cobas® HBV RNA

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

For Research Use only

Not for use in diagnostic procedures

cobas® HBV RNA	P/N: 09700064190
cobas® HBV RNA Control Kit	P/N: 09700048190
For use on the cobas® 5800 System:	
cobas® Negative Control Kit	P/N: 09051554190
For use on the cobas® 6800/8800 Systems:	
cobas® Negative Control Kit	P/N: 09051554190 or P/N: 07002220190

Table of Contents

Intended use

cobas® HBV RNA for use on the **cobas®** 5800/6800/8800 Systems (**cobas®** HBV RNA) is an automated real-time RT-PCR assay for the in vitro quantitative detection of circulating HBV RNA in EDTA plasma and serum.

cobas® HBV RNA is intended for research use only and is not for use in diagnostic procedures.

cobas® HBV RNA can be used to support scientific research to understand the potential use of this new biomarker.

Summary and explanation of the test

Explanation of the test

cobas® HBV RNA is a quantitative test that is run on the **cobas®** 5800/6800/8800 Systems. **cobas®** HBV RNA enables the detection and quantitation of HBV RNA in EDTA plasma and serum specimens. The RNA level is quantified against a non-HBV RNA quantitation standard (RNA-QS), which is introduced into each specimen during sample processing. The RNA-QS also functions as a 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

The **cobas®** HBV RNA test is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas®** 5800 System is designed as one integrated instrument. The **cobas®** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas®** 5800 or **cobas®** 6800/8800 Systems software, which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.

Armored RNA quantitation standard (QS) molecules are added during sample preparation. Nucleic acids (from samples and QS) are released by addition of proteinase and lysis reagent to the sample during universal sample preparation steps. The released nucleic acids bind to the silica surface of the added magnetic glass particles. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Unbound substances and impurities, such as denatured proteins, cellular debris, and potential PCR inhibitors (such as hemoglobin) from the lysed sample are reduced with subsequent wash reagent steps and purified nucleic acids are eluted from the glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid HBV RNA from the sample is achieved by the use of organism-specific forward and reverse primers, which were selected to specifically hybridize to highly conserved regions of the HBV pre-genomic nucleic acid. A thermostable DNA polymerase enzyme is used for both reverse-transcription and amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas®** HBV RNA master mix contains detection probes which are specific for HBV and QS nucleic acid. The specific HBV and 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 fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease 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 is concomitantly increased. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified HBV RNA target and the QS are possible.

Reagents and materials

cobas® HBV RNA reagents and controls

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

Table 1 cobas® HBV RNA

Store at 2-8°C
192 test cassette (P/N 09700064190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 mL
RNA Quantitation Standard (RNA QS)	Tris buffer, <0.05% EDTA, <0.001% non-HBV related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), 0.002% Poly rA RNA (synthetic), <0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
HBV RNA Master Mix Reagent 2 (HBV RNA 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 RNA primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for HBV RNA and the RNA 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 RNA Control Kit
Store at 2–8°C
(P/N 09700048190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
HBV RNA Low Positive Control (HBV RNA L(+)C)	< 0.001% armored HBV 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 Ag; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, CMV DNA, CHIKV/DENV RNA, ZIKA RNA and Babesia RNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5 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-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
HBV RNA High Positive Control (HBV RNA H(+)C)	< 0.001% armored HBV 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 Ag; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, CMV DNA, CHIKV/DENV RNA, ZIKA RNA and Babesia RNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5 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-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance
**Hazardous substance

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

Store at 2-8°C

For use with core software 1.4 or higher on **cobas**® 6800/8800 Systems (P/N 07002220190)

-or-

For use with core software 1.0.2 or higher on **cobas**® 5800 System, 1.4 or higher on **cobas**® 6800/8800 Systems (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 Ag; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, CMV DNA, CHIKV/DENV RNA, ZIKA RNA and Babesia RNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	 **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (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 severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

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

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance or mixture

Reagent storage and handling requirements

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

When reagents are not loaded on the **cobas®** 5800/6800/8800 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 RNA	2–8°C
cobas® HBV RNA Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas® omni Lysis Reagent	2–8°C
cobas® omni MGP Reagent	2–8°C
cobas® omni Specimen Diluent	2–8°C
cobas® omni Wash Reagent	15–30°C

Reagent storage requirements for the cobas® 5800 System

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

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

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® HBV RNA	Date not passed	90 days from first usage[b,c]	Max 40 runs[c]	Max 36 days[c]
cobas® HBV RNA Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[c]
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[c]
cobas® omni Lysis Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® omni MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® omni Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas® omni Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a] Single use reagents

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

[c] The performance has not been established for suggested days and time, but is based on similar reagents used on the same system.

Reagent storage requirements for the cobas® 6800/8800 Systems

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

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

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

[a] Single use reagents

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

[c] The performance has not been established for suggested days and time, but is based on similar reagents used on the same system.

Additional materials required for the cobas® 5800 System

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

Material	P/N
cobas® omni Processing Plate 24	08413975001
cobas® omni Amplification Plate 24	08499853001
cobas® omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1mL	04639642001
Tip CORE TIPS with Filter, 300uL	07345607001
cobas® omni Liquid Waste Container	07094388001
cobas® omni Lysis Reagent	06997538190
cobas® omni MGP Reagent	06997546190
cobas® omni Specimen Diluent	06997511190
cobas® omni Wash Reagent	06997503190
Solid Waste Bag or Solid Waste Bag With Insert	07435967001 or 08030073001
cobas® omni Secondary Tubes 13x75 (optional)	06438776001

Additional materials required for the cobas® 6800/8800 Systems

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

Material	P/N
cobas® omni Processing Plate	05534917001
cobas® omni Amplification Plate	05534941001
cobas® omni Pipette Tips	05534925001
cobas® omni Liquid Waste Container	07094388001
cobas® omni Lysis Reagent	06997538190
cobas® omni MGP Reagent	06997546190
cobas® omni Specimen Diluent	06997511190
cobas® omni Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001
cobas® omni Secondary Tubes 13x75 (optional)	06438776001

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® HBV RNA analysis package for the **cobas**® 5800 System must be installed on the **cobas**® 5800 instrument. The x800 Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

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

Table 10 Instrumentation

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

For additional information, please refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides.

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 Research Use Only. Not for use in diagnostic procedures.
- **cobas**® HBV RNA has not been evaluated for use as a screening test for the presence of HBV RNA in blood or blood products.
- 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.[1,2] Only personnel proficient in handling infectious materials and the use of **cobas**® HBV RNA and the **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with freshly prepared or ready to use 0.6% sodium or potassium hypochlorite solution (bleach) or follow appropriate site procedures.
- **cobas**® HBV RNA 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, antibody to HBc, HIV Ag. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, CMV DNA, CHIKV/DENV RNA, ZIKA RNA and Babesia RNA. 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.**
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. 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 contamination 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 RNA test kits, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.

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

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® HBV RNA 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 freshly prepared or ready to use 0.6% sodium or potassium hypochlorite solution (bleach) or follow appropriate site procedures. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800/6800/8800 Systems, follow the instructions in the **cobas**® 5800 or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

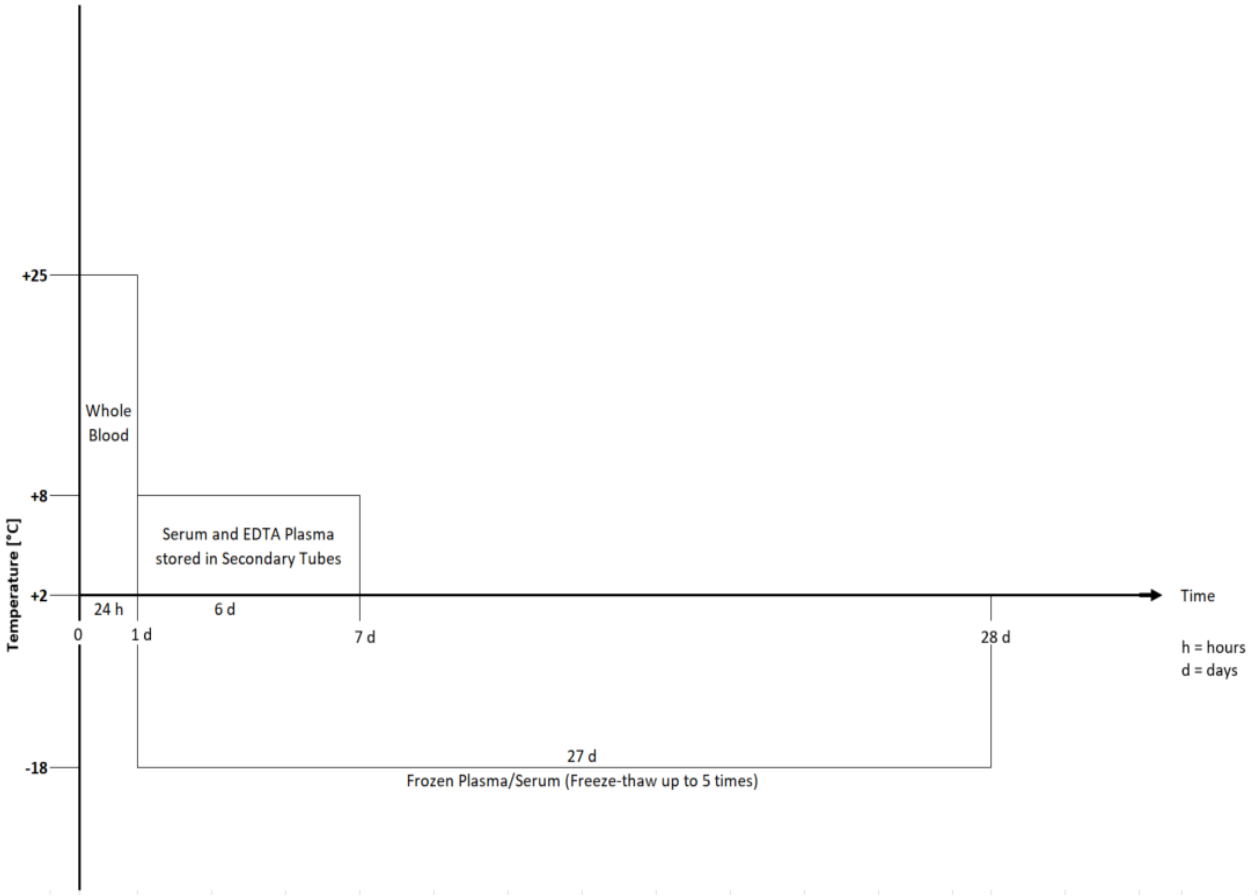
Do not store centrifuged plasma and serum samples in primary tubes.

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.

Samples

- Whole blood should be collected in SST™ Serum Preparation Tubes, BD Vacutainer® PPT™ Plasma Preparation Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions. Refer to Figure 1.
- Whole blood collected in SST™ Serum Preparation 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 2-25°C prior to plasma/serum preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation plasma/serum samples may be stored for up to 6 days at 2-8 °C in secondary tubes or at ≤ -18°C for up to 27 days in secondary tubes.
- Plasma/serum samples are stable for up to five freeze/thaw cycles when frozen at ≤ -18°C.

Figure 1 Sample storage conditions



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

Instructions for use

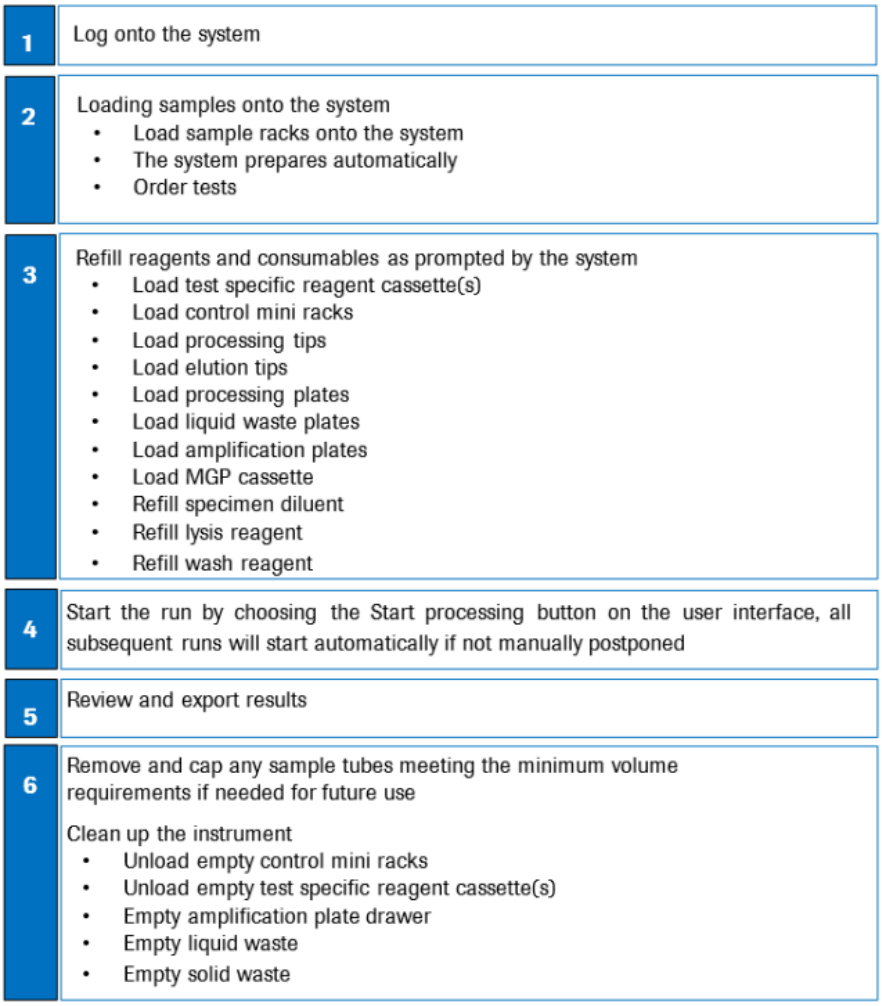
Procedural notes

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

Running cobas® HBV RNA on the cobas® 5800 System

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

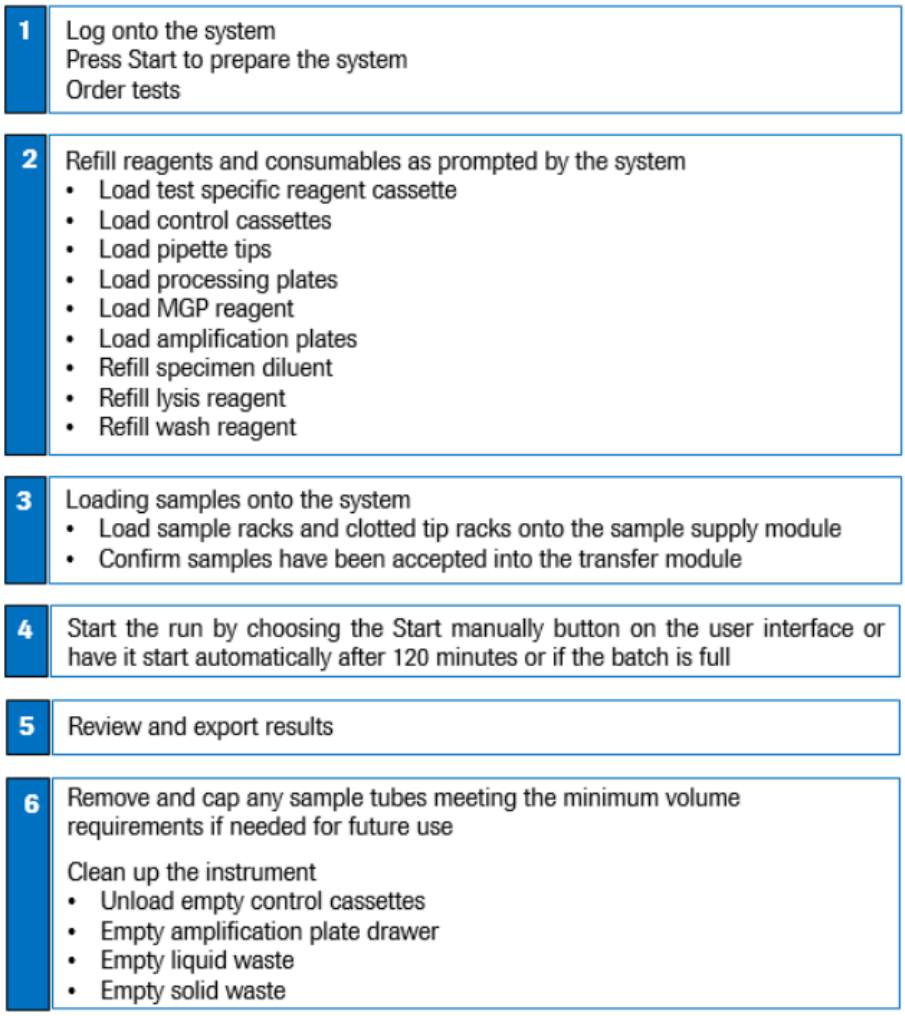
Figure 2 **cobas**® HBV RNA test procedure on the **cobas**® 5800 System



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

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

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



Results

The **cobas®** 5800/6800/8800 Systems automatically determine the HBV RNA concentration for the samples and controls. The HBV RNA concentration is expressed in copies per milliliter (cp/mL).

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

- One negative control [(–) C] and two positive controls, a low positive control [HBV RNA L(+) C] and a high positive control [HBV RNA H(+) C] are processed at least every 72 hours and with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas®** 5800 System software and/or report, check for flags and their associated results to ensure the result validity.

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

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

Control results on the cobas® 5800 System

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

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

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

- One negative control [(–) C] and two positive controls, a low positive control [HBV RNA L(+) C] and a high positive control [HBV RNA H(+) C] is processed with each batch.
- In the **cobas®** 6800/8800 Systems 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 RNA L(+) C, HBV RNA H(+) C. The negative control result is displayed as (–) C and the low and high positive controls are displayed as HBV RNA L(+) C and HBV RNA H(+) C.

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

Control flags on the cobas® 6800/8800 Systems

Table 11 Control flags for negative and positive controls

Negative Control	Flag	Result	Interpretation
(–) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Flag**	**Result**	**Interpretation**
HBV RNA 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.
HBV RNA 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 control batch is invalid, repeat testing of all samples of affected batch.

Interpretation of results

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

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

Table 12 Target results for individual target result interpretation

Results	Interpretation
Target Not Detected	HBV RNA not detected. Report results as “HBV RNA not detected”.
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “HBV RNA detected, less than (Titer Min)”. Titer Min = 10 cp/mL
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 RNA detected”.
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “HBV RNA detected, greater than (Titer Max)”. Titer Max = 1.0E+09 cp/mL

[a] Sample result > Titer Max refers to HBV RNA 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 RNA-negative human EDTA plasma/serum and the test should be repeated. Multiply the reported result by the dilution factor.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.

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

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

Interpretation of results on the cobas® 6800/8800 Systems

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

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

Procedural limitations

- **cobas**® HBV RNA has been evaluated only for use in combination with the **cobas**® HBV RNA Control Kit, **cobas**® NHP Negative Control Kit, **cobas**® **omni** MGP Reagent, **cobas**® **omni** Lysis Reagent, **cobas**® **omni** Specimen Diluent, and **cobas**® **omni** Wash Reagent for use on the **cobas**® 5800/6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma and serum. Testing of other sample types with **cobas**® HBV RNA may result in inaccurate results. Plasma/serum RNA load measurements are not directly comparable to those of other sample types.
- Quantitation of HBV RNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Results for this assay are quantified in units of cp/mL. It needs to be considered if comparing results to other assays that other assays may not quantify in the same units, or may not be comparable due to the lack of an international standard for HBV RNA.
- As with any molecular test, mutations within the target regions of **cobas**® HBV RNA could affect primer and/or probe binding resulting in the under-quantitation of viral RNA or failure to detect the presence of viral RNA.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- **cobas**® HBV RNA is not intended for use as a screening test for the presence of HBV RNA in blood or blood products.

Additional information

Key test features

Sample type	EDTA plasma, serum
Minimum amount of sample required	350 µL*
Sample processing volume	200 µL

*Dead volume of 0.150 ml is identified for the **cobas® omni** Secondary Tubes. Other tubes compatible with the **cobas®** 5800/6800/8800 Sytems (consult User Assistance Guide) may have different dead volume and require more or less minimal volume. Contact your local Roche service representative for further information.

Symbols

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

Table 13 Symbols used in labeling for Roche PCR diagnostics products

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

Technical support

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

Manufacturer and distributors

Table 14 Manufacturer and distributors

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

Made in USA

Distributed by	Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457, USA (For Technical Assistance call the Roche Response Center toll-free: 1-800-526-1247)

Trademarks and patents

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

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References

1. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 6th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 300859, revised December 2020.

2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 2.0 06/2024	Return minimum sample volume to original level in **Instructions for use** and **Key test features** section. Updated **cobas**® branding. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.