cobas® HBV

Quantitative nucleic acid test
for use on the cobas® 4800 System

For in vitro diagnostic use

cobas® HBV	120 Tests	P/N: 06979564190
cobas® HBV/HCV/HIV-1 Control Kit	10 Sets	P/N: 06979572190
cobas® 4800 System Sample Preparation Kit 2	240 Tests 960 Tests	P/N: 06979513190 P/N: 06979521190
cobas® 4800 System Wash Buffer Kit	240 Tests 960 Tests	P/N: 05235863190 P/N: 05235871190
cobas® 4800 System Specimen Diluent 2	240 Tests	P/N: 06979556190
cobas® 4800 System Lysis Kit 2	240 Tests 960 Tests	P/N: 06979530190 P/N: 06979548190

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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 using the automated **cobas®** 4800 System for specimen processing, amplification and detection.

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 and negative run control on the **cobas®** 4800 System with the **cobas®** HBV, **cobas®** HCV, and **cobas®** HIV-1 tests.

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 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 nucleic acid test performed on the **cobas®** 4800 System. **cobas®** HBV enables the detection and quantitation of HBV DNA in EDTA plasma or serum of infected patients. Probes are used to detect and quantify, but not discriminate HBV genotypes A, B, C, D, E, F, G and H and the most predominant precore mutant. The viral load is quantified against a lambda phage DNA quantitation standard (DNA QS), which is introduced into each sample during sample preparation. The DNA QS also functions as an internal control to monitor 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®** 4800 System consists of the **cobas®** x 480 instrument and the **cobas®** z 480 analyzer. Automated data management is performed by the **cobas®** 4800 software which assigns test results for all tests as target not detected, < LLoQ (lower limit of quantitation), > ULoQ (upper limit of quantitation) or HBV DNA detected, a value in the linear range LLoQ ≤ x ≤ ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acids from patient samples, external controls and added lambda phage DNA QS molecules are simultaneously extracted. In summary, viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acids bind to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors are removed with subsequent wash buffer steps and purified nucleic acids are eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acids from the patient 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 is used for PCR amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[14,17,18] Any contaminating amplicons from previous PCR runs are inactivated as PCR templates by AmpErase, which is present in the master mix, prior to the first denaturation step of PCR. AmpErase catalyzes the removal of uracil from DNA, but has no activity on naturally occurring DNA, which does not contain uracil. Amplicon formed during subsequent cycles of PCR are not inactivated since AmpErase is inactive at the annealing and denaturation temperatures of PCR.

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 act 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 the 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 is possible.

Materials and reagents

Reagents

All unopened reagents and controls shall be stored as recommended in the **Reagent storage and handling requirements** table.

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HBV 120 Tests (P/N: 06979564190)	**MMX R1** (**cobas**® Master Mix Reagent 1) Manganese acetate, potassium hydroxide, < 0.1% sodium azide	10 × 1.75 mL	N/A
	HBV MMX R2 (**cobas**® HBV Master Mix Reagent 2) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTP, < 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 Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	10 × 0.5 mL	N/A
	DNA QS (**cobas**® HBV DNA Quantitation Standard) Tris buffer, < 0.05% EDTA, < 0.001% non-HBV construct containing non-HBV primer binding and a unique probe region (non-infectious DNA), 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	10 × 1.75 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® HBV/HCV/HIV-1 Control Kit 10 Sets (P/N: 06979572190)	**HBV/HCV/HIV-1 L(+)C** (**cobas**® HBV/HCV/HIV-1 Low Positive Control) < 0.001% 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 HIV 1/2, antibody to HCV, 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[b]	10 × 0.75 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)
	HBV/HCV/HIV-1 H(+)C (**cobas**® HBV/HCV/HIV-1 High Positive Control) < 0.001% 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 HIV 1/2, antibody to HCV, 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[b]	10 × 0.75 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)

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
	(–) C (**cobas**® Negative Control) Normal human plasma, non-reactive by licensed tests for antibody to HIV 1/2, antibody to HCV, 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[b]	10 × 0.75 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)

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Sample Preparation Kit 2 240 Tests (P/N: 06979513190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 × 8 mL	N/A
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 × 17 mL	N/A
cobas® 4800 System Sample Preparation Kit 2 960 Tests (P/N: 06979521190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 × 16 mL	N/A
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 × 17 mL	N/A
cobas® 4800 System Wash Buffer Kit 240 Tests (P/N: 05235863190)	**WB** Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 × 55 mL	N/A
cobas® 4800 System Wash Buffer Kit 960 Tests (P/N: 05235871190)	**WB** Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 × 200 mL	N/A
cobas® 4800 System Specimen Diluent 2 240 Tests (P/N: 06979556190)	**SD 2** Tris buffer, 0.1% methyl-4 hydroxybenzoate, <0.1% sodium azide	10 × 8 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 240 Tests (P/N: 06979530190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase[b]	10 x 1.0 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing mist or vapours. P280: Wear protective gloves. P284: Wear respiratory protection. P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/ doctor. 39450-01-6 Proteinase, Tritirachium album serine

	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate	10 x 27 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 960 Tests (P/N: 06979548190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, <0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase[b]	10 x 1.0 mL	 **DANGER** H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261: Avoid breathing mist or vapours. P280: Wear protective gloves. P284: Wear respiratory protection. P304 + P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/ doctor. 39450-01-6 Proteinase, Tritirachium album serine

	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate	10 x 84 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

[a] Product safety labeling primarily follows EU GHS guidance

[b] Hazardous substance

Reagent storage and handling requirements

Reagent	Storage Temperature	Storage Time
cobas® HBV	2–8°C	Stable until the expiration date indicated
cobas® HBV/HCV/HIV-1 Control Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Sample Preparation Kit 2	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Wash Buffer Kit	15–25°C	Stable until the expiration date indicated
cobas® 4800 System Specimen Diluent 2	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Lysis Kit 2	2–8°C	Stable until the expiration date indicated

Do not freeze reagents.

Additional materials required

Materials	P/N
cobas® 4800 System Extraction (deepwell) Plate 2.0 mL	06884008001
cobas® 4800 System AD (microwell) Plate 0.3 mL	05232724001
Sealing foil applicator	04900383001
CORE Tips, 1000 µL, rack of 96	04639642001
200 mL Reagent Reservoir	05232759001
50 mL Reagent Reservoir	05232732001
24-position carrier	04639502001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
Lab gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.
Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500	Any appropriate centrifuge is acceptable.

For more information regarding the materials sold separately, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas**® x 480 instrument **cobas**® z 480 analyzer Control Unit
cobas® 4800 System Application Software (Core) Version 2.2.0 or higher
cobas® 4800 System **cobas**® HBV AP v1.2.0 or higher

Note: Contact your local Roche representative for a detailed order list for sample racks, tip racks, reagent racks and plate carriers accepted on the instruments.

Supported sample tubes

The test accepts commonly used primary and secondary tubes.

The following sample tubes are supported:

Primary tubes

Nominal Diameter (mm)	Sample input volume – processed (centrifuged) whole blood		Tube Additive	
	400 µL processing volume	200 µL processing volume	EDTA Plasma	Serum
11-14	1800 µL or more	1000 µL or more	With or without gel	With gel
14.5-16	More than 4000 µL	More than 4000 µL	With or without gel	With gel

For specific sample tube order information, and minimum sample input volumes for specific primary tubes, contact your local Roche representative.

Secondary tubes

Nominal Diameter (mm)	Sample input volume	
	400 µL processing volume	200 µL processing volume
11-16	1000 µL or more (specific secondary tubes have a minimum input volume of less than 1000 µL)	750 µL or more (specific secondary tubes have a minimum input volume of less than 750 µL)

For specific sample tube order information, and minimum sample input volumes for specific secondary tubes, contact your local Roche representative.

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 analytical sensitivity of this test, care should be taken to keep reagents, samples and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- **cobas**® HBV is only intended for quantitation of HBV viral load and is not intended for initial clinical diagnosis of HBV infection.
- **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.
- 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 biohazardous materials and the use of **cobas**® HBV and the **cobas**® 4800 System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions.
- **cobas**® HBV/HCV/HIV-1 Control Kit contains plasma derived from human blood. The source material has been tested by a licensed antibody test and found to be non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg and antibody to HBc. Testing by PCR methods showed no detectable HIV-1 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.
- Prevent exposure of MGP to sources of magnetic field.
- **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.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas**® x 480 instrument or **cobas**® z 480 analyzer, consult the **cobas**® 4800 System - User Assistance. If contamination is suspected, perform cleaning and weekly maintenance as described in the **cobas**® 4800 System - User Assistance.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

Note: **For specific instructions, see "Sample collection, transport and storage".**

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling sample and kit reagents, and after removing the lab gloves.
- Wear eye protection, lab coats and lab gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.

- 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.
- Maintain a consistent temperature in the laboratory that conforms to the environmental specifications of the system, as provided in the **cobas**® 4800 System - User Assistance.

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 bottle and vial to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® 4800 Lysis Buffer 2 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, **cobas**® 4800 Sample Preparation Kit 2 and **cobas**® 4800 System Specimen Diluent 2 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**® 4800 Lysis Buffer 2, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

Contamination

- Lab gloves must be worn and must be changed between handling samples and **cobas**® HBV reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls. Wear lab gloves, lab coats, and eye protection when handling samples and kit reagents.
- Avoid microbial and ribonuclease contamination of reagents.
- False positive results may occur if carryover of samples is not prevented during sample handling.

Integrity

- Do not use kits after their expiry dates.
- Do not pool reagents.
- Do not use disposable items after their expiry dates.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **cobas**® HBV, **cobas**® 4800 System Sample Preparation Kit 2 and **cobas**® 4800 System Specimen Diluent 2 contain sodium azide (see "**Warnings and precautions**"). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of solutions containing sodium azide down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Note: For disposal of liquid waste, refer to the appropriate cobas® 4800 System - User Assistance.

Spillage and cleaning

- **cobas**® 4800 Lysis Buffer 2 contains guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® x 480 instrument, follow the instructions in the **cobas**® 4800 System - User Assistance to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas**® x 480 instrument or the **cobas**® z 480 analyzer. Clean the **cobas**® x 480 instrument or the **cobas**® z 480 analyzer according to procedures described in the **cobas**® 4800 System - User Assistance.

Sample collection, transport, and storage

Note: Handle all samples 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 (15-30°C) 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.

Sample collection

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.

Note: The user must follow the guidance provided by the tube manufacturer for serum/plasma preparation.

Sample transport storage and stability

- 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 and subsequent testing.
- Upon separation, plasma/serum samples may be stored in secondary tubes for up to 24 hours at 2°C to 30°C, up to 72 hours at 2°C to 8°C or up to 6 weeks at ≤ -18°C. Separated plasma/serum samples in secondary tubes are stable for up to three freeze/thaw cycles when stored 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.

Instructions for use

Running the test

Sample processing volume

The default sample processing volume for **cobas**® HBV is 400 µL. For low volume samples, a sample processing volume of 200 µL may be chosen. Only in this case, **cobas**® 4800 System Specimen Diluent 2 as an additional reagent has to be loaded onto the system. The user will be prompted to do so by the software wizard, if the sample type "Diluted serum or plasma" was chosen during the work order creation.

Figure 1: cobas® HBV workflow

1	Start the system
2	Perform instrument maintenance
3	Remove samples and reagents from storage
4	Start run
5	Scan parameter cards
6	Load samples
7	With LIS: confirm work order Without LIS: create work order
8	Load consumables (deepwell plate, microwell plate, tip racks)
9	Load reagents
10	Start sample preparation run
11	Unload and seal microwell plate
12	Load microwell plate into analyzer
13	Remove samples, used reagents, and deepwell plate
14	Review results
15	With LIS: send results to LIS
16	Unload analyzer

Note: Refer to the cobas® 4800 System - User Assistance for detailed operating instructions.

Run Size

The generic sample preparation reagents (**cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2 and **cobas**® 4800 System Wash Buffer Kit) are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and samples to be run. **cobas**® HBV is available in a single kit size sufficient to test up to 120 (10×12) samples, including controls and samples. The **cobas**® HBV/HCV/HIV-1 Control Kit is available in a single kit size and can support all run configurations. For each test batch, one HBV/HCV/HIV-1 Low Positive Control, one HBV/HCV/HIV-1 High Positive Control and one Negative Control must be used. For a single test run, the maximum number of samples allowed is 93 samples and 3 controls.

Figure 1 summarizes the procedure.

Note: ***For optimal use of reagents, the generic sample preparation reagents can be used for a run containing 1-21 total samples (10×24 test kit size) or 1-93 total samples (10×96 test kit size). However, different kit sizes of the cobas® 4800 System Wash Buffer Kit, cobas® 4800 System Sample Preparation Kit 2 and cobas® 4800 System Lysis Kit 2 cannot be combined. For example, if a 96-test Wash Buffer reagent bottle is scanned at the start of the run, 96-test size reagents from the other sample preparation reagent kit must also be used.***

Workflow

cobas® HBV is performed using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas**® x 480 instrument followed by amplification/detection on the **cobas**® z 480 analyzer. **cobas**® HBV cannot be run in mixed-batch mode with other tests. Refer to the **cobas**® 4800 System - User Assistance for details.

1. Perform the system startup by following the instructions in the **cobas**® 4800 System - User Assistance.
2. Perform maintenance actions by following the instructions in the **cobas**® 4800 System - User Assistance.
3. Collect all reagents and consumables needed. All reagents except HBV MMX R2 and MMX R1 must be at ambient temperature prior to loading on the **cobas**® x 480 instrument. The HBV MMX R2 and MMX R1 reagents may be taken directly from 2-8°C storage as they equilibrate to ambient temperature on board the **cobas**® x 480 instrument by the time they are used in the process.

Note: ***All reagents and reagent reservoirs are barcoded and designed for one time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs.***

4. Start a new run and select the workflow type as HBV.
5. Follow the software wizard guide and scan the barcode on the control ranges and calibration coefficients parameter cards.

Note: ***Scan parameter cards from unexpired reagents. The software does not check reagent expiry dates in parameter cards. Check the expiry date printed in the parameter card or in the reagent kits before scanning the corresponding barcode ID.***

6. Load the samples. Primary or secondary sample tubes can be loaded and minimum sample volume depends on the tube type and size. Refer to the supported sample tubes section for more details.
7. Create the work order. There are three ways to create a work order:
   - By using the sample editor before the sample rack is loaded into the **cobas**® x 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary. When selecting the requested results, select "HBV".
   - By following the software wizard for the new run and loading samples into **cobas**® x 480 instrument when prompted. The sample barcodes will be automatically scanned, and the requested results for each sample must be defined. When selecting the requested results, select "HBV".
   - By using your institution's LIS system.

   Refer to the **cobas**® 4800 System - User Assistance for more details. When selecting the requested results, check "HBV". Load samples and define/select workorder or use LIS as appropriate.
8. Load the consumables as instructed by the software wizard. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.

9. Load the reagents.

   Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:

   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir
   - Place the filled reagent reservoir into the designated position on the reagent carrier

Note: The cobas® 4800 System has an internal clock to monitor the length of time the reagents are on-board. Once LYS 2 or WB is scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the Workplace Tab. The system will not allow the run to start if the on-board timer has expired.

Note: To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial immediately prior to dispensing into the reagent reservoir.

10. Load amplification/detection reagent vials (HBV MMX R2, MMX R1 and DNA QS), control vials [HBV/HCV/HIV-1 L(+)C, HBV/HCV/HIV-1 H(+)C and (–) C] and generic reagent vials (P2 and SD2 as required) directly onto the reagent carrier.

Note: In order to prevent unnecessary run aborts and contamination, it is required to flick down the reagent vials to avoid formation of bubbles/liquid films. Controls should be opened starting with the ones closest to you (from position 24 to 1). Change lab gloves after handling positive controls.

11. Start the sample preparation run. After a successful sample preparation run, the "Sample Preparation results" button and the Unload button become available. If desired, select "Sample Preparation results" button to review the results then select "Unload" to unload the plate carriers. Alternatively, select "Unload" to unload the plate carrier without reviewing the results. See the **cobas**® 4800 System - User Assistance.
12. After unloading the microwell plate, follow the instructions in the **cobas**® 4800 System - User Assistance for sealing and transferring the plate to the **cobas**® z 480 analyzer.
13. Load the microwell plate into the analyzer and start the amplification and detection run as instructed in the **cobas**® 4800 System - User Assistance.

Note: The cobas® 4800 System has an internal clock to monitor the length of time after addition of the prepared samples to activated master mix. Amplification and detection should be started as soon as possible but no later than 40 minutes after the end of the cobas® x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system will abort the run if the timer has expired.

14. Remove samples, used reagents, and deepwell plate as instructed in the **cobas**® 4800 System - User Assistance.
15. After the amplification and detection run is completed, follow the instructions in the **cobas**® 4800 System - User Assistance to review and accept results.
16. If working with LIS, send results to the LIS.
17. Follow the instructions in the **cobas**® 4800 System - User Assistance to unload the microwell plate from the **cobas**® z 480 analyzer.

Results

The **cobas**® 4800 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/HCV/HIV-1 L(+)C and a high positive control HBV/HCV/HIV-1 H(+)C, are processed with each batch.
- In the **cobas**® 4800 Software and/or report, check for batch validity.
- Invalidation of results is performed automatically by the **cobas**® 4800 Software based on negative and positive control failures.

Control result interpretation

Table 1: Control result interpretation for negative and positive controls

Negative Control	**Result**	**Interpretation**
(–) C	Target Not Detected	Control is valid. HBV DNA not detected.
	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Result**	**Interpretation**
HBV/HCV/HIV-1 L(+)C	Titer	Control is valid. Calculated titer is within the control range.
	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
HBV/HCV/HIV-1 H(+)C	Titer	Control is valid. Calculated titer is within the control range.
	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

Interpretation of results

Note: All assay and batch validation is determined by the cobas® 4800 Software.

Note: A valid batch may include both valid and invalid sample results.

For a valid batch, sample results are interpreted as shown in Table 2.

Table 2: Target results for individual target result interpretation

cobas® HBV	Result Report and 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 = 1.00E+01 IU/mL (400 µL and 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 (400 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.

List of result flags

The following table lists all flags which are relevant for result interpretation.

Table 3: List of flags

Flag code	Description	Recommended action
R4800	The target is invalid due to calculation failure.	The target is invalid due to calculation failure. 1. Rerun the sample. 2. If the problem persists, contact Roche Service.
R4801	The quantitation standard is invalid.	The quantitation standard is invalid for a sample. 1. Rerun the sample. 2. If the problem persists, contact Roche Service.
R4802	An external control is invalid.	An external control is invalid.[a] 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R4803	The quantitation standard is invalid.	The quantitation standard is invalid for an external control. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R4804	The external control is out of range.	The external control is out of range.[b] 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service .
X3	Error: Clot was detected Sample was not processed.	Make sure that the samples were handled according to the Instructions for Use. 1. Check the sample for clots. 2. Rerun the sample.
X4	Error: Pipetting error occurred. Sample was not processed.	Insufficient sample volume or mechanical error during pipetting is the most likely reason. 1. Make sure that there is enough sample volume. 2. Check whether the tip eject plate is placed correctly. 3. Rerun the sample.

[a] This is a sample flag and it occurs when an external control in the run is called invalid.

[b] This flag includes all scenarios in which the external control is invalid (target calling or titer).

Note: For all system flags refer to the cobas® 4800 System – User Assistance.

Procedural limitations

1. **cobas**® HBV has been evaluated only for use in combination with the **cobas**® HBV/HCV/HIV-1 Control Kit, **cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2, **cobas**® 4800 System Wash Buffer Kit and **cobas**® 4800 System Specimen Diluent 2.
2. Reliable results are dependent on adequate sample collection, transport, storage and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert) and the **cobas**® 4800 System - User Assistance.
3. This test has been validated only for use with EDTA plasma and serum. Testing of other sample types may result in inaccurate results.
4. 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.
5. 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.
6. The predictive value of an assay depends on the prevalence of the disease in any particular population.
7. The addition of AmpErase enzyme into the **cobas**® HBV Master Mix enables selective amplification of target nucleic acid; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
8. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
9. Only the **cobas**® x 480 instrument and **cobas**® z 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
10. 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.
11. Cross-contamination can cause false positive results. The sample to sample cross-contamination rate of **cobas**® HBV has been determined in a non-clinical study to be 0.0% with a one-sided 95% confidence interval of 1.3%. Run to run cross-contamination has not been observed.
12. **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)

WHO International Standard

The limit of detection of **cobas**® HBV was determined by analysis of serial dilutions of the WHO International Standard for HBV DNA for Nucleic Acid Amplification Technology Assays (2nd WHO International Standard, NIBSC code 97/750) genotype A obtained from NIBSC, in HBV-negative EDTA plasma or serum using sample processing volumes of 400 µL and 200 µL. Panels of six concentration levels plus a negative sample were tested over three lots of the **cobas**® HBV reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum from both sample processing volumes are shown in Table 4 to Table 7. The study demonstrates that **cobas**® HBV detected HBV DNA at a concentration of 4.4 IU/mL in EDTA plasma and 2.8 IU/mL in serum with a hit rate of ≥ 95% by PROBIT for the 400 µL sample processing volume and at a concentration of 7.6 IU/mL in EDTA plasma and 5.5 IU/mL in serum with a hit rate of ≥ 95% by PROBIT for the 200 µL sample processing volume.

Table 4: Limit of detection in EDTA plasma (400 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate
25.0	126	126	100.0%
15.0	126	126	100.0%
10.0	126	126	100.0%
5.0	126	122	96.8%
2.0	125	94	75.2%
0.5	126	38	30.2%
0.0	36	0	0.0%
LoD by PROBIT at 95% hit rate	4.4 IU/mL 95% confidence range: 3.6 – 5.7 IU/mL		

Table 5: Limit of detection in serum (400 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate
25.0	125	125	100.0%
15.0	126	126	100.0%
10.0	126	126	100.0%
5.0	126	126	100.0%
2.0	126	109	86.5%
0.5	126	54	42.9%
0.0	36	0	0.0%
LoD by PROBIT at 95% hit rate	2.8 IU/mL 95% confidence range: 2.3 - 3.8 IU/mL		

Table 6: Limit of detection in EDTA plasma (200 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate
25.0	126	126	100.0%
15.0	126	125	99.2%
10.0	126	125	99.2%
5.0	126	109	86.5%
2.0	126	71	56.4%
0.5	126	18	14.3%
0.0	36	0	0.0%
LoD by PROBIT at 95% hit rate	7.6 IU/mL 95% confidence range: 6.3–9.6 IU/mL		

Table 7: Limit of detection in serum (200 µL)

Input titer concentration (HBV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate
25.0	126	126	100.0%
15.0	126	126	100.0%
10.0	126	126	100.0%
5.0	126	115	91.3%
2.0	126	90	71.4%
0.5	126	26	20.6%
0.0	36	0	0.0%
LoD by PROBIT at 95% hit rate	5.5 IU/mL 95% confidence range: 4.5 – 7.0 IU/mL		

Linear range

Linearity of **cobas**® HBV was determined by analysis with a dilution series consisting of ≥ 14 panel members with the predominant HBV genotype (GT A) spanning the assay linear range. High titer panel members were prepared from a high titer lambda DNA stock whereas the lower titer panel members were prepared from a high titer clinical sample (CS). The linearity panel was designed to have an approximately 2 $\log_{10}$ titer overlap between the two material sources.

With 400 µL sample processing volume, **cobas**® HBV is linear for EDTA plasma and serum from 10.0 IU/mL to 1.0E+09 IU/mL and shows a maximum deviation from the better fitting non-linear regression of less or equal than ±0.06 $\log_{10}$. Across the linear range, the accuracy of the test was within ±0.08 $\log_{10}$ for EDTA plasma and within ±0.12 $\log_{10}$ for serum.

With 200 µL sample processing volume, **cobas**® HBV is linear for EDTA plasma and serum from 10.0 IU/mL to 1.0E+09 IU/mL and shows a maximum deviation from the better fitting non-linear regression of less or equal than ±0.05 $\log_{10}$. Across the linear range, the accuracy of the test was within ±0.08 $\log_{10}$ for EDTA plasma and within ±0.16 $\log_{10}$ for serum.

See Figure 2 to Figure 5 for representative results.

Figure 2: Linearity in EDTA plasma (400 µL)

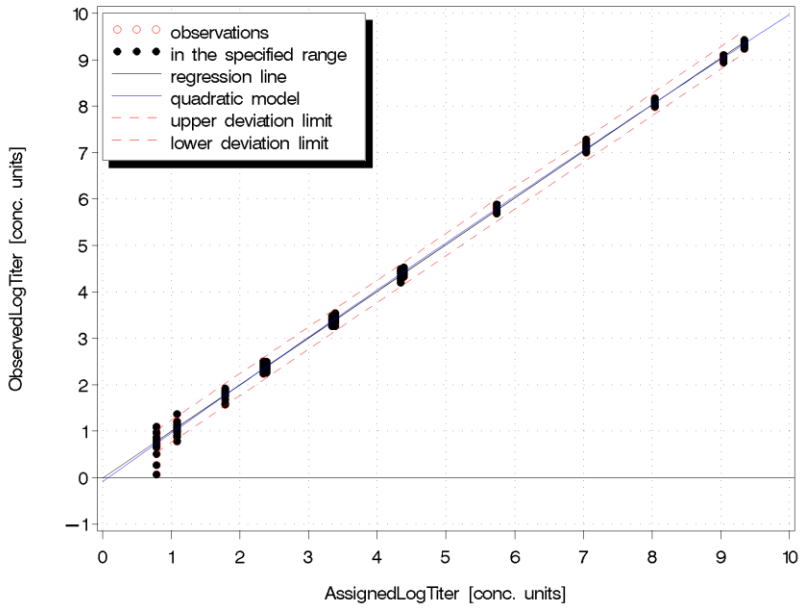


Figure 3: Linearity in serum (400 µL)

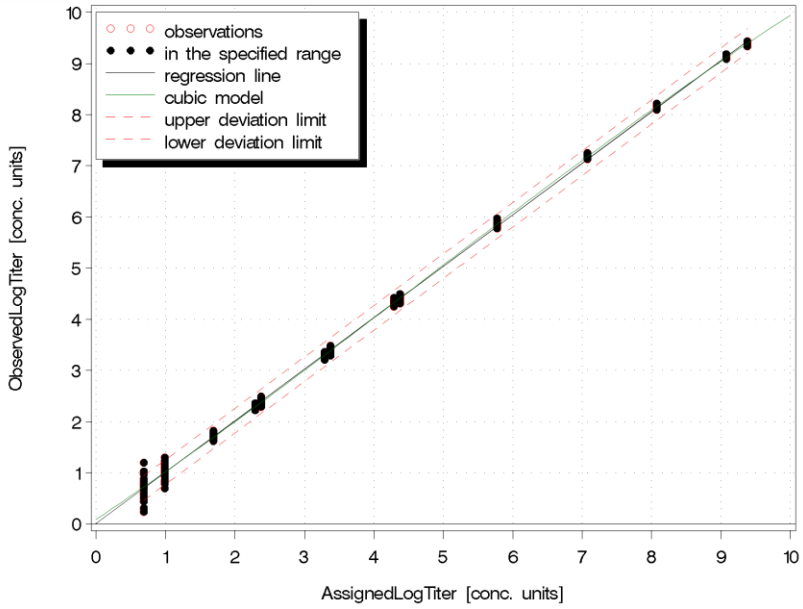


Figure 4: Linearity in EDTA plasma (200 µL)

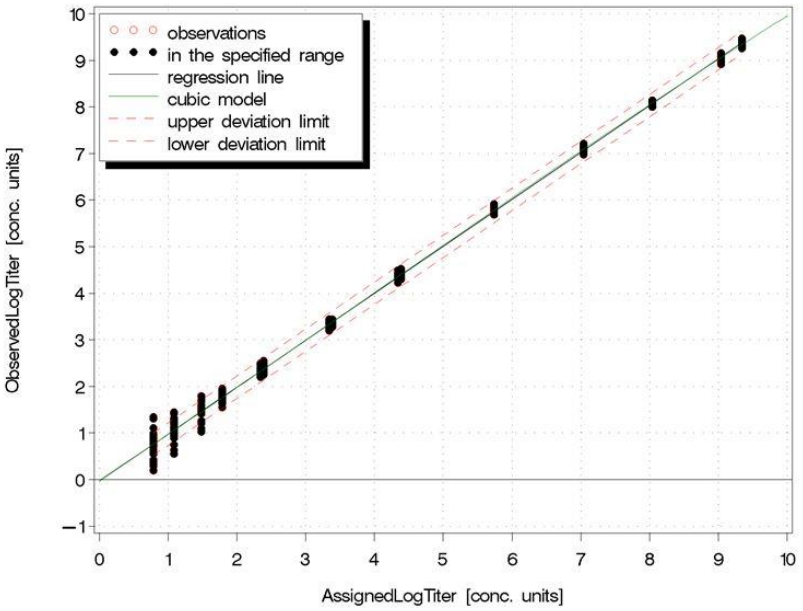
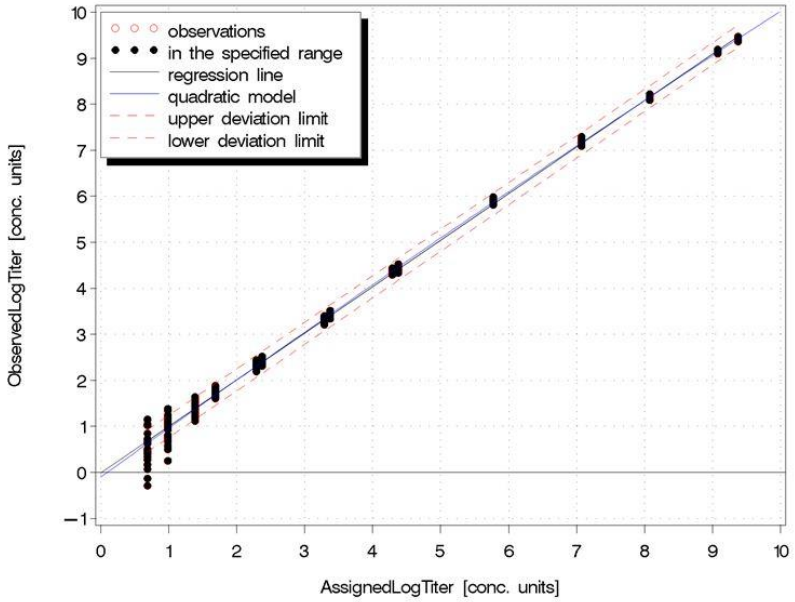


Figure 5: Linearity in serum (200 µL)



Precision – within laboratory

Precision of **cobas®** HBV was determined by analysis of serial dilutions of clinical HBV genotype (GT A) samples (CS) and of high titer lambda DNA stock HBV (lambda DNA) in HBV-negative EDTA plasma and serum. Seven dilution levels were tested in 72 replicates for each level, matrix and sample processing volume across three lots of **cobas®** HBV reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas®** HBV procedure on the **cobas®** 4800 System. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 8 to Table 11.

cobas® HBV showed high precision for three lots of reagents tested across a concentration range of 2.5E+01 IU/mL to 1.0E+09 IU/mL with both 200 µL and 400 µL sample processing volumes.

Table 8: Within-laboratory precision of cobas® HBV (EDTA plasma samples – sample processing volume of 400 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
1.0E+09	1.10E+09	Lambda DNA	0.07	0.08	0.05	0.07
1.0E+07	1.10E+07	Lambda DNA	0.05	0.05	0.05	0.05
5.0E+05	5.49E+05	Lambda DNA	0.06	0.04	0.05	0.05
2.0E+04	2.44E+04	CS	0.06	0.05	0.05	0.05
2.0E+03	2.44E+03	CS	0.08	0.06	0.06	0.07
2.0E+02	2.44E+02	CS	0.06	0.07	0.06	0.06
2.5E+01	3.05E+01	CS	0.14	0.13	0.09	0.12

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

Table 9: Within-laboratory precision of cobas® HBV (serum samples – sample processing volume of 400 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
1.0E+09	1.19E+09	Lambda DNA	0.04	0.04	0.03	0.04
1.0E+07	1.19E+07	Lambda DNA	0.05	0.05	0.04	0.05
5.0E+05	5.93E+05	Lambda DNA	0.03	0.04	0.03	0.03
2.0E+04	1.95E+04	CS	0.04	0.03	0.03	0.03
2.0E+03	1.95E+03	CS	0.03	0.02	0.03	0.03
2.0E+02	1.95E+02	CS	0.05	0.04	0.03	0.04
2.5E+01	2.44E+01	CS	0.16	0.08	0.14	0.13

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

Table 10: Within-laboratory precision of cobas® HBV (EDTA plasma – sample 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
1.0E+09	1.10E+09	Lambda DNA	0.04	0.06	0.04	0.05
1.0E+07	1.10E+07	Lambda DNA	0.04	0.07	0.05	0.05
5.0E+05	5.49E+05	Lambda DNA	0.03	0.04	0.04	0.04
2.0E+04	2.44E+04	CS	0.04	0.05	0.06	0.05
2.0E+03	2.44E+03	CS	0.05	0.07	0.05	0.06
2.0E+02	2.44E+02	CS	0.05	0.07	0.04	0.06
2.5E+01	3.05E+01	CS	0.30	0.14	0.22	0.23

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

Table 11: Within-laboratory precision of cobas® HBV (serum – sample 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
1.0E+09	1.19E+09	Lambda DNA	0.02	0.02	0.02	0.02
1.0E+07	1.19E+07	Lambda DNA	0.03	0.04	0.04	0.04
5.0E+05	5.93E+05	Lambda DNA	0.02	0.03	0.04	0.03
2.0E+04	1.95E+04	CS	0.03	0.02	0.03	0.03
2.0E+03	1.95E+03	CS	0.04	0.03	0.03	0.03
2.0E+02	1.95E+02	CS	0.06	0.15	0.05	0.10
2.5E+01	2.44E+01	CS	0.14	0.18	0.15	0.16

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

Genotype verification

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

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

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

HBV DNA clinical specimens for eight different genotypes (B, C, D, E, F, G, H, G1896A-precore mutant) were diluted in EDTA plasma and serum to the EDTA plasma LoD concentration of the predominant genotype (HBV GT A) based on 95% Hit Rate LoD analysis (5.0 IU/mL). Hit rate analysis was performed with 42 replicates for each genotype and sample matrix. These results verify that **cobas**® HBV detected HBV for HBV genotypes B, C, D, E, F, G, H and precore mutant (PC) at the concentration of 5 IU/mL with an upper one-sided 95% confidence interval being greater to the expected hit rate of 95%.

Table 12: LoD verification of HBV genotypes B–H and precore mutant in 400 µL EDTA plasma

Genotype	Hit rate	Upper One Sided 95% Confidence Interval
B	97.6%	99.9%
C	95.2%	99.2%
D	100.0%	100.0%
E	100.0%	100.0%
F	100.0%	100.0%
G	100.0%	100.0%
H	90.5%	96.7%
PC	100.0%	100.0%

Table 13: LoD verification of HBV genotypes B–H and precore mutant in 400 µL serum

Genotype	Hit rate	Upper One Sided 95% Confidence Interval
B	100.0%	100.0%
C	100.0%	100.0%
D	100.0%	100.0%
E	100.0%	100.0%
F	100.0%	100.0%
G	100.0%	100.0%
H	97.6%	99.9%
PC	100.0%	100.0%

Verification of linear range for genotypes B through H and precore mutant

The dilution series used in the verification of genotypes linearity study of **cobas**® HBV consists of nine panel members spanning the intended linear range. High titer panel members were prepared from a high titer lambda DNA stock whereas the lower titer panel members were made from a high titer clinical sample (CS). The linearity panel was designed to have a minimum overlap of 2 $\log_{10}$ titer between the two material sources. The linear range of **cobas**® HBV spanned from the LLoQ (10.0 IU/mL for a sample processing volume of 400 µL) to the ULoQ (1.0E+09 IU/mL) and included at least two medical decision points. Twelve replicates per level were tested in EDTA plasma.

The linear range of **cobas**® HBV was verified for all eight genotypes (B, C, D, E, F, G, H, precore mutant). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ±0.08 $\log_{10}$.

Analytical specificity

The analytical specificity of **cobas**® HBV was evaluated by diluting a panel of pathogens (Table 14) with HBV DNA positive and HBV DNA negative EDTA plasma. The pathogens were added to negative EDTA plasma and tested with and without HBV DNA. Negative results were obtained with **cobas**® HBV for all pathogen samples without HBV target and positive results were obtained on all of the pathogen samples with HBV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HBV samples containing potentially cross-reacting organisms was within ±0.12 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 14: Pathogens tested for cross-reactivity

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

Analytical specificity – interfering substances

Elevated levels of potentially interfering endogenous substances (triglycerides (27.9 - 30.1 g/L), conjugated bilirubin (0.18 - 0.22 g/L), unconjugated bilirubin (0.19 - 0.2 g/L), albumin (57.8 - 60.6 g/L), hemoglobin (1.8 - 2.3 g/L) and human DNA (2 mg/L) in samples were tested in presence and absence of HBV DNA. The tested substances were shown not to interfere with the test performance of **cobas**® HBV. Moreover, the presence of markers for the autoimmune diseases systemic lupus erythematosus (SLE), rheumatoid factor (RF) and antinuclear antibody (ANA) was confirmed to not cause interference.

The mean $\log_{10}$ titer of each of the positive HBV samples containing potentially interfering endogenous substances and autoimmune disease markers was between -0.05 $\log_{10}$ and 0.07 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

In addition, drug compounds listed in Table 15 were tested at three times the C_{max} in presence and absence of HBV DNA.

Table 15: Drug compounds tested for interference with the quantitation of HBV DNA by cobas® HBV

Class of drug	Generic drug name	
Immune Modulators	Peginterferon α-2a	Ribavirin
	Peginterferon α-2b	
HIV Entry Inhibitor	Maraviroc	
HIV Integrase Inhibitors	Elvitegravir/Cobicistat	Raltegravir
Non-nucleoside HIV Reverse Transcriptase Inhibitors	Efavirenz	Nevirapine
	Etravirine	Rilpivirine
HIV Protease inhibitors	Atazanavir	Nelfinavir
	Darunavir	Ritonavir
	Fosamprenavir	Saquinavir
	Lopinavir	Tipranavir
HCV Protease Inhibitors	Boceprevir	Telaprevir
	Simeprevir	
Reverse Transcriptase or DNA Polymerase Inhibitors	Abacavir	Ganciclovir
	Aciclovir	Lamivudine
	Adefovir dipivoxil	Sofosbuvir
	Cidofovir	Telbivudine
	Emtricitabine	Tenofovir
	Entecavir	Valganciclovir
	Foscarnet	Zidovudine
Compounds for Treatment of Opportunistic Infections	Azithromycin	Pyrazinamide
	Clarithromycin	Rifabutin
	Ethambutol	Rifampicin
	Fluconazole	Sulfamethoxazole
	Isoniazid	Trimethoprim

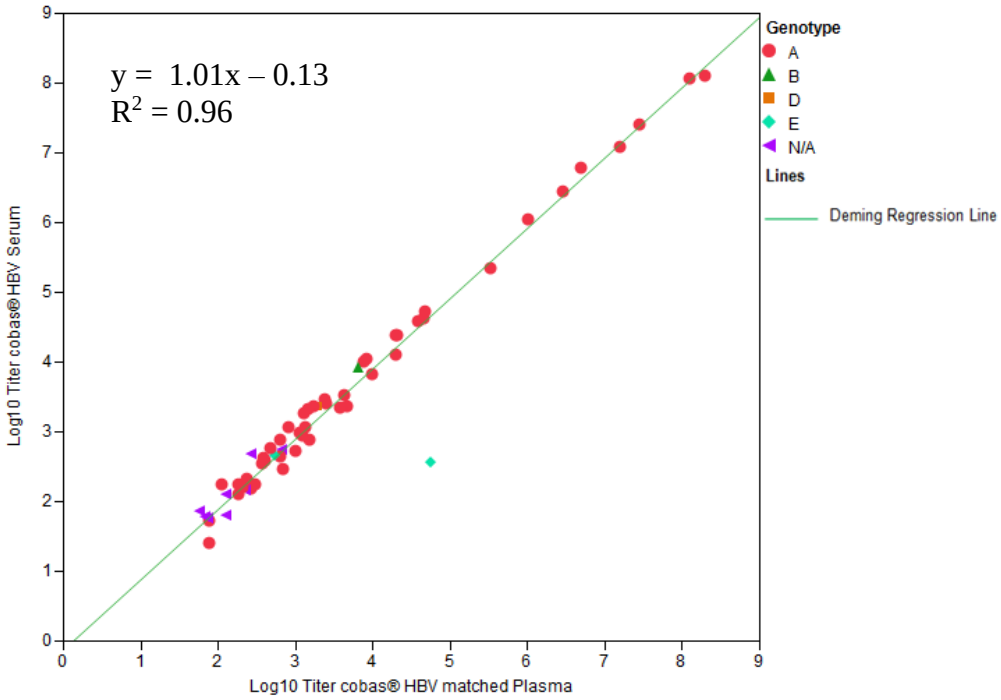
All potentially interfering drug compounds have been shown to not interfere with the test performance. Negative results were obtained with **cobas®** HBV for all samples without HBV target and positive results were obtained on all of the samples with HBV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HBV samples containing potentially interfering drug compounds was between -0.02 $\log_{10}$ and 0.03 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Matrix equivalency – EDTA plasma versus serum

One hundred nineteen paired EDTA plasma and serum samples were analyzed for matrix equivalency. Of these, 59 paired samples were HBV positive samples. The HBV positive samples covered genotypes A, B, D and E across the linear range.

The mean titer deviation measured for the matching EDTA plasma and serum samples was -0.10 $\log_{10}$ (95% Confidence Interval: -0.18; -0.01) (Figure 6).

Figure 6: 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 spiked with HBV target. These samples were tested at a target concentration of approximately 3 × LoD (15.0 IU/mL).

The results of this study determined that all replicates were valid and positive for the HBV resulting in a whole system failure rate of 0.0%. The two-sided 95% exact confidence interval was 0.0% for the lower bound and 3.6% for the upper bound [0.0%: 3.6%].

Cross contamination

The cross-contamination rate for **cobas**® HBV was determined by testing 230 replicates of HBV-negative EDTA-plasma samples and 235 replicates of a high titer HBV samples at 1.4E+09 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 230 replicates of the negative samples were valid and detected negative, resulting in a cross-contamination rate of 0.0% with a one-sided 95% confidence interval of 1.3%.

Clinical performance evaluation

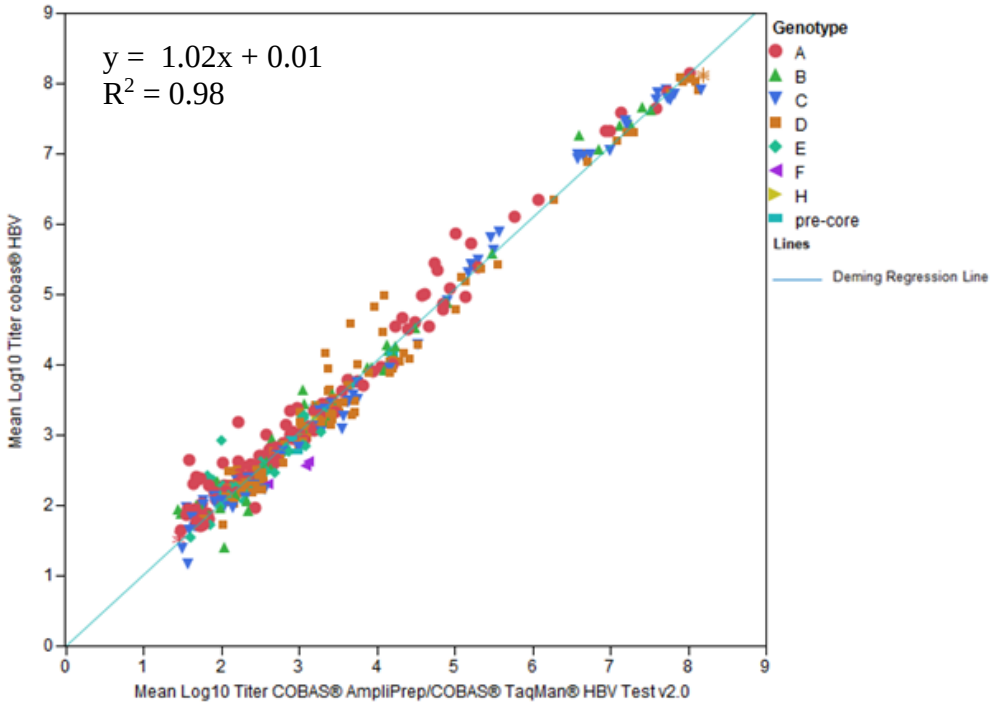
Performance evaluation of cobas® HBV compared to the COBAS® AmpliPrep/COBAS® TaqMan® HBV Quantitative Test, v2.0

The performance of **cobas**® HBV and the COBAS® AmpliPrep/COBAS® TaqMan® HBV Quantitative Test, v2.0 (TaqMan® HBV Test, v2.0) was compared by analysis of serum and EDTA plasma samples from HBV-infected patients. A total of 215 EDTA plasma and 170 serum samples across all HBV genotypes (except genotype G), analyzed in duplicate, were

valid and within the quantitation range of both tests. The Deming regression analysis was performed. The mean titer deviation of the samples tested with the two tests was 0.06 $\log_{10}$ (95% Confidence Interval: 0.04; 0.09).

The Deming regression results are shown in Figure 7. The color represents the genotype.

Figure 7: Regression analysis of cobas® HBV vs TaqMan® HBV Test, v2.0, EDTA plasma and serum samples



Specificity

The specificity of **cobas**® HBV was determined by analyzing HBV-negative EDTA plasma and serum samples from individual donors. Six hundred fifteen individual EDTA plasma and six hundred thirteen individual serum samples (1228 total results) were tested with three lots of **cobas**® HBV reagents. Six hundred fifteen samples in EDTA plasma and 613 samples in serum tested negative for HBV DNA. In the test panel the specificity of **cobas**® HBV was 100.0% in plasma and in serum (one-sided 95% confidence interval of 99.5%).

Additional information

Key assay features

Sample type	EDTA plasma, serum
Sample processing volume	400 µL or 200 µL
Analytical sensitivity	EDTA plasma: 4.4 IU/mL (400 µL) Serum: 2.8 IU/mL (400 µL) 7.6 IU/mL (200 µL) 5.5 IU/mL (200 µL)
Linear range	400 µL: 10.0 IU/mL – 1.0E+09 IU/mL 200 µL: 10.0 IU/mL – 1.0E+09 IU/mL
Specificity	100.0% (one-sided 95% confidence interval: 99.5%)
Genotypes detected	HBV genotypes A-H, G1896A-precore mutant

Symbols

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

Table 16: Symbols used in labeling for Roche PCR diagnostic 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

Table 17: Manufacturer

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

Made in USA

Trademarks and patents

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

Copyright

CE 0123 IVD

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Document revision

Document Revision Information	
Doc Rev. 7.0 02/2024	Updated Lysis Kits 2 hazard information. Updated the harmonized symbol page. Added **Technical support** section. Updated to current economic operators. Updated **Trademarks and patents** section, including the link. Added Made in statement. Updated **cobas**® branding. Added Rx only symbol. Please contact your local Roche Representative if you have any questions.
Doc Rev. 8.0 08/2024	Updated Wash Buffer kits hazard information. Removed Rx Only from front page. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.
Doc Rev. 9.0 10/2024	Revised to comply with IVDR. Added NIBSC code for WHO International Standard. Specified primer binding region. Clinical performance evaluation section was added. Added intended use for **cobas**® HBV/HCV/HIV-1 Control Kit. Please contact your local Roche Representative if you have any questions.

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