Rx Only

cobas® HBV

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

For in vitro diagnostic use

cobas® HBV	P/N: 09040820190
cobas® HBV/HCV/HIV-1 Control Kit	P/N: 09040773190
cobas® NHP Negative Control Kit	P/N: 09051554190

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Intended use

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.

The **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.

Summary and explanation of the test

Background

Hepatitis B virus (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 360 million are chronically infected carriers.[1-3] 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.[4] 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.[4] However, targeted screening programs have shown prevalence rates in excess of 15% in certain high-risk immigrant populations.[5] Patients with chronic HBV infection are at high risk of long-term complications of infection, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[6-9] Serologic markers are commonly used as diagnostic and/or prognostic indicators of acute or chronic HBV infection.[10] 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.[4]

The most common marker of HBV infection is the presence of HBsAg.[10] Although carriers may clear HBsAg and develop antibody to HBsAg, there still appears to be a risk of serious liver complications later in life.[11,12] 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.[11,12] 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.[9]

HBV DNA in EDTA plasma and serum can be quantitated by nucleic acid amplification technologies, such as PCR.[13-16] 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.[17,18]

Explanation of the test

cobas® HBV is a quantitative test performed on the **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 genotypes 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.

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**® 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**® 6800/8800 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 $\leq x \leq$ 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).[16,19,20] 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.[14,15] 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 through Table 4, Table 7 and Table 8.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product

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% (W/V) proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains subtilisin. 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, < 0.1% 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
(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% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 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 dust/fume/gas/mist/vapours/spray. 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-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H - isothiazol-3- one [EC no. 220-239- 6] (3:1)
HBV/HCV/HIV-1 High Positive Control (HBV/HCV/HIV-1 H(+)C)	< 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage), < 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. 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

Table 3 **cobas®** NHP Negative Control Kit

(NHP-NC)

Store at 2-8°C
(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/fumes/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 Polidocanol3483-12-3 (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 and handling requirements

Reagents must be stored and handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **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

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 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**® 6800/8800 Systems.

Table 6 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[a]	90 days from first usage	Max 40 runs	Max 40 hours
cobas® HBV/HCV/HIV-1 Control Kit	Date not passed[a]	Not applicable[b]	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed[a]	Not applicable[b]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed[a]	30 days from loading[c]	Not applicable	Not applicable

[a] Reagents are not expired.
[b] Single use reagents
[c] Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Additional materials required

Table 7 Materials and consumables for use on **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 Solid Waste Update	07435967001 and 07094361001 or 08030073001 and 08387281001

Instrumentation and software required

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 8 Instrumentation

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

For additional information, please refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide.

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.[21,22] Only personnel proficient in handling infectious materials and the use of **cobas**® HBV and **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 hypochlorite in distilled or deionized water (dilute household bleach 1:10) 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. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.

- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution or acids. 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 hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

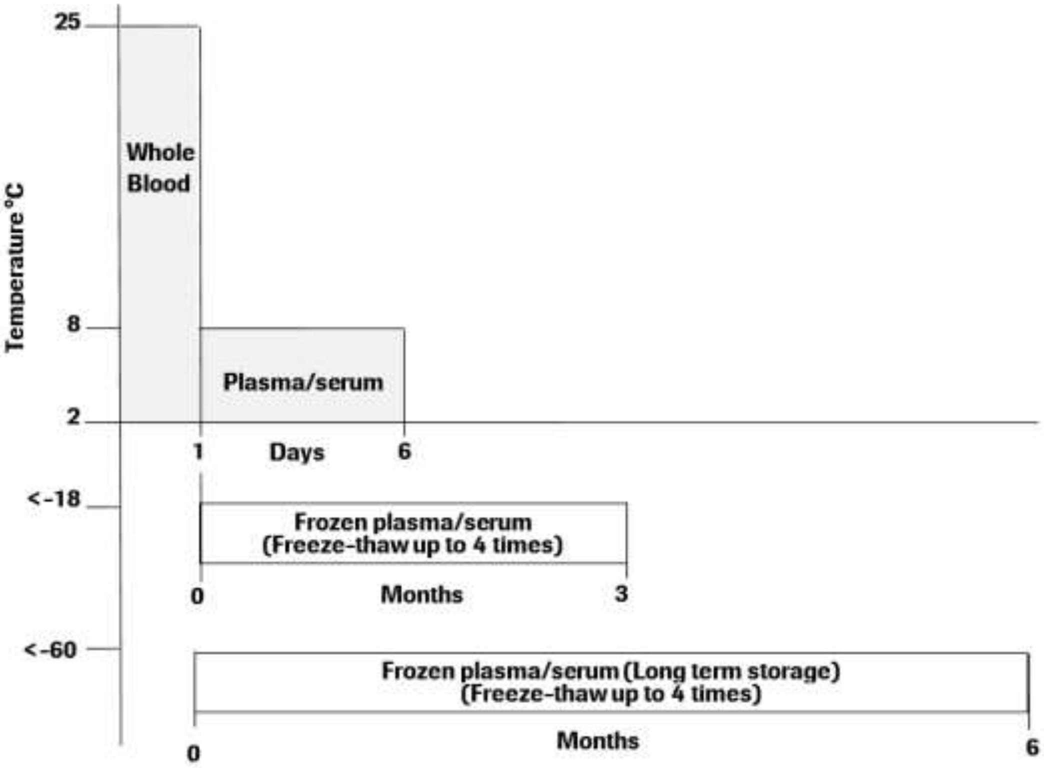
Sample collection, transport, and storage

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 Table 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.
- 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.

Figure 1 Sample storage conditions



Instructions for use

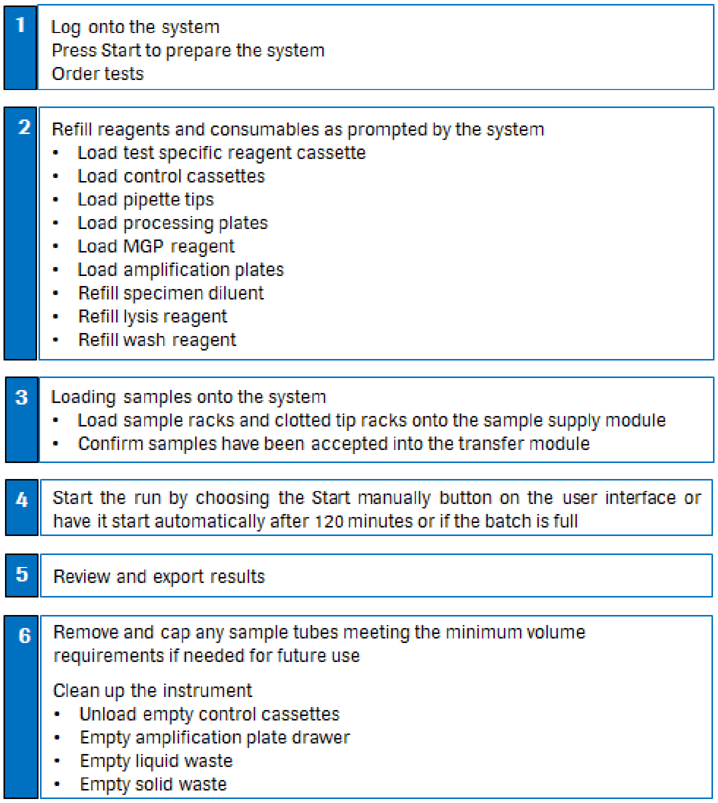
Procedural notes

- 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**® 6800/8800 Systems User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® HBV

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 2 below summarizes the procedure.

Figure 2 **cobas**® HBV test procedure



1
Log onto the system
Press Start to prepare the system
Order tests

2
Refill reagents and consumables as prompted by the system
- Load test specific reagent cassette
- Load control cassettes
- Load pipette tips
- Load processing plates
- Load MGP reagent
- Load amplification plates
- Refill specimen diluent
- Refill lysis reagent
- Refill wash reagent

3
Loading samples onto the system
- Load sample racks and clotted tip racks onto the sample supply module
- Confirm samples have been accepted into the transfer module

4
Start the run by choosing the Start manually button on the user interface or have it start automatically after 120 minutes or if the batch is full

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 cassettes
- Empty amplification plate drawer
- Empty liquid waste
- Empty solid waste

Results

The **cobas**® 6800/8800 System automatically determines 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

- 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

Table 9 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**® 6800/8800 software and/or report. The result interpretation should be as follows:

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

Table 10 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.

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**® 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.
- Drug interference studies were performed in vitro, and may not assess the potential interferences that might be seen after the drugs are metabolized in vivo.
- Due to inherent differences among technologies, it is recommended that before switching to the **cobas**® HBV, users should perform method comparison studies in their laboratory to assess 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

Limit of Detection (LoD)

The limit of detection (LoD) of **cobas**® HBV was determined for the WHO International Standard (i.e., genotype A), genotypes B through H, and the predominant precore mutant. The determined LoD with 500 µL sample processing volume is 6.6 IU/mL for EDTA plasma and 3.5 IU/mL for serum by PROBIT analysis. For the 200 µL sample processing volume, LoD was determined with the WHO International Standard to be 15.5 IU/mL for EDTA plasma and 12.5 IU/mL for serum by PROBIT analysis. The same LoD was verified at the same level for genotypes B through H and the predominant precore mutant.

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 processing volume, over three lots of **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 11. The study demonstrates that **cobas**® HBV detected HBV DNA for the WHO International Standard at a concentration of 2.7 IU/mL for LoD by PROBIT with a hit rate of 95% for the 500 µL sample processing volume and at a concentration of 15.5 IU/mL for LoD by PROBIT 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 2.4 IU/mL for LoD by PROBIT with a hit rate of 95% for the 500 µL sample processing volume and at a concentration of 12.5 IU/mL for LoD by PROBIT with a hit rate of 95% for the 200 µL sample processing volume.

Table 11 HBV DNA WHO International Standard limit of detection in EDTA plasma and serum (500 µL and 200 µL)

Matrix	**LoD by PROBIT at 95% hit rate 500 µL**	**95% confidence interval 500 µL**
Plasma	2.7 IU/mL	2.4 – 3.1 IU/mL
Serum	2.4 IU/mL	2.0 – 2.7 IU/mL
Matrix	**LoD by PROBIT at 95% hit rate 200 µL**	**95% confidence interval 200 µL**
Plasma	15.5 IU/mL	14.4 – 16.9 IU/mL
Serum	12.5 IU/mL	11.6 – 13.8 IU/mL

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). Dilution of clinical specimens or contrived plasmids were made in HBV-negative human EDTA plasma and serum for a final sample processing volume 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 12 and Table 13, respectively. The study demonstrates that **cobas**® HBV detected all HBV genotypes tested with a similar LoD as HBV genotype A.

Table 12 HBV DNA genotypes B through H and the predominant precore mutant limit of detection in EDTA plasma (500 µL)

Genotype	95% LoD by PROBIT	95% Confidence Interval
GT B	3.5 IU/mL	2.95 IU/mL - 4.32 IU/mL
GT C	4.1 IU/mL	3.32 IU/mL - 5.82 IU/mL
GT D	4.5 IU/mL	3.59 IU/mL - 6.49 IU/mL
GT E	3.2 IU/mL	2.76 IU/mL - 3.98 IU/mL
GT F	1.9 IU/mL	1.66 IU/mL - 2.24 IU/mL
GT G	2.5 IU/mL	2.17 IU/mL - 3.02 IU/mL
GT H	6.6 IU/mL	5.33 IU/mL - 8.77 IU/mL
precore mutant	2.4 IU/mL	2.08 IU/mL - 2.90 IU/mL

Table 13 HBV DNA genotypes B through H and the predominant precore mutant limit of detection in serum (500 µL)

Genotype	95% LoD by PROBIT	95% Confidence Interval
GT B	3.3 IU/mL	2.76 IU/mL - 4.30 IU/mL
GT C	3.3 IU/mL	2.83 IU/mL - 4.23 IU/mL
GT D	2.6 IU/mL	2.17 IU/mL - 3.42 IU/mL
GT E	2.7 IU/mL	2.25 IU/mL- 3.49 IU/mL
GT F	2.0 IU/mL	1.72 IU/mL - 2.45 IU/mL
GT G	2.1 IU/mL	1.75 IU/mL - 2.66 IU/mL
GT H	3.5 IU/mL	2.89 IU/mL - 4.60 IU/mL
precore mutant	1.6 IU/mL	1.43 IU/mL - 2.03 IU/mL

The limit of detection of **cobas**® HBV was verified for the 200 µL sample processing volume. 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, 21 replicates per lot. The results from EDTA plasma and serum using 200 µL are shown in Table 14. These results verify that **cobas**® HBV detected HBV DNA for seven different genotypes and the predominant precore mutant at concentrations of 12.5 IU/mL with a hit rate of ≥ 93.65% with an upper one-sided 95% confidence interval of ≥ 97.80%.

Table 14 HBV DNA genotypes B through H and the predominant precore mutant verification of limit of detection in EDTA plasma and serum (200 µL)

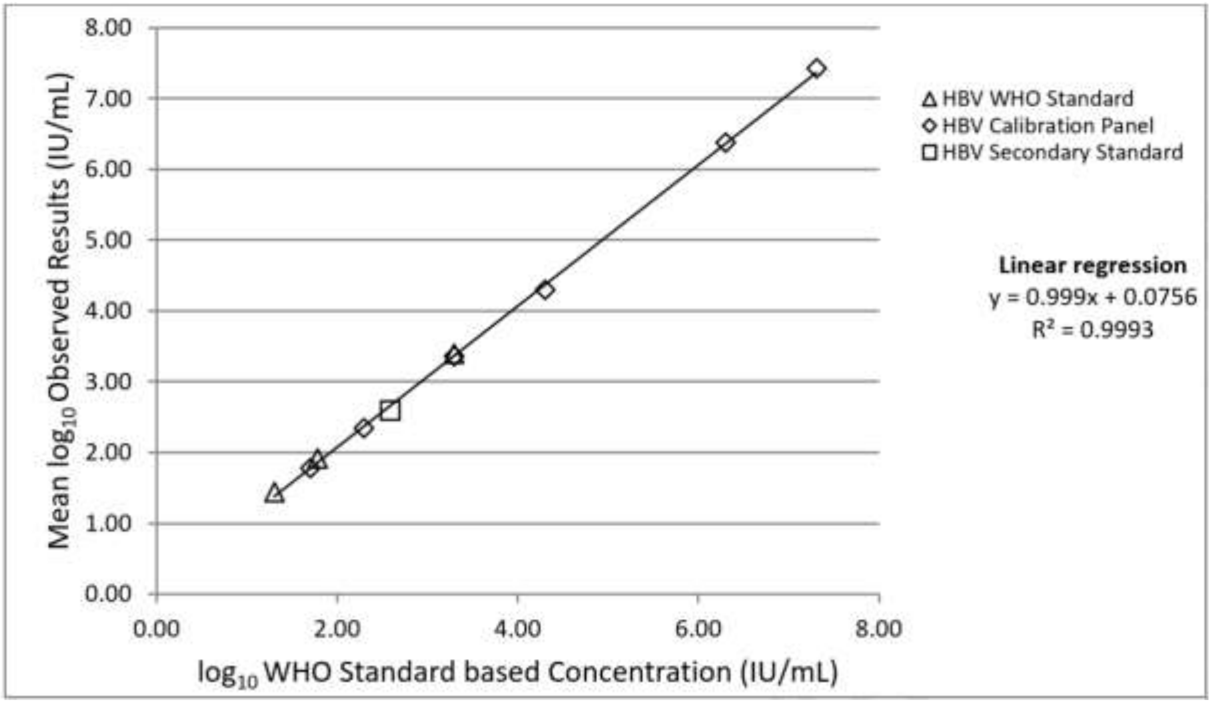
Genotype	LoD by Hit Rate
GT B	12.5 IU/mL
GT C	12.5 IU/mL
GT D	12.5 IU/mL
GT E	12.5 IU/mL
GT F	12.5 IU/mL
GT G	12.5 IU/mL
GT H	12.5 IU/mL
precore mutant	12.5 IU/mL

Traceability to the 2nd WHO International Standard for Hepatits B Virus DNA for Nucleic Acid Amplification Techniques (NAT)-based assays

Several standards and controls have been used during development of this test to provide traceability to the WHO standard [the 2nd WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Amplification Techniques (NIBSC 97/750)].[23] The standards used during development of the test include the HBV WHO Standard, the RMS HBV Secondary Standard, and the RMS HBV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the HBV WHO Standard was from 2.00E+01 IU/mL to 2.00E+03 IU/mL (1.30 – 3.30 $\log_{10}$ IU/mL), the RMS HBV Secondary Standard was tested at 3.89E+02 IU/mL (2.59 $\log_{10}$ IU/mL), and the RMS HBV Calibration Panel was tested from 5.00E+01 to 2.00E+07 IU/mL (1.70 - 7.30 $\log_{10}$ IU/mL).

All materials behaved similarly and demonstrated co-linear dilution performance across the linear range of **cobas**® HBV (Figure 3). Based on these results, the calibration and standardization process of **cobas**® HBV provides quantitation values for the calibration panel, the RMS HBV Secondary Standard, and the HBV WHO Standard that are similar to the expected values with deviation of not more than 0.14 $\log_{10}$ IU/mL. The maximum deviation was obtained around the test LLoQ using a combined regression analyses for the RMS HBV Calibration Panel the RMS HBV Secondary Standard and the HBV WHO Standard.

Figure 3 Traceability to WHO International Standard (mean observed $\log_{10}$ titer versus $\log_{10}$ WHO standard based titer) using **cobas**® HBV



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 processing volume and 25 IU/mL in 200 µL sample processing 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 sample processing 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 sample processing 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 through Figure 7 for representative results.

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

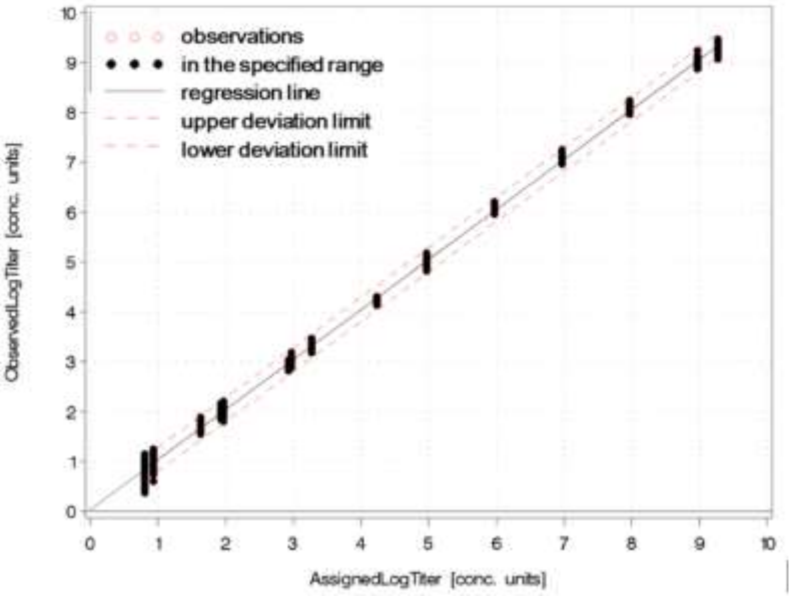


Figure 5 Linear range determination in EDTA plasma (200 µL)

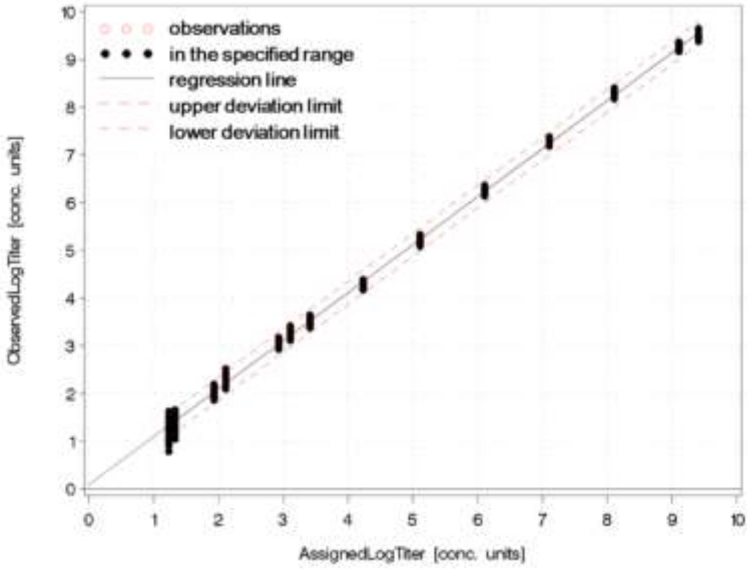


Figure 6 Linear range determination in serum (500 µL)

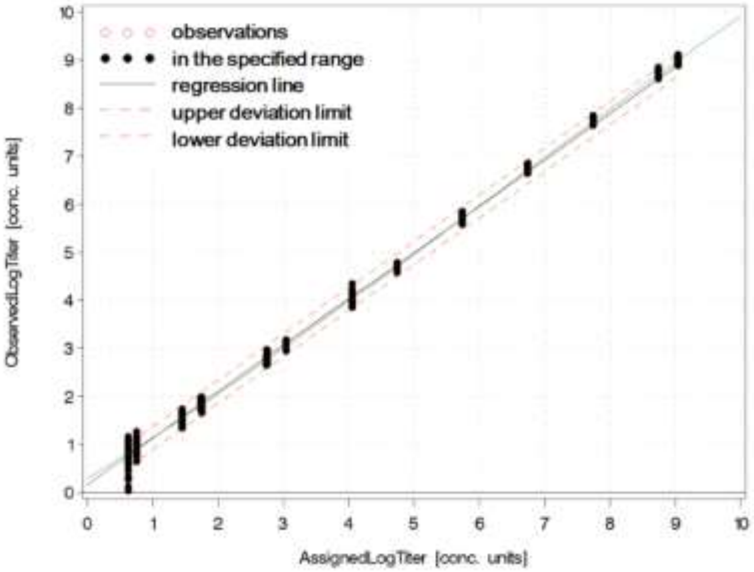
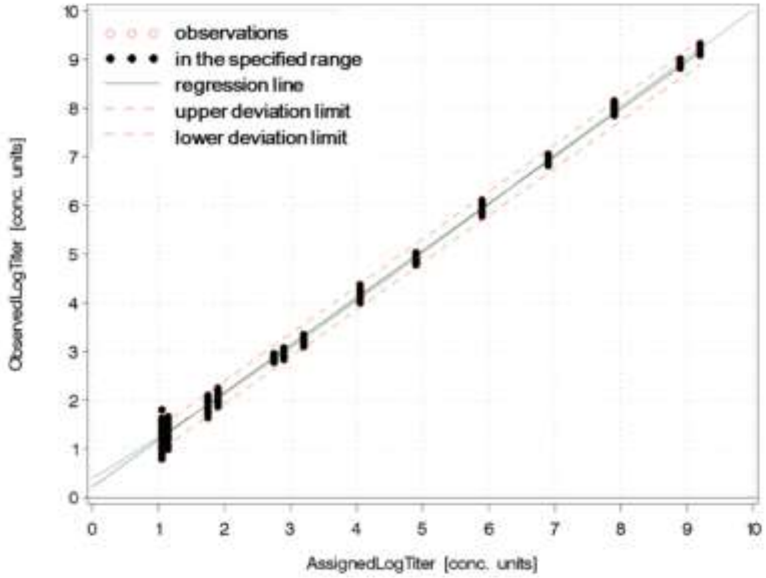


Figure 7 Linear range determination in serum (200 µL)



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 $\log_{10}$.

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 sample processing volume across three lots of **cobas**® HBV test reagents across a concentration range of 5.00E+01 IU/mL to 1.0E+09 IU/mL using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas**® HBV procedure on fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. Results are shown in Table 15 through Table 18 below.

Table 15 Within-laboratory precision of **cobas**® HBV (EDTA plasma samples – sample processing volume of 500 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Lot 1 SD	Lot 2 SD	Lot 3 SD	All lots 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 16 Within-laboratory precision of **cobas**® HBV (serum samples - sample processing volume of 500 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Lot 1 SD	Lot 2 SD	Lot 3 SD	All lots 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 17 Within-laboratory precision of **cobas**® HBV (EDTA plasma samples – sample processing volume of 200 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Lot 1 SD	Lot 2 SD	Lot 3 SD	All lots 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 18 Within-laboratory precision of **cobas**® HBV (serum samples – sample processing volume of 200 µL)*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Source material	Lot 1 SD	Lot 2 SD	Lot 3 SD	All lots 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.

Performance with HBV DNA-negative samples

The performance of **cobas**® HBV with HBV DNA-negative samples 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 results of all specimens tested with **cobas**® HBV was 100% "Target Not Detected" (with a two-sided 95% confidence interval of 99.4% – 100%).

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 (Table 19). 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 19 Microorganisms tested for cross-reactivity

Viruses	Bacteria	Yeast
Adenovirus type 5	*Propionibacterium acnes*	*Candida albicans*
Cytomegalovirus	*Staphylococcus aureus*	-
Hepatitis A Virus	-	-
Hepatitis C Virus	-	-
Hepatitis D Virus	-	-
Human Immunodeficiency Virus-1	-	-
Human T-Cell Lymphotropic Virus types 1 and 2	-	-
Human Herpes Virus type-6	-	-
Herpes Simplex Virus type-1 and 2	-	-
West Nile Virus	-	-
St. Louis encephalitis Virus	-	-
Dengue virus types 1, 2, 3, and 4	-	-
FSME virus (strain HYPR)	-	-
Yellow Fever Virus	-	-
Human Papillomavirus	-	-
Varicella-Zoster Virus	-	-
Influenza A	-	-
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 20 were tested at three 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 ± 0.14 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 20 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
Immune Modulator	Ribavirin
Immune Modulator	Peginterferon α-2b
HIV Entry Inhibitor	Maraviroc
HIV Integrase Inhibitor	Elvitegravir/Cobicistat
HIV Integrase Inhibitor	Raltegravir
Non-nucleoside HIV Reverse Transcriptase Inhibitor	Efavirenz
Non-nucleoside HIV Reverse Transcriptase Inhibitor	Etravirine
Non-nucleoside HIV Reverse Transcriptase Inhibitor	Nevirapine
Non-nucleoside HIV Reverse Transcriptase Inhibitor	Rilpivirine
HIV Protease Inhibitor	Atazanavir
HIV Protease Inhibitor	Tipranavir
HIV Protease Inhibitor	Darunavir
HIV Protease Inhibitor	Fosamprenavir
HIV Protease Inhibitor	Lopinavir
HIV Protease Inhibitor	Nelfinavir
HIV Protease Inhibitor	Ritonavir
HIV Protease Inhibitor	Saquinavir
HCV Protease Inhibitor	Boceprevir
HCV Protease Inhibitor	Simeprevir
HCV Protease Inhibitor	Telaprevir
Reverse Transcriptase or DNA Polymerase Inhibitors	Abacavir
Reverse Transcriptase or DNA Polymerase Inhibitors	Emtricitabine
Reverse Transcriptase or DNA Polymerase Inhibitors	Entecavir
Reverse Transcriptase or DNA Polymerase Inhibitors	Foscarnet
Reverse Transcriptase or DNA Polymerase Inhibitors	Cidofovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Lamivudine
Reverse Transcriptase or DNA Polymerase Inhibitors	Ganciclovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Tenofovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Adefovir dipivoxil
Reverse Transcriptase or DNA Polymerase Inhibitors	Telbivudine
Reverse Transcriptase or DNA Polymerase Inhibitors	Zidovudine
Reverse Transcriptase or DNA Polymerase Inhibitors	Aciclovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Valganciclovir
Reverse Transcriptase or DNA Polymerase Inhibitors	Sofosbuvir
Compounds for Treatment of Opportunistic Infections	Azithromycin
Compounds for Treatment of Opportunistic Infections	Clarithromycin
Compounds for Treatment of Opportunistic Infections	Ethambutol

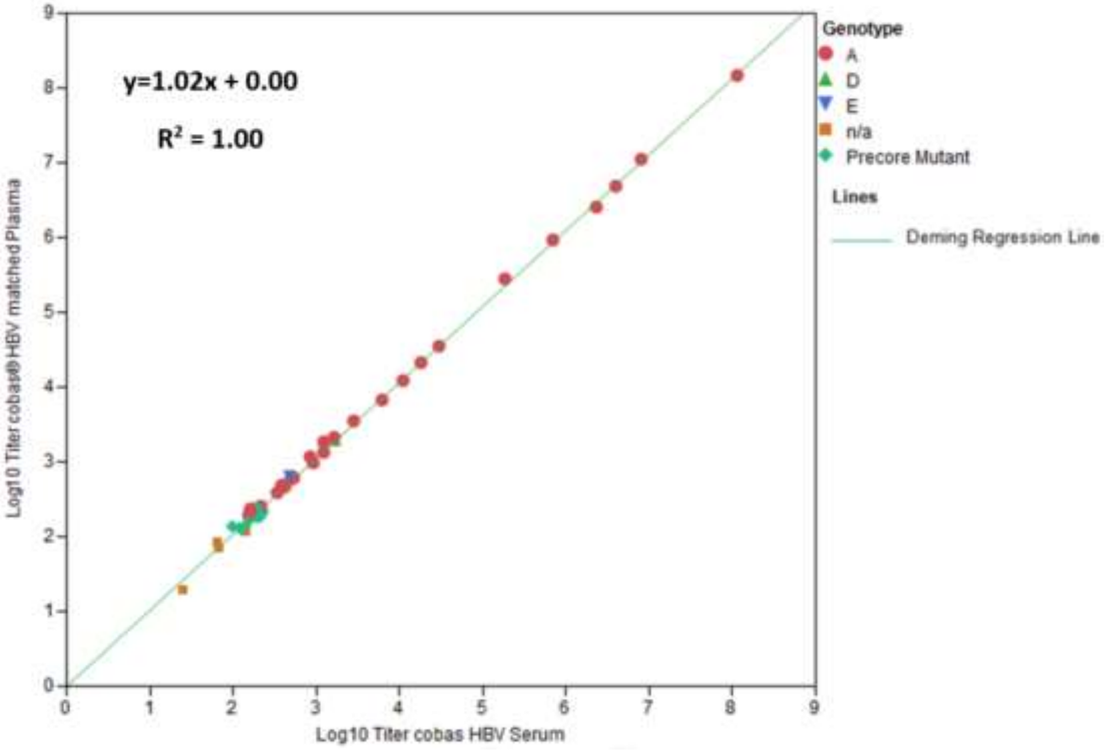
Class of drug	Generic drug name
Compounds for Treatment of Opportunistic Infections	Fluconazole
Compounds for Treatment of Opportunistic Infections	Isoniazid
Compounds for Treatment of Opportunistic Infections	Pyrazinamide
Compounds for Treatment of Opportunistic Infections	Rifabutin
Compounds for Treatment of Opportunistic Infections	Rifampicin
Compounds for Treatment of Opportunistic Infections	Sulfamethoxazole
Compounds for Treatment of Opportunistic Infections	Trimethoprim

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 8).

Figure 8 Matrix equivalency performance between EDTA plasma and serum



Cross contamination

The cross-contamination rate for **cobas**® HBV was determined by testing 240 replicates of a normal, virus-negative (HIV-1, 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 21 through Table 24 below.

Lot-to-lot variability

Lot-to-lot variability testing was performed for genotypes A and C at 1 test site, using three reagent lots. Two operators at the site tested each lot for 6 days. Two runs were performed each day.

Table 21 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected $\log_{10}$ HBV DNA concentration for the **cobas**® 6800 System.

Table 21 Attributable percentage of total variance (%TV), total precision standard deviation (SD), 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)

Geno-type	Expected HBV DNA Conc. ($\log_{10}$ IU/mL)	Observed Mean[a] HBV DNA Conc. ($\log_{10}$ IU/mL)	No. of Tests[b]	Lot %TV[c] (CV%)[d]	Oper-ator %TV[c] (CV%)[d]	Day %TV[c] (CV%)[d]	Run %TV[c] (CV%)[d]	Within-Run %TV[c] (CV%)[d]	Total Precision SD[e]	Total Precision 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
A	2.70	2.72	108	52% (11.96)	0% (0.00)	0% (0.00)	0% (0.00)	48% (11.56)	0.072	16.69
A	3.70	3.64	108	60% (14.29)	0% (0.00)	4% (3.55)	1% (1.57)	36% (11.01)	0.080	18.53
A	4.70	4.65	107	47% (13.05)	0% (0.00)	3% (3.22)	1% (2.32)	49% (13.29)	0.082	19.14
A	5.70	5.67	107	53% (13.66)	2% (2.59)	0% (0.00)	0% (0.00)	45% (12.54)	0.081	18.80
A	6.70	6.71	105	50% (11.66)	0% (0.00)	0% (0.00)	5% (3.82)	44% (10.92)	0.071	16.48
A	7.70	7.41	108	55% (13.08)	0% (0.00)	0% (0.00)	4% (3.59)	40% (11.18)	0.076	17.65
A	8.70	8.41	107	51% (12.52)	0% (0.00)	0% (0.00)	10% (5.61)	38% (10.75)	0.075	17.51
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
C	2.70	2.71	105	53% (13.92)	2% (2.63)	3% (3.48)	0% (0.00)	41% (12.27)	0.082	19.16

Geno-type	Expected HBV DNA Conc. ($\log_{10}$ IU/mL)	Observed Mean[a] HBV DNA Conc. ($\log_{10}$ IU/mL)	No. of Tests[b]	Lot %TV[c] (CV%)[d]	Oper-ator %TV[c] (CV%)[d]	Day %TV[c] (CV%)[d]	Run %TV[c] (CV%)[d]	Within-Run %TV[c] (CV%)[d]	Total Precision SD[e]	Total Precision CV%[d]
C	3.70	3.64	107	61% (11.67)	0% (0.00)	0% (0.80)	0% (0.00)	39% (9.37)	0.065	15.02
C	4.70	4.65	106	47% (11.44)	0% (0.00)	0% (0.00)	0% (0.00)	53% (12.25)	0.073	16.82
C	5.70	5.69	107	60% (14.76)	0% (0.00)	1% (1.51)	0% (0.00)	39% (11.86)	0.082	19.08
C	6.70	6.69	107	48% (11.79)	0% (0.00)	2% (2.31)	0% (0.00)	50% (12.13)	0.074	17.14
C	7.70	7.38	107	51% (11.22)	0% (0.00)	0% (0.00)	1% (1.57)	48% (10.94)	0.068	15.80
C	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] %TV = Percent contribution to Total Variance

[d] CV% = Lognormal percent coefficient of variation = sqrt(10^[SD^2 * ln(10)] - 1) * 100

[e] Calculated using the total variability from the SAS MIXED procedure.

DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; SD = standard deviation; sqrt = square root; Conc. = concentration.

In Table 22 below, the negative percent agreement (NPA) for the **cobas**® 6800 System using negative panel member tests was 100%.

Table 22 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 23 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 23 Attributable percentage of total variance (%TV), total precision standard deviation (SD), and lognormal CV(%) of HBV DNA concentration (log$_{10}$ IU/mL) by genotype and positive panel member (reproducibility)

Genotype	Expected HBV DNA Conc. (log$_{10}$ IU/mL)	Observed Mean[a] HBV DNA Conc. (log$_{10}$ IU/mL)	No. of Tests[b]	Lot %TV[c] (CV%)[d]	Operator %TV[c] (CV%)[d]	Day %TV[c] (CV%)[d]	Run %TV[c] (CV%)[d]	Within-Run %TV[c] (CV%)[d]	Total Precision SD[e]	Total Precision 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
A	2.70	2.66	108	34% (9.53)	0% (0.00)	0% (0.00)	16% (6.40)	50% (11.52)	0.070	16.33
A	3.70	3.60	108	34% (7.49)	2% (1.90)	7% (3.42)	0% (0.00)	56% (9.58)	0.055	12.80
A	4.70	4.62	107	13% (5.40)	0% (0.00)	0% (0.00)	12% (5.28)	75% (13.05)	0.065	15.12
A	5.70	5.63	107	37% (7.82)	1% (1.26)	0% (0.00)	0% (0.00)	62% (10.04)	0.055	12.81
A	6.70	6.67	106	20% (5.99)	3% (2.16)	4% (2.57)	15% (5.16)	60% (10.48)	0.059	13.59
A	7.70	7.37	108	3% (2.70)	2% (2.06)	0% (0.00)	0% (0.00)	95% (15.12)	0.067	15.50
A	8.70	8.36	107	12% (4.32)	0% (0.00)	0% (0.00)	2% (1.53)	86% (11.46)	0.053	12.36
A	7.70	7.34	106	0% (0.00)	3% (2.38)	0% (0.00)	13% (5.12)	84% (13.11)	0.062	14.30
A	8.70	8.36	107	4% (2.24)	0% (0.00)	16% (4.35)	10% (3.46)	70% (9.09)	0.047	10.91
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
C	2.70	2.67	105	19% (5.94)	3% (2.22)	0% (0.00)	0% (0.00)	79% (12.27)	0.060	13.84
C	3.70	3.61	107	14% (4.49)	0% (0.00)	7% (3.15)	0% (0.00)	78% (10.48)	0.051	11.84
C	4.70	4.62	106	24% (6.45)	0% (0.00)	0% (0.00)	0% (0.00)	76% (11.59)	0.057	13.29

Genotype	Expected HBV DNA Conc. (log$_{10}$ IU/mL)	Observed Mean[a] HBV DNA Conc. (log$_{10}$ IU/mL)	No. of Tests[b]	Lot %TV[c] (CV%)[d]	Operator %TV[c] (CV%)[d]	Day %TV[c] (CV%)[d]	Run %TV[c] (CV%)[d]	Within-Run %TV[c] (CV%)[d]	Total Precision SD[e]	Total Precision CV%[d]
C	5.70	5.65	107	18% (5.96)	0% (0.00)	3% (2.29)	0% (0.00)	80% (12.68)	0.061	14.22
C	6.70	6.65	107	23% (6.35)	6% (3.26)	0% (0.00)	1% (1.33)	70% (11.10)	0.057	13.29
C	7.70	7.34	106	0% (0.00)	3% (2.38)	0% (0.00)	13% (5.12)	84% (13.11)	0.062	14.30
C	8.70	8.36	107	4% (2.24)	0% (0.00)	16% (4.35)	10% (3.46)	70% (9.09)	0.047	10.91
C	7.70	7.34	106	0% (0.00)	3% (2.38)	0% (0.00)	13% (5.12)	84% (13.11)	0.062	14.30
C	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] %TV = Percent contribution to Total Variance

[d] CV% = Lognormal percent coefficient of variation = sqrt(10^[SD^2 * ln(10)] - 1) * 100

[e] Calculated using the total variability from the SAS MIXED procedure.

DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; SD = standard deviation; sqrt = square root; Conc. = concentration.

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 24 below.

Table 24 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 25).

Table 25 Treatment groups

Clinical Study	**HBeAg Status**	**Treatment**	**Treatment Arm**
Pharmaceutical Clinical Trial[24]	HBeAg (+)	entecavir monotherapy	Arm I
Pharmaceutical Clinical Trial[24]	HBeAg (+)	entecavir + tenofovir	Arm II
Pharmaceutical Clinical Trial[24]	HBeAg (-)	entecavir monotherapy	Arm III (includes up to 17 subjects from clinical practice)
Pharmaceutical Clinical Trial[24]	HBeAg (-)	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 26 below shows the demographic and baseline characteristics of subjects whose samples were tested on the **cobas**® 6800 System.

The demographic distribution of the subjects in this study was consistent with that of patients with chronic HBV in the US,[4,5] HBeAg (+) and HBeAg (-) subjects were enrolled in this study, and data for these populations were analyzed separately.

Table 26 Demographics and baseline characteristics of subjects

Characteristics	Unit	Statistics
Number of Subjects	Total, N	396
Age Category (years), < 40	n (%)	186 (47.0%)
Age Category (years), ≥ 40	n (%)	210 (53.0%)
Age, Mean ± SD	Years	42 ± 15.2
Age, Median	Years	42
Age, Range	Years	17 - 81
Gender, Male	n (%)	276 (69.7%)
Gender, Female	n (%)	120 (30.3%)
Race, Asian	n (%)	204 (51.5%)
Race, Black / African American	n (%)	14 (3.5%)
Race, White / Caucasian	n (%)	169 (42.7%)
Race, Other	n (%)	9 (2.3%)
Genotype, A	n (%)	64 (16.2%)
Genotype, A & G	n (%)	1 (0.3%)
Genotype, B	n (%)	62 (15.7%)
Genotype, C	n (%)	74 (18.7%)
Genotype, D	n (%)	105 (26.5%)
Genotype, E	n (%)	4 (1.0%)
Genotype, F	n (%)	10 (2.5%)
Genotype, Mixed	n (%)	1 (0.3%)
Genotype, Unknown	n (%)	75 (18.9%)
Normal ALT at Baseline, Yes	n (%)	23 (5.8%)
Normal ALT at Baseline, No	n (%)	361 (91.2%)
Normal ALT at Baseline, Unknown	n (%)	12 (3.0%)
Baseline ALT, Mean ± SD	(IU/L)	140 ± 169.9
Baseline ALT, Median	(IU/L)	96
Baseline ALT, Range	(IU/L)	14 - 1583
HBV DNA at Baseline Mean ± SD	($\log_{10}$ IU/mL)	6.6 ± 2.38
HBV DNA at Baseline, Median	($\log_{10}$ IU/mL)	7.4
HBV DNA at Baseline, Range	($\log_{10}$ IU/mL)	-0.0 - 10.1
HBV DNA Category, < 2.0 x 10^3 IU/mL	n (%)	41 (10.4%)
HBV DNA Category, 2.0 x 10^3 to 2.0 x 10^4 IU/mL	n (%)	13 (3.3%)
HBV DNA Category, > 2.0 x 10^4 IU/mL	n (%)	330 (83.3%)
HBV DNA Category, Unknown	n (%)	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 < 10^8 IU/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 27 and Table 28 below).

Table 27 Probability of achieving virologic response at Week 96 given baseline HBV DNA <10^8 IU/mL by treatment arm

On-Treatment Visit	Treatment Arm	Evaluable Subjects	PPV Estimate (95% CI)	PPV n / N	NPV Estimate (95% CI)	NPV n / N	OR 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)
Baseline	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)
Baseline	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)
Baseline	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)
Baseline	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 an 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 an 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 28 Probability of achieving virologic response at Week 96 given virologic response at a specific on-treatment visit by treatment arm

On-Treatment Visit	Treatment Arm	Eligible Subjects	PPV Estimate (95% CI)	PPV n / N	NPV Estimate (95% CI)	NPV n / N	OR Estimate (95% CI)
Week 12	Arm I	103	79.6 (70.8, 86.3)	82 / 103	NC	0	3.90 (0.08, 202.63)
Week 12	Arm II	100	97.0 (91.5, 99.0)	97 / 100	NC	0	32.33 (0.54, 1921.79)
Week 12	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)
Week 12	Arm IV	48	95.8 (86.0, 98.8)	46 / 48	NC	0	23.00 (0.36, 1485.21)
Week 12	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)
Week 24	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)
Week 24	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)
Week 24	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)
Week 24	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)
Week 48	Arm II	97	95.9 (89.9, 98.4)	93 / 97	NC	0	23.25 (0.41, 1328.83)
Week 48	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)
Week 48	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)
Week 48	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 29.

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

On-Treatment Visit	Treatment Arm	Eligible Subjects	PPV Estimate (95% CI)	PPV n / N	NPV Estimate (95% CI)	NPV n / N	OR Estimate (95% CI)
Week 12	Arm I	101	62.4 (52.6, 71.2)	63 / 101	NC	0	1.66 (0.03, 85.30)
Week 12	Arm II	100	43.0 (33.7, 52.8)	43 / 100	NC	0	0.75 (0.01, 38.79)
Week 12	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)
Week 12	Arm IV	49	32.7 (21.2, 46.6)	16 / 49	NC	0	0.48 (0.01, 25.57)
Week 12	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)
Week 24	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)
Week 24	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)
Week 24	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)
Week 24	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)
Week 48	Arm II	97	43.3 (33.9, 53.2)	42 / 97	NC	0	0.76 (0.01, 39.29)
Week 48	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)
Week 48	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)
Week 48	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 30). 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 30 Probability of HBeAg loss at Week 96 given virologic response at a specific on-treatment visit by treatment arm

On-Treatment Visit	Treatment Arm	Eligible Subjects	PPV Estimate (95% CI)	PPV n / N	NPV Estimate (95% CI)	NPV n / N	OR Estimate (95% CI)
Week 12	Arm I	102	46.1 (36.7, 55.7)	47 / 102	NC	0	0.85 (0.02, 43.91)
Week 12	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)
Week 24	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)
Week 48	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 processing volume	500 µL or 200 µL		
Analytical sensitivity		500 µL	200 µL
	EDTA plasma	6.6 IU/mL	15.5 IU/mL
	Serum	3.5 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		
Performance with HBV DNA-negative samples	100% "Target Not Detected" (with a two-sided 95% confidence interval of 99.4% – 100%)		
Genotypes detected	HBV Genotype A-H, and predominant precore mutant		

Symbols

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

Table 31 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB	Age or Date of Birth		Date of manufacture
SW	Ancillary Software	D	Distributed by
Assigned Range [copies/mL]	Assigned Range (copies/mL)	⊗	Do not re-use
Assigned Range [IU/mL]	Assigned Range (IU/mL)	♀	Female
EC REP	Authorized representative in the European Community		For IVD perfomance evaluation only
BARCODE	Barcode Data Sheet	GTIN	Global Trade Item Number
LOT	Batch code	IVD	*In vitro* diagnostic medical device
☣	Biological risks	LLR	Lower Limit of Assigned Range
REF	Catalogue number	♂	Male
Collect Date	Collect date		Manufacturer
i	Consult instructions for use	CONTROL −	Negative control
Σ	Contains sufficient for <*n*> tests	NON STERILE	Non sterile
CONTENT	Contents of kit	#	Patient number
CONTROL	Control	?	Patient Name

	Peel here
CONTROL | +	Positive control
QS copies / PCR	QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.
QS IU/PCR	QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SN	Serial number
Site	Site
Procedure | Standard	Standard Procedure
STERILE | EO	Sterilized using ethylene oxide
	Store in the dark
	Temperature limit
	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
	This way up
UDI	Unique Device Identification
Procedure | UltraSensitive	Ultrasensitive Procedure
ULR	Upper Limit of Assigned Range
Urine Fill Line	Urine Fill Line
Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.
	Use-by date
	Device for near-patient testing
	Device Not for Near Patient Testing
	Device for self-testing
	Device not for self-testing

Technical support

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

Manufacturer and distributors

Table 32 Manufacturer and distributors

🏭	Roche Molecular Systems, Inc. 1080 US Highway 202 South Branchburg, NJ 08876 USA www.roche.com Made in USA	
D	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457 USA (For Technical Assistance call the Roche Response Center toll-free: 1-800-526-1247)	Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany

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All other product names and trademarks are the property of their respective owners.

Carryover prevention technology in the AmpErase enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

See http://www.roche-diagnostics.us/patents

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Document revision

Document Revision Information	
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