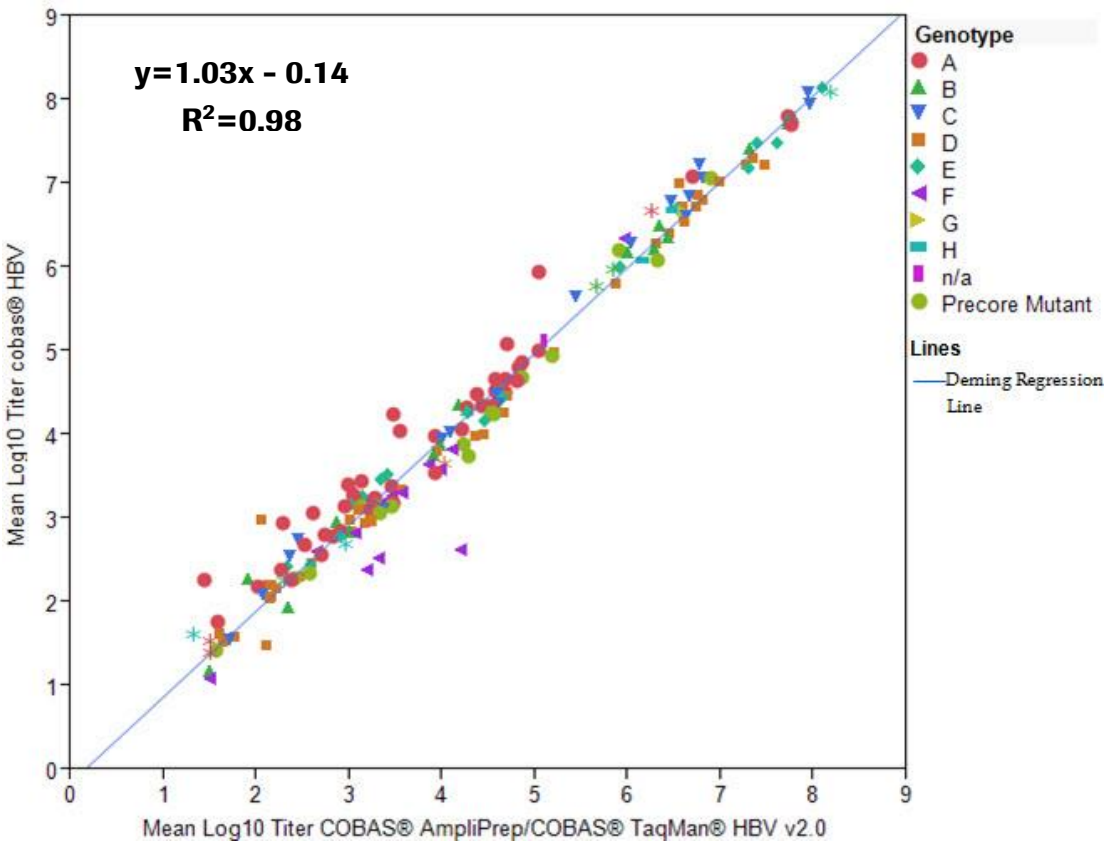
Method correlation

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

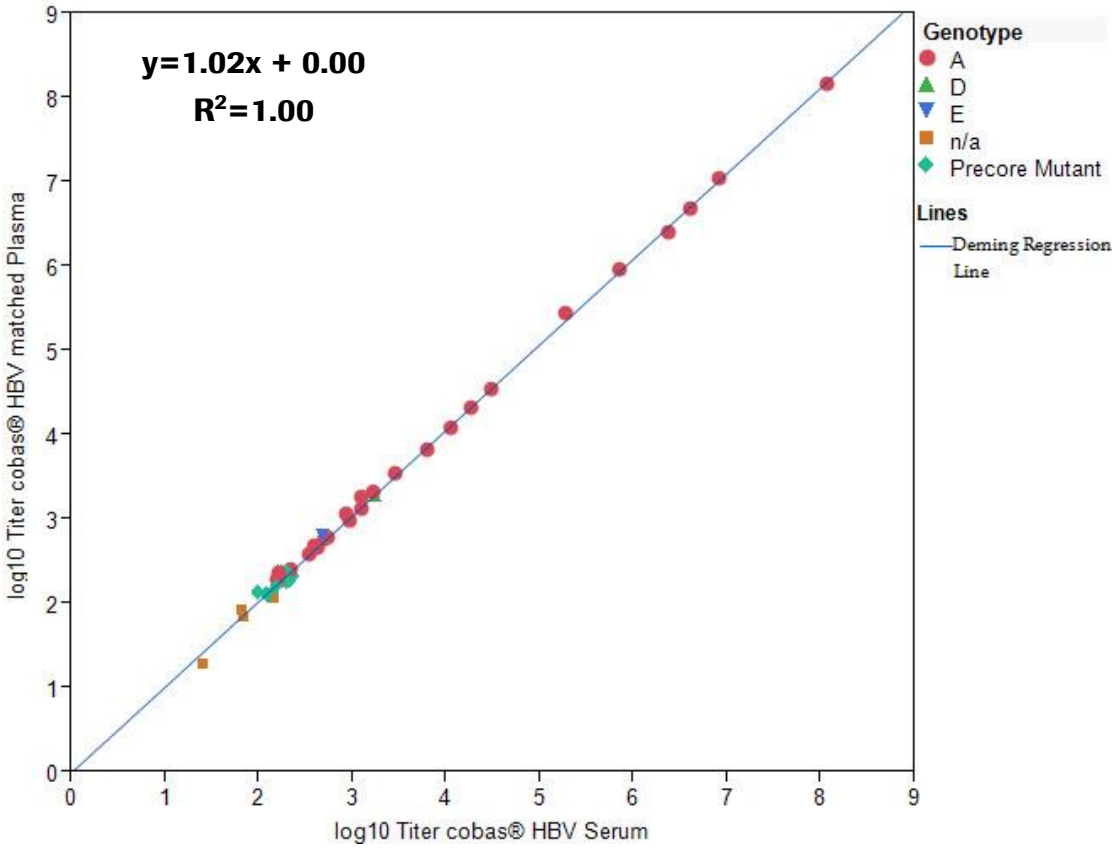


Matrix equivalency – EDTA plasma versus serum

Fifty paired EDTA plasma and serum samples were analyzed for matrix equivalency. The HBV positive samples covered most genotypes and 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%].

Clinical performance evaluation

Reproducibility study

The reproducibility and lot-to-lot variability of the **cobas**® HBV were evaluated in EDTA plasma on the **cobas**® 6800 system using a mixed model to estimate the total variance.

The evaluation results are summarized in Table 29 through Table 32 below.

Lot-to-lot variability

Lot-to-lot variability testing was performed for genotypes A and C at one test site, using three reagent lots. Two operators at the site tested each lot for six days. Two runs were performed each day.

Table 29 below shows attributable percentages of total variance, total precision standard deviations (SDs), and lognormal coefficient of variability (CVs) by genotype and expected $\log_{10}$ HBV DNA concentration for the **cobas**® 6800 system.

Table 29 Attributable percentage of total variance, total precision standard deviation, and lognormal CV(%) of HBV DNA concentration ($\log_{10}$ IU/mL) by genotype and positive panel member (lot-to-lot) on the **cobas**® 6800 system (reproducibility)

-	HBV DNA Concentration ($\log_{10}$ IU/mL)		-	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
A	1.48	1.50	107	13% (12.90)	0% (0.00)	0% (0.00)	0% (0.00)	87% (34.68)	0.157	37.27
	2.70	2.72	108	52% (11.96)	0% (0.00)	0% (0.00)	0% (0.00)	48% (11.56)	0.072	16.69
	3.70	3.64	108	60% (14.29)	0% (0.00)	4% (3.55)	1% (1.57)	36% (11.01)	0.080	18.53
	4.70	4.65	107	47% (13.05)	0% (0.00)	3% (3.22)	1% (2.32)	49% (13.29)	0.082	19.14
	5.70	5.67	107	53% (13.66)	2% (2.59)	0% (0.00)	0% (0.00)	45% (12.54)	0.081	18.80
	6.70	6.71	105	50% (11.66)	0% (0.00)	0% (0.00)	5% (3.82)	44% (10.92)	0.071	16.48
	7.70	7.41	108	55% (13.08)	0% (0.00)	0% (0.00)	4% (3.59)	40% (11.18)	0.076	17.65
	8.70	8.41	107	51% (12.52)	0% (0.00)	0% (0.00)	10% (5.61)	38% (10.75)	0.075	17.51

-	HBV DNA Concentration (log_{10} IU/mL)		-	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
C	1.48	1.49	107	23% (13.62)	1% (2.83)	0% (0.00)	0% (0.00)	76% (25.26)	0.124	29.05
	2.70	2.71	105	53% (13.92)	2% (2.63)	3% (3.48)	0% (0.00)	41% (12.27)	0.082	19.16
	3.70	3.64	107	61% (11.67)	0% (0.00)	0% (0.80)	0% (0.00)	39% (9.37)	0.065	15.02
	4.70	4.65	106	47% (11.44)	0% (0.00)	0% (0.00)	0% (0.00)	53% (12.25)	0.073	16.82
	5.70	5.69	107	60% (14.76)	0% (0.00)	1% (1.51)	0% (0.00)	39% (11.86)	0.082	19.08
	6.70	6.69	107	48% (11.79)	0% (0.00)	2% (2.31)	0% (0.00)	50% (12.13)	0.074	17.14
	7.70	7.38	107	51% (11.22)	0% (0.00)	0% (0.00)	1% (1.57)	48% (10.94)	0.068	15.80
	8.70	8.42	106	56% (13.92)	0% (0.00)	0% (0.00)	4% (3.54)	40% (11.72)	0.080	18.62

Note: Results with detectable viral load are included in this table; Within the range of assay are results from 1.0E+01 IU/mL to 1.0E+09 IU/mL.

[a] Calculated using the SAS MIXED procedure.
[b] Number of valid tests with detectable viral load.
[c] Calculated using the total variability from the SAS MIXED procedure.
[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100

CV(%) = percent coefficient of variation; DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; SD = standard deviation; sqrt = square root.

In Table 30 below, the negative percent agreement (NPA) for the **cobas®** 6800 system using negative panel member tests was 100%.

Table 30 Negative percent agreement using the negative panel member (lot-to-lot)

Expected HBV DNA Concentration	No. of Valid Tests	Positive Results	Negative Results	Negative Percent Agreement[a]	95% CI[b]
Negative	106	0	106	100.00	(96.58, 100.00)

[a] NPA = (number of negative results / total number of valid tests in negative panel member) * 100.
[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.
CI = confidence interval; DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; NPA = negative percent agreement.

Reproducibility

Reproducibility testing was performed at three sites for genotypes A and C, using one reagent lot. Two operators at each site tested for 6 days. Two runs were performed each day.

Table 31 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected log_{10} HBV DNA concentration on the **cobas**® 6800 system.

Table 31 Attributable percentage of total variance, total precision standard deviation, and lognormal CV(%) of HBV DNA concentration (log_{10} IU/mL) by genotype and positive panel member (reproducibility)

-	HBV DNA Concentration (log_{10} IU/mL)		-	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Site	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
A	1.48	1.48	107	1% (4.21)	0% (0.00)	5% (7.75)	1% (3.56)	93% (34.98)	0.153	36.41
	2.70	2.66	108	34% (9.53)	0% (0.00)	0% (0.00)	16% (6.40)	50% (11.52)	0.070	16.33
	3.70	3.60	108	34% (7.49)	2% (1.90)	7% (3.42)	0% (0.00)	56% (9.58)	0.055	12.80
	4.70	4.62	107	13% (5.40)	0% (0.00)	0% (0.00)	12% (5.28)	75% (13.05)	0.065	15.12
	5.70	5.63	107	37% (7.82)	1% (1.26)	0% (0.00)	0% (0.00)	62% (10.04)	0.055	12.81
	6.70	6.67	106	20% (5.99)	3% (2.16)	4% (2.57)	15% (5.16)	60% (10.48)	0.059	13.59
	7.70	7.37	108	3% (2.70)	2% (2.06)	0% (0.00)	0% (0.00)	95% (15.12)	0.067	15.50
	8.70	8.36	107	12% (4.32)	0% (0.00)	0% (0.00)	2% (1.53)	86% (11.46)	0.053	12.36

-	HBV DNA Concentration (log_{10} IU/mL)		-	Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Site	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
C	1.48	1.48	107	2% (11.79)	1% (7.06)	0% (0.00)	0% (0.00)	97% (84.30)	0.324	86.20
	2.70	2.67	105	19% (5.94)	3% (2.22)	0% (0.00)	0% (0.00)	79% (12.27)	0.060	13.84
	3.70	3.61	107	14% (4.49)	0% (0.00)	7% (3.15)	0% (0.00)	78% (10.48)	0.051	11.84
	4.70	4.62	106	24% (6.45)	0% (0.00)	0% (0.00)	0% (0.00)	76% (11.59)	0.057	13.29
	5.70	5.65	107	18% (5.96)	0% (0.00)	3% (2.29)	0% (0.00)	80% (12.68)	0.061	14.22
	6.70	6.65	107	23% (6.35)	6% (3.26)	0% (0.00)	1% (1.33)	70% (11.10)	0.057	13.29
	7.70	7.34	106	0% (0.00)	3% (2.38)	0% (0.00)	13% (5.12)	84% (13.11)	0.062	14.30
	8.70	8.36	107	4% (2.24)	0% (0.00)	16% (4.35)	10% (3.46)	70% (9.09)	0.047	10.91

Note: Results with detectable viral load are included in this table; Within the range of assay are results from 1.0E+01 IU/mL to 1.0E+09 IU/mL.

[a] Calculated using the SAS MIXED procedure.
[b] Number of valid tests with detectable viral load.
[c] Calculated using the total variability from the SAS MIXED procedure.
[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100.
CV(%) = percent coefficient of variation; HBV = hepatitis B virus; DNA = deoxyribonucleic acid; No. = number; SD = standard deviation; sqrt = square root.

The NPA was 100% (106/106; 95% CI: 96.58% to 100%) using negative panel member tests on the **cobas®** 6800 system as presented in Table 32 below.

Table 32 Negative percent agreement using the negative panel member (reproducibility) on the **cobas®** 6800 system

Expected HBV DNA Concentration	No. of Tests	Positive Results	Negative Results	Negative Percent Agreement[a]	95% CI[b]
Negative	106	0	106	100.00	(96.58, 100.00)

[a] NPA = (number of negative results/total number of valid tests in negative panel member) * 100.
[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.
CI = confidence interval; DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; NPA = negative percent agreement.

Clinical utility

The study was designed to evaluate the ability of the assay to predict clinical outcome.

Residual specimens obtained from approximately 300 subjects who were randomized to receive treatment for 100 weeks with entecavir plus tenofovir or entecavir monotherapy during a pharmaceutical clinical trial were tested. In addition specimens from approximately 70 HBeAg (-) chronic HBV-infected subjects from routine clinical practice who received treatment with tenofovir monotherapy were tested (Table 33).

Table 33 Treatment groups

Clinical Study	HBeAg Status	Treatment	Treatment Arm
Pharmaceutical Clinical Trial[21]	HBeAg (+)	entecavir monotherapy	Arm I
		entecavir + tenofovir	Arm II
	HBeAg (-)	entecavir monotherapy	Arm III (includes up to 17 subjects from clinical practice)
		entecavir + tenofovir	Arm IV
Clinical Practice	HBeAg (-)	tenofovir monotherapy	Arm V

HBeAg = Hepatitis B e antigen.

Testing with **cobas**® HBV was performed at three sites. Each site was equipped with one **cobas**® 6800 system. Three kit lots of reagents were used in the study; each sample was tested with one kit lot. Table 34 below shows the demographic and baseline characteristics of subjects whose samples were tested on the **cobas**® 6800 system, both HBeAg (+) and HBeAg (-) subjects were enrolled in this study, and data for these populations were analyzed separately.

Table 34 Demographics and baseline characteristics of subjects

Characteristics	Statistics
Total, N	396
Age Category (years), n (%)	-
< 40	186 (47.0%)
>= 40	210 (53.0%)
Age (years)	-
Mean ± SD	42 ± 15.2
Median	42
Range	17 - 81
Gender, n (%)	-
Male	276 (69.7%)
Female	120 (30.3%)
Race, n (%)	-
Asian	204 (51.5%)
Black / African American	14 (3.5%)
White / Caucasian	169 (42.7%)
Other	9 (2.3%)

Genotype, n (%)	-
A	64 (16.2%)
A & G	1 (0.3%)
B	62 (15.7%)
C	74 (18.7%)
D	105 (26.5%)
E	4 (1.0%)
F	10 (2.5%)
Mixed	1 (0.3%)
Unknown	75 (18.9%)
Normal ALT at Baseline, n (%)	-
Yes	23 (5.8%)
No	361 (91.2%)
Unknown	12 (3.0%)
Baseline ALT (IU/L)	-
Mean ± SD	140 ± 169.9
Median	96
Range	14 - 1583
HBV DNA (log_{10} IU/mL) at Baseline	-
Mean ± SD	6.6 ± 2.38
Median	7.4
Range	-0.0 - 10.1
HBV DNA Category, n (%)	-
< 2.0 x 10^3 IU/mL	41 (10.4%)
2.0 x 10^3 to 2.0 x 10^4 IU/mL	13 (3.3%)
> 2.0 x 10^4 IU/mL	330 (83.3%)
Unknown	12 (3.0%)

ALT = alanine aminotransferase; HBV = hepatitis B virus; DNA = deoxyribonucleic acid; SD = standard deviation.

Prediction of response to antiviral therapy

Definitions:

- Week 12 virologic response (VR) = HBV DNA 2 $\log_{10}$ decrease from baseline
- Week 24 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-))
- Week 48 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-))
- Week 96 VR = HBV DNA < 50 IU/mL (VR endpoint)
- No-VR endpoint= HBV DNA > 50 IU/mL at Week 96
- Biochemical Response (BR) = normalization of ALT compared to baseline; for male ALT < 30 IU/L and for female ALT < 19 IU/L
- HBeAg loss = conversion from HBeAg (+) to HBeAg (-) status during therapy

Predicting virologic response at Week 96

In this study, baseline HBV DNA concentration and VRs at Weeks 12, 24, and 48 of treatment were used to evaluate the ability to predict outcome (VR, BR, or HBeAg loss) at Week 96 of therapy. VR96 (HBV DNA<50 IU/mL) was assessed using HBV DNA results from an approved test.

When **cobas**® HBV was used to measure HBV DNA, a baseline HBV DNA concentration of <10^8IU/mL and VRs at Weeks 12, 24, and 48 were shown to be highly predictive of VR96 for all the treatment arms in this study (PPVs 79.6% to 100%) (Table 35 and Table 36 below).

Table 35 Probability of achieving virologic response at Week 96 given baseline HBV DNA <10^8 IU/mL by treatment arm

-			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Evaluable Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Baseline	Arm I	103	93.5 (82.5, 97.8)	43 / 46	31.6 (21.0, 44.5)	18 / 57	6.62 (1.81, 24.20)
-	Arm II	102	96.2 (87.0, 98.9)	50 / 52	4.0 (1.1, 13.5)	2 / 50	1.04 (0.14, 7.69)
-	Arm III	49	100.0 (92.1, 100.0)	45 / 45	25.0 (4.6, 69.9)	1 / 4	30.00 (0.83, 1087.42)
-	Arm IV	48	97.9 (88.9, 99.6)	46 / 47	100.0 (20.7, 100.0)	1 / 1	92.00 (1.81, 4686.43)
-	Arm V	30	90.0 (74.4, 96.5)	27 / 30	NC	0	9.00 (0.15, 541.69)

Notes: Positive Predictive Value (PPV) = TP / (TP + FP) or the probability of being a VR96 given the subject was a virologic responder at a specific visit.
Negative Predictive Value (NPV) = TN / (FN + TN) or the probability of not being a VR96 given the subject was not a virologic responder at a specific visit.
Odds Ratio (OR) = (TP • TN) / (FP • FN).
95% CIs for PPV and NPV are calculated based on Wilson Score CI.
0.5 was added to empty cells (TP, TN, FP, or FN = 0), prior to calculation of OR and corresponding 95% CI.
Week 96 VR = HBV DNA < 50 IU/mL (VR endpoint) from the COBAS® Ampliprep/ COBAS® Taqman® HBV Test, version 2
Baseline HBV DNA Concentration < 1E8 IU/mL as determined on the **cobas**® 6800 system.
Arm I: entecavir monotherapy (HBeAg (+)).
Arm II: entecavir + tenofovir (HBeAg (+)).
Arm III: entecavir monotherapy (HBeAg (-)).
Arm IV: entecavir + tenofovir (HBeAg (-)).
Arm V: tenofovir monotherapy (HBeAg (-)).
CI = confidence interval; DNA = deoxyribonucleic acid; FN = false negative; FP = false positive; HBeAg = Hepatitis B e antigen; HBV = hepatitis B virus; NC = not calculable (as there were no subjects with virologic non-responses at that visit); TN = true negative; TP = true positive;
VR = Virologic Response; VR96 = Virologic Response at Week 96.

Table 36 Probability of achieving virologic response at Week 96 given virologic response at a specific on-treatment visit by treatment arm

-			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Week 12	Arm I	103	79.6 (70.8, 86.3)	82 / 103	NC	0	3.90 (0.08, 202.63)
-	Arm II	100	97.0 (91.5, 99.0)	97 / 100	NC	0	32.33 (0.54, 1921.79)
-	Arm III	48	97.8 (88.7, 99.6)	45 / 46	0.0 (0.0, 65.8)	0 / 2	11.25 (0.28, 445.33)
-	Arm IV	48	95.8 (86.0, 98.8)	46 / 48	NC	0	23.00 (0.36, 1485.21)
-	Arm V	21	85.7 (48.7, 97.4)	6 / 7	7.1 (1.3, 31.5)	1 / 14	0.46 (0.02, 8.69)
Week 24	Arm I	103	96.1 (89.2, 98.7)	74 / 77	69.2 (50.0, 83.5)	18 / 26	55.50 (13.37, 230.39)
-	Arm II	102	96.7 (90.8, 98.9)	89 / 92	10.0 (1.8, 40.4)	1 / 10	3.30 (0.31, 35.08)
-	Arm III	47	100.0 (89.8, 100.0)	34 / 34	7.7 (1.4, 33.3)	1 / 13	5.67 (0.18, 179.94)
-	Arm IV	49	97.7 (87.9, 99.6)	42 / 43	16.7 (3.0, 56.4)	1 / 6	8.40 (0.45, 156.19)
-	Arm V	20	94.1 (73.0, 99.0)	16 / 17	33.3 (6.1, 79.2)	1 / 3	8.00 (0.35, 184.38)
Week 48	Arm I	101	89.9 (81.9, 94.6)	80 / 89	91.7 (64.6, 98.5)	11 / 12	97.78 (11.28, 847.86)
-	Arm II	97	95.9 (89.9, 98.4)	93 / 97	NC	0	23.25 (0.41, 1328.83)
-	Arm III	46	100.0 (91.6, 100.0)	42 / 42	25.0 (4.6, 69.9)	1 / 4	28.00 (0.77, 1015.78)
-	Arm IV	48	97.8 (88.4, 99.6)	44 / 45	33.3 (6.1, 79.2)	1 / 3	22.00 (0.98, 494.79)
-	Arm V	28	92.3 (75.9, 97.9)	24 / 26	50.0 (9.5, 90.5)	1 / 2	12.00 (0.53, 273.05)

Notes: Positive Predictive Value (PPV) = TP / (TP + FP) or the probability of being a VR96 given the subject was a virologic responder at a specific visit.

Negative Predictive Value (NPV) = TN / (FN + TN) or the probability of not being a VR96 given the subject was not a virologic responder at a specific visit.

Odds Ratio (OR) = (TP • TN) / (FP • FN).

95% CIs for PPV and NPV are calculated based on Wilson Score CI.

0.5 was added to empty cells (TP, TN, FP, or FN = 0), prior to calculation of OR and corresponding 95% CI.

VR96 is achieved if the subject has HBV DNA < 50 IU/mL from the COBAS® TaqMan® HBV Test For Use with the High Pure System at Week 96.

Week 12 VR = HBV DNA > 2 $\log_{10}$ decrease from baseline; Week 24 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-)); Week 48 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-)).

Arm I: entecavir monotherapy (HBeAg (+)).

Arm II: entecavir + tenofovir (HBeAg (+)).

Arm III: entecavir monotherapy (HBeAg (-)).

Arm IV: entecavir + tenofovir (HBeAg (-)).

Arm V: tenofovir monotherapy (HBeAg (-)).

CI = confidence interval; DNA = deoxyribonucleic acid; FN = false negative; FP = false positive; HBeAg = Hepatitis B e antigen; HBV = hepatitis B virus; NC = not calculable (as there were no subjects with virologic non-responses at that visit); TN = true negative; TP = true positive; VR = Virologic Response; VR96 = Virologic Response at Week 96.

Predicting biochemical response at Week 96

The probability of achieving a biochemical response at Week 96 given an on-treatment VR at Week 12, Week 24 or Week 48 is summarized in Table 37.

The value of VR at Week 12, Week 24, or Week 48 as a predictor of BR96 varied by VR week and treatment arm.

Table 37 Probability of achieving biochemical response at Week 96 given virologic response at a specific on-treatment visit by treatment arm

-			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Week 12	Arm I	101	62.4 (52.6, 71.2)	63 / 101	NC	0	1.66 (0.03, 85.30)
-	Arm II	100	43.0 (33.7, 52.8)	43 / 100	NC	0	0.75 (0.01, 38.79)
-	Arm III	49	50.0 (36.1, 63.9)	23 / 46	66.7 (20.8, 93.9)	2 / 3	2.00 (0.17, 23.62)
-	Arm IV	49	32.7 (21.2, 46.6)	16 / 49	NC	0	0.48 (0.01, 25.57)
-	Arm V	21	40.0 (16.8, 68.7)	4 / 10	90.9 (62.3, 98.4)	10 / 11	6.67 (0.60, 74.51)
Week 24	Arm I	102	66.2 (55.1, 75.8)	51 / 77	60.0 (40.7, 76.6)	15 / 25	2.94 (1.16, 7.45)
-	Arm II	103	44.6 (34.8, 54.7)	41 / 92	81.8 (52.3, 94.9)	9 / 11	3.62 (0.74, 17.68)
-	Arm III	51	47.2 (32.0, 63.0)	17 / 36	33.3 (15.2, 58.3)	5 / 15	0.45 (0.13, 1.57)
-	Arm IV	50	38.6 (25.7, 53.4)	17 / 44	100.0 (61.0, 100.0)	6 / 6	7.56 (0.40, 144.09)
-	Arm V	24	42.1 (23.1, 63.7)	8 / 19	80.0 (37.6, 96.4)	4 / 5	2.91 (0.27, 31.22)
Week 48	Arm I	100	65.2 (54.8, 74.3)	58 / 89	81.8 (52.3, 94.9)	9 / 11	8.42 (1.71, 41.41)
-	Arm II	97	43.3 (33.9, 53.2)	42 / 97	NC	0	0.76 (0.01, 39.29)
-	Arm III	49	52.3 (37.9, 66.2)	23 / 44	40.0 (11.8, 76.9)	2 / 5	0.73 (0.11, 4.81)
-	Arm IV	49	37.0 (24.5, 51.4)	17 / 46	100.0 (43.9, 100.0)	3 / 3	3.52 (0.17, 74.51)
-	Arm V	28	33.3 (18.0, 53.3)	8 / 24	75.0 (30.1, 95.4)	3 / 4	1.50 (0.13, 16.82)

Notes: Positive Predictive Value (PPV) = TP / (TP + FP) or the probability of being a BR96 given the subject was a virologic responder at a specific visit.
Negative Predictive Value (NPV) = TN / (FN + TN) or the probability of not being a BR96 given the subject was not a virologic responder at a specific visit.
Odds Ratio (OR) = (TP • TN) / (FP • FN).
95% CIs for PPV and NPV are calculated based on Wilson Score CI.
0.5 was added to empty cells (TP, TN, FP or FN = 0), prior to calculation of OR and corresponding 95% CI.
Arm I: entecavir monotherapy (HBeAg (+)).
Arm II: entecavir + tenofovir (HBeAg (+)).
Arm III: entecavir monotherapy (HBeAg (-)).
Arm IV: entecavir + tenofovir (HBeAg (-)).
Arm V: tenofovir monotherapy (HBeAg (-)).

Biochemical Response is defined as normalization of ALT (ALT < 30 IU/L for males and ALT < 19 IU/L for females) at Week 96 as compared to baseline for subjects with elevated ALT at baseline.
Week 12 VR = HBV DNA > 2 $\log_{10}$ decrease from baseline. Week 24 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-)).
Week 48 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-)).
ALT = alanine aminotransferase; CI = confidence interval; DNA = deoxyribonucleic acid; FN = false negative; FP = false positive; HBeAg = Hepatitis B e antigen; HBV = hepatitis B virus; NC = not calculable (as there were no subjects with virologic non-responses at that visit); TN = true negative; TP = true positive; VR = Virologic Response; BR96 = Biochemical Response at Week 96.

Predicting HBeAg loss

HBeAg loss could only be evaluated in subjects who were HBeAg (+) at baseline.

Absence of VR at Week 24 was highly predictive of persistence of HBeAg (NPVs were ≥ 80.0% for both Arms I and II), and absence of VR at Week 48 also predicted HBeAg persistence in Arm I (NPV was 100%) (Table 38). As all subjects on the combination regimen (Arm II) had achieved VRs by Week 48, it was not possible to calculate an NPV at this time point for this group.

Table 38 Probability of HBeAg loss at Week 96 given virologic response at a specific on-treatment visit by treatment arm

-			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Week 12	Arm I	102	46.1 (36.7, 55.7)	47 / 102	NC	0	0.85 (0.02, 43.91)
-	Arm II	101	41.6 (32.5, 51.3)	42 / 101	NC	0	0.71 (0.01, 36.60)
Week 24	Arm I	103	52.6 (41.6, 63.3)	41 / 78	80.0 (60.9, 91.1)	20 / 25	4.43 (1.51, 13.00)
	Arm II	104	44.1 (34.4, 54.2)	41 / 93	81.8 (52.3, 94.9)	9 / 11	3.55 (0.73, 17.33)
Week 48	Arm I	101	51.1 (41.0, 61.2)	46 / 90	100.0 (74.1, 100.0)	11 / 11	23.00 (1.31, 403.28)
-	Arm II	98	40.8 (31.6, 50.7)	40 / 98	NC	0	0.69 (0.01, 35.48)

Note: Positive Predictive Value (PPV) = TP / (TP + FP) or the probability of HBeAg loss at Week 96 given the subject was a virologic responder at a specific visit.
Negative Predictive Value (NPV) = TN / (FN + TN) or the probability of no HBeAg loss at Week 96 given the subject was not a virologic responder at a specific visit.
Odds Ratio (OR) = (TP • TN) / (FP • FN).
95% CIs for PPV and NPV are calculated based on Wilson Score CI.
0.5 was added to empty cells (TP, TN, FP or FN = 0), prior to calculation of OR and corresponding 95% CI.
Arm I: entecavir monotherapy (HBeAg (+)).
Arm II: entecavir + tenofovir (HBeAg (+)).
HBeAg loss is achieved if there is loss of HBeAg during therapy.
Week 12 VR = HBV DNA > 2 $\log_{10}$ decrease from baseline; Week 24 VR = HBV DNA < 2000 IU/mL (HBeAg (+));
Week 48 VR = HBV DNA < 2000 IU/mL (HBeAg (+)).
CI = confidence interval; DNA = deoxyribonucleic acid; FN = false negative; FP = false positive; HBeAg = Hepatitis B e antigen; HBV = hepatitis B virus; NC = not calculable (as there were no subjects with virologic non-responses at that visit); TN = true negative; TP = true positive; VR = Virologic Response; BR96 = Biochemical Response at Week 96.

The results demonstrated that **cobas**® HBV is useful for monitoring of viral load in subjects with chronic HBV infection at the start of and during antiviral treatment. This study demonstrated that HBV DNA concentration measurement at baseline, a decrease in HBV DNA concentration at Week 12, or HBV DNA concentrations below specific thresholds at Weeks 24 or 48 during treatment predicted response to therapy; the study identified subjects who achieved Virologic Response, Biochemical Response, or loss of HBeAg at Week 96 of therapy.

Conclusion

cobas® HBV can quantitate the level of HBV DNA to monitor and predict response to antiviral therapy. The results of this study demonstrate the clinical utility of this test for determining early on-treatment response to therapy in the management of patients with chronic HBV infection.

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		

Symbols

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

Table 39 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 For USA: Caution: 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 and importer

Table 40 Manufacturer and importer

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

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

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EC REP Roche Diagnostics GmbH
Sandhofer Str. 116
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CE 2797

IVD

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Document revision

Document Revision Information	
Doc Rev. 3.1 12/2024	Added system software version 2.0 information for **cobas**® 6800/8800 systems. Please contact your local Roche Representative if you have any questions.
Doc Rev. 4.0 03/2025	Revised to comply with IVDR, including use of EU Importer and summary of safety and performance. Removed Rx Only from front page. Updated the HxV Control kits hazard information. Updated the harmonized symbol page. Added **cobas**® 5800 specific information. Corrected typographical errors in **Table 25** and **Table 26**. Added intended use for **cobas**® HBV/HCV/HIV-1 Control Kit. Updated **cobas**® branding. Added system software version 2.0 information for **cobas**® 6800/8800 systems. P/Ns of consumables removed, detailed information on consumables are referenced in the **cobas**® 5800 and **cobas**® 6800/8800 systems User Assistance. Please contact your local Roche Representative if you have any questions.

The summary of the safety and performance report can be found using the following link:
https://ec.europa.eu/tools/eudamed