**PROCEDURE: Coro Molecular Microbiology Cobas HBV**

1. **PRINCIPLE**
   1. Intended use
      1. cobas® HBV is a quantitative test performed on the cobas® 6800 System. cobas® HBV enables the detection and quantitation of HBV DNA in EDTA plasma of infected patients for use in laboratories that support clinical trials as well as routine clinical practice in the management of patients with HBV. A single probe is used to detect and quantify, but not discriminate genotypes A-H. The viral load is quantified against a non-HBV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample preparation. The DNA-QS also functions to monitor for the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.
      2. cobas®HBV is an in vitro nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) DNA in human EDTA plasma of HBV-infected individuals.
      3. 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.
      4. 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.
   2. Summary and Background
      1. Hepatitis B virus (HBV) is one of several viruses known to cause viral hepatitis. Over 2 billion people throughout the world have been exposed to HBV and over 360 million are chronically infected carriers. 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. 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. However, targeted screening programs have shown prevalence rates in excess of 15% in certain high-risk immigrant populations. Patients with chronic HBV infection are at high risk of long-term complications of infection, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Serologic markers are commonly used as diagnostic and/or prognostic indicators of acute or chronic HBV infection. 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. Although carriers may clear HbsAg and develop antibody to HbsAg, there still appears to be a risk of serious liver complications later in life. 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. 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.
      3. HBV DNA in EDTA plasma can be quantitated by nucleic acid amplification technologies, such as PCR. 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.
   3. Sample Preparation
      1. cobas® HBV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800 System consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800 software which assigns test results for all tests as target not detected, < 10 IU/ml (lower limit of quantitation), > 1,000,000,000 IU/ml (upper limit of quantitation) or HBV DNA detected, a value in the linear range 10 -1,000,000,000 IU/ml. Results can be reviewed directly on the system screen, exported, or printed as a report.
      2. Nucleic acid from patient samples, external controls and added lambda DNA (DNA-QS) molecules are simultaneously extracted.
      3. Viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.
   4. Selective Amplification
      1. Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly conserved regions of HBV. Selective amplification of DNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HBV genome. A thermostable DNA polymerase enzyme is used for amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR mix, during the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.
      2. cobas® HBV master mix contains detection probes which are specific for the HBV target sequences and the QS nucleic acid, respectively. The specific HBV and DNA-QS detection probes are each labeled with one of two unique fluorescent dyes which acts as a reporter. Each probe also has a second dye which acts as a quencher. The two reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified HBV target and the DNA-QS. When not bound to the target sequence, the fluorescent signal of the intact probe is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified HBV target and the DNA-QS are possible.
2. **AVAILABILITY**
   1. Specimens may be submitted 7 days a week/ 24 hour a day.
   2. Testing is performed on Tuesdays.
3. **TEST CODE**
   1. HBVL1
4. **SPECIMEN REQUIREMENTS**
   1. Specimen Collection

**Caution**: Handle all specimens as if they are capable of transmitting infectious agents.

**Note**: Store all samples at specified temperatures.

**Note**: Sample stability is affected by elevated temperatures.

**Note**: If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) until completely thawed and then centrifuge to collect all sample volume at the bottom of the tube.

* + 1. Blood should be collected in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions.
    2. Ensure sufficient whole blood collection to allow usage of the processing volume for EDTA plasma of 500 µL (for a total minimum sample requirement of 650 µL).
  1. Specimen Transport
     1. Separate plasma from whole blood within 6 hours of collection by centrifugation at 800-1600 x G for 20 minutes at room temperature.

**Note: Per PI:** Whole blood collected 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.

* 1. Specimen Storage
     1. Upon separation plasma samples may be stored for up to 6 days at 2°C to 8°C or up to 12 weeks at ≤ -18°C.
     2. Per Lab Protocol:
        1. RIH: Freeze samples at -80°C immediately after centrifugation and transport to Coro Molecular Microbiology Lab.
        2. TMH: Store plasma at 2-8°C and send to RIH Micro. RIH Micro will freeze plasma and transport to Coro Molecular Microbiology Lab.
        3. Coro Molecular Micro Lab will store all plasma samples at -80°C.
     3. For long-term storage (up to 6 months), temperatures at ≤ -60°C are recommended.
     4. Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.
     5. Specimens should be handled as infectious using safe laboratory procedures.

1. **MATERIALS AND REAGENTS**
   1. Reagents
      1. cobas® HBV includes the following components:

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| --- | --- | --- |
| **cobas**® **HBV reagents and controls** | | |
| **cobas**® **HBV** | | |
| **Proteinase Solution** | **PASE** | Tris buffer  < 0.05% EDTA  Calcium chloride  Calcium acetate  8% (w/v) proteinase |
| **DNA Quantitation Standard** | **DNA-QS** | Tris buffer  < 0.05% EDTA  < 0.001% non-HBV lambda DNA construct containing non-HBV primer binding and a unique probe region (non-infectious DNA)  0.002% Poly rA RNA (synthetic)  < 0.1% sodium azide |
| **Elution Buffer** | **EB** | Tris buffer  0.2% methyl-4 hydroxybenzoate |
| **Master Mix Reagent 1** | **MMX-R1** | Manganese acetate  Potassium hydroxide  < 0.1% sodium azide |
| **HBV Master Mix Reagent 2** | **HBV MMX-R2** | Tricine buffer  Potassium acetate  18% dimethyl sulfoxide, glycerol  < 0.1% Tween 20, EDTA  < 0.12% dATP, dCTP, dGTP, dUTPs  < 0.01% upstream and downstream HBV primers  < 0.01% Quantitation Standard forward and reverse primers  < 0.01% fluorescent-labeled oligonucleotide probes specific for HBV and the HBV Quantitation Standard  < 0.01% oligonucleotide aptamer  < 0.1% Z05D DNA polymerase  < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial)  < 0.1% sodium azide |
| **cobas**® **HBV/HCV/HIV-1 Control Kit** | | |
| **HBV/HCV/HIV-1**  **Low Positive Control** | **HBV/HCV/HIV-1 L(+)C** | < 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage)  < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein  < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein  Normal human plasma non-reactive by licensed tests for antibody to HCV antibody to HIV-1/2, HBsAg, antibody to HBc, HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods  0.1% ProClin® 300 preservative |
| **HBV/HCV/HIV-1**  **High Positive Control** | **HBV/HCV/HIV-1 H(+)C** | < 0.001% armored HIV-1 Group M RNA (non-infectious RNA in MS2 bacteriophage)  < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein  < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein  Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.  0.1% ProClin® 300 preservative |
| **cobas**® **NHP Negative Control Kit** | | |
| **Normal Human Plasma Negative Control** | **NHP-NC** | Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.  < 0.1% ProClin® 300 preservative |
| **cobas**® **omni reagents for sample preparation** | | |
| **cobas omni MGP Reagent** | **MGP** | Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide |
| **cobas omni Specimen Diluent** | **SPEC DIL** | Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide |
| **cobas omni Lysis Reagent** | **LYS** | 42.56% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate |
| **cobas omni Wash Reagent** | **WASH** | Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate |

* + 1. The following supplies are needed but not supplied in the cobas® HBV Kit**.**

|  |  |
| --- | --- |
| **Instrumentation and Software required** | |
|  | **cobas**®6800 System (Option Moveable) (P/N 05524245001 and 06379672001) |
|  | **cobas**®6800 System (Fix) (P/N 05524245001 and 06379664001) |
|  | Sample Supply Mode (P/N 06301037001) |
| **Materials and consumables for use on the cobas**® **6800/8800 Systems)\***  *\*The 1G server is provided with the system.* | |
|  | **cobas omni** Processing Plate (P/N 05534917001) |
|  | **cobas omni** Amplification Plate (P/N 05534941001) |
|  | **cobas omni** Pipette Tips (P/N 05534925001) |
|  | **cobas omni** Liquid Waste Container (P/N 07094388001) |
|  | **cobas omni** Lysis Reagent (P/N 06997538190) |
|  | **cobas omni** MGP Reagent (P/N 06997546190) |
|  | **cobas omni** Specimen Diluent (P/N 06997511190) |
|  | **cobas omni** Wash Reagent (P/N 06997503190) |
|  | Solid Waste Bag (P/N 07435967001) |
|  | Solid Waste Container (P/N 07094361001) |

* 1. Reagent Precautions
     1. Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of reagents or controls.
     2. 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.
     3. 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.
     4. cobas® HBV 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.
     5. 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.
     6. Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.
  2. Reagent Storage and Handling
     1. Do not freeze reagents or controls.
     2. When reagents are not loaded on the cobas® 6800/8800 Systems, store them at the corresponding temperature specified below:
        1. Store cobas® HBV, cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, cobas omni Lysis Reagent, cobas omni MGP Reagent, and cobas omni Specimen Diluent at 2-8 °C.
        2. Store the cobas omni Wash Reagent at 15-25 °C.
     3. Reagents loaded onto the **cobas®** 6800 System are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas®** 6800 Systems allow reagents to be used only if all of the conditions shown in the following table are met. The system automatically prevents use of expired reagents. The following table allows the user to understand the reagent handling conditions enforced by the **cobas®** 6800 System.

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Open-kit stability** | **Number of runs for which this kit can be used** | **On-board stability** |
| **cobas®** HBV | 30 days from first usage | Max 10 runs | Max 8 hours |
| **cobas®** HBV/HCV/HIV-1 Control Kit | N/A | N/A | Max 8 hours |
| **cobas®** NHP Negative Control Kit | N/A | N/A | Max 10 hours |
| **cobas omni** Lysis Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** MGP Reagent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Specimen Diluent | 30 days from loading\* | N/A | N/A |
| **cobas omni** Wash Reagent | 30 days from loading\* | N/A | N/A |

* + 1. Do not use reagents after their expiration dates.
    2. Do not pool reagents. Gloves must be worn and must be changed between handling specimens and cobas® 6800 reagents to prevent contamination. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
    3. Handle all reagents with caution and avoid contact with skin, eyes, or mouth. Refer to the package insert for any known toxicity.
       1. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. 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 of these reagents occur, dilute with water before wiping dry.
       2. If spills occur on the cobas® 6800 System, follow the instructions in the cobas® 6800 Operating Manual and Coro Molecular Micro Operating Procedure.
       3. Reagents required to perform this assay are located:

Equipment Room Refrigerator MM R6 and MM R7

1. **QUALITY CONTROL**
   1. Quality Control Information
      1. One negative control (–) C and two positive controls, a low positive control HBV L(+)C and a high positive control HBV H(+)C, are processed with each batch.
      2. Store controls at 2-8°C n refrigerator MM R6 and MM R7. Controls are stable until the expiration date indicated.
      3. Record QC results on the sheets provided. Include date of testing, kit lot #, control lot #s, expiration dates, and results.
      4. Batch validity is checked within the cobas 6800 software (monitor) and is printed with the run report.
      5. The batch is valid if no flags appear for all three controls, which include one negative control and two positive controls.
      6. Validation of results is performed automatically by the cobas® 6800 software based on negative and positive control results.
      7. New lot numbers/shipment of HBV kits are QC’d using control kits that have passed QC.
      8. New lot numbers/ shipments of Positive and Negative Control kits are run using HBV kits that have passed QC.
      9. Verification of Performance is run on new lot numbers of HBV kits and/or every 6 months, after Software Upgrades, and after major System Upgrades.
         1. A purchased Verification Panel is run which tests the linear range of the assay.
         2. Results are plotted and the R² value is determined.
         3. An unacceptable result would be an R² value <0.9900 or 2 panel members with a >0.5 Log difference from the expected result and would warrant an investigation and repeat.
      10. Environmental testing is performed monthly
          1. The hood, instruments, and bench space is swabbed and placed in an aliquot of plasma dilution matrix and placed on the run of patient samples.
          2. For any positive result, clean all areas and retest.
   2. Acceptable Limits
      1. The HBV L(+) and HBV H(+) Control results must be ‘Valid.’ The (–) Control result must be ‘Valid.’
      2. For any run, valid results must be obtained for both the Positive and Negative Control for the cobas® 6800 Software to display the reportable cobas® HBV results from that run.
      3. QC statistics are calculated monthly to define analytic imprecision and to monitor trends over time.
2. Refer to Viral Load Monitoring Statistics on the M drive Molecular folder.
   1. Corrective Actions
      1. Assay will require repeating if either positive or negative controls are not valid.
      2. If the HBV L(+) and HBV H(+) Control or the (-) Control results are consistently invalid, contact your local Roche Support Network Customer Support Center for technical assistance.
   2. Control Flags
      1. Control flags for negative and positive controls:

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

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

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

1. **TEST PROCEDURE**
   1. Procedure Notes
      1. Do not use cobas® HBV reagents, cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, or cobas omni reagents after their expiry dates.
      2. Do not reuse consumables. They are for one-time use only.
      3. Refer to the cobas® 6800 Systems Operator’s Manual for proper maintenance of instruments.
   2. Running the Test

**Note:** Refer to Cobas 6800 Systems Operator Manual on M drive/Molecular Folder/ Roche 6800.

**Note**: Refer to Coro Molecular Micro Cobas 6800 Operating Procedure for detailed procedure

**Note**: Refer to Appendix A of Cobas Operating Procedure for Cobas 6800 Quick Start Guide

**Note**: Refer to Appendix B of Cobas Operating Procedure for Cobas 6800 Maintenance

**Note**: Clean benchtops with 10% bleach followed by 70% alcohol pre and post running the assay.

* + 1. Remove frozen plasma samples from freezer to thaw and come to room temperature. They may be placed in DI H2O.
    2. Required sample volume is 650 ul.
    3. Go to the 6800 **Monitor Tab** and check the taskbar and messages at the top left on the monitor screen.
       1. Address any issues or maintenance due.
    4. Refill reagents and consumables as prompted by the system:
       1. Load wash reagent, lysis reagent and diluent.
       2. Load processing plates and amplification plates.
       3. Load Magnetic Glass Particles.
       4. Load test specific reagents.
       5. Load control cassettes.
       6. Load tip racks.
       7. Replace rack for clotted tips.
    5. Set the system to “Ready”.
       1. In the task overview, ensure that there is no maintenance overdue.
       2. On the **Monitoring** tab, Choose the **Start** button.
          1. The system changes to **Preparing** status.
       3. Wait for the system to change to **Ready** status before you start loading. This may take 15 minutes.
    6. Organize the Viral Load runs for the day and make Tasklists.
    7. Load sample racks onto rack trays.
       1. Cobas 6800 has LIS Order Download
       2. For any samples without LIS barcode use Rack Based Ordering, i.e. environmental samples.
          1. Designated HBV racks have Pink labels on them
          2. Sample ID must be entered in Manual Barcode Entry tab
    8. Bring racks and samples to hood for loading racks with samples.
       1. Vortex samples and discard caps
       2. Check for bubbles and remove if needed
       3. Samples can be centrifuged to collect all sample volume at bottom of tube if needed
       4. Add sample plasma tubes to sample racks
    9. Load trays with sample racks onto the Sample Supply module and go to the “Batches” tab.

**Recommendation:** Load the HBV samples after the HIV/HCV run has moved to the Processing Chamber.

* + - 1. Monitor the “error lane” for any problems
      2. After the sample barcodes are read it will make the “batch” and list the number of HBVs in the batch.
         1. **Make sure that number matches the expected number of tests**.
         2. Resolve any discrepancies
    1. Hit the “**Start Manually**” button to begin processing.
       1. At this point you may go to the Routine Tab> Test Order Status to see the finish time.
    2. Monitor the instrument during processing in the Transfer Module.
       1. Address any errors or issues.

**Note: Do not walk away from instrument until samples are moved to the Processing module**

* + 1. Unload racks and samples when finished pipetting
       1. Recap tubes in the hood with new caps and store in freezer boxes
    2. Unload consumables at the end of processing:
       1. Remove amplification plates from the analytic module.
       2. Unload empty control cassettes.
       3. Empty solid waste.
       4. Empty liquid waste.

1. **RESULTS INTERPRETATION**
   1. Reporting Results- Refer to Appendix A for LIS resulting instructions.

**Note:** All assay and run validation is performed by the cobas® 6800 Software.

**Note:** A valid run may include both valid and invalid specimen results.

* + 1. The cobas® 6800 System automatically determine the HBV RNA concentration for the samples and controls. The HBV RNA concentration is expressed in International Units per milliliter (IU/mL).
    2. For a valid batch, check each individual sample for flags in the cobas® 6800 software and/or report. The result interpretation should be as follows:

|  |  |
| --- | --- |
| **Results** | **Interpretation** |
| Target Not Detected | HBV DNA not detected.  Report results as “Not detected.” |
| < Titer Min | Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “<10 IU/mL  Titer min = 10 IU/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) IU/mL”. |
| > Titer Maxa | Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “>1000000000.”  Titer max = 1.00E+09 IU/mL |
| 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, depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.* | |

* + 1. Release Results
       1. Select all test results to be released
       2. Choose the **Release button**
          1. It is possible to release an Invalid result so use caution when releasing.
       3. Test results are sent to the SOFT Instrument Menu for posting.
    2. Invalid Patient Results
       1. An Invalid sample will be retested on the next run. If it repeats as Invalid report as Indeterminate- Suggest repeat.

1. **PROCEDURAL NOTES**
   1. Warnings and Precautions
      1. 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.
         1. For in vitro diagnostic use only.
         2. cobas® HBV has not been evaluated for use as a screening test for the presence of HBV in blood or blood products or as a diagnostic test to confirm the presence of HBV infection.
         3. 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-A4.20,21 Only personnel proficient in handling infectious materials and the use of cobas® HBV and cobas® 6800 Systems should perform this procedure.
         4. All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10).
         5. cobas® HBV/HCV/HIV-1 Control Kit and cobas® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
         6. Do not freeze whole blood or any samples stored in primary tubes.
         7. Use only supplied or specified required consumables to ensure optimal test performance.
         8. Safety Data Sheets (SDS) are available on request from your local Roche representative.
         9. 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.
         10. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
   2. Limitations
      1. cobas® HBV has been evaluated only for use in combination with the cobas® HBV/HCV/HIV-1 Control Kit, cobas® NHP Negative Control Kit, cobas omni MGP Reagent, cobas omni Lysis Reagent, cobas omni Specimen Diluent, and cobas omni Wash Reagent for use on the cobas® 6800 Systems.
      2. Reliable results depend on proper sample collection, storage and handling procedures.
      3. This test has been validated in lab only for use with EDTA plasma. Testing of other sample types may result in inaccurate results.
      4. Quantitation of HBV RNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods.
      5. Though rare, mutations within the highly conserved regions of a viral genome covered by cobas® HBV may affect primer and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus.
      6. Drug interference studies were performed in vitro and may not assess the potential interferences that might be seen after the drugs are metabolized in vivo.
      7. 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.
      8. cobas® HBV is not intended for use as a screening test for the presence of HBV in donated blood or blood products, or as a diagnostic test to confirm the presence of HBV infection.
   3. Interference
      1. Analytical specificity – Interfering substances
         1. Elevated levels of triglycerides (34.5 g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) in samples have been tested in the presence and absence of HBV DNA. The tested endogenous interferences were shown not to interfere with the test performance of cobas® HBV.
         2. The presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody were tested.
         3. Drug compounds listed in the following table were tested at three times the Cmax in presence and absence of HBV DNA.
         4. All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with cobas® HBV for all samples without HBV target and positive results were obtained on all of the samples with HBV target. Furthermore, the mean log10 titer of each of the positive HBV samples containing potentially interfering substances was within ± 0.14 log10 of the mean log10 titer of the respective positive spike control.

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

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**Appendix A**

**HBV Viral Load SCC Soft Resulting**

Test ID: **HBVL1**

Template: **HBVLD**

Workstation: **RMOLM**

1. Print a “Resulting Worklist” by Template: HBVLD
   1. Status= Pending and Nonverified
   2. Start date= go back 1 month
   3. End date= current date
   4. Received box- unchecked
   5. Check list for any old outstanding orders- investigate and resolve any issues.
   6. List will be in Order sequence number from low to high. Except:
      1. STATs will go to top of list
      2. Add ons or any order that has been changed will stay at the bottom
      3. Print list:
         1. Click Printer icon
         2. Choose Worklist- Layout Horizontal or Vertical
         3. Print to Local Printer J73
   7. Use this list to check against specimens in the freezer
2. Create a Tasklist
   1. Follow procedure for “Creating a Tasklist” in Soft Manual under TASKLIST
      1. Template= HBVLD
   2. Number the specimens according to the tasklist beginning with #1 and ending with #93
   3. Print the worklist and check it against the samples to verify both are in the same order.
3. Posting Results using LIS Interface
   1. View and Review results from the cobas 6800
   2. Click on all results to be released and click the **RELEASE** button
   3. Results will transfer to the Soft Instrument Menu
   4. From SoftLab, go to “Interfaces”, and “Instrument Menu”
   5. Select Cobas 6800 from the Instrument Menu
   6. Select “Loadlist and Today’s Results”, “Not Posted”, “By Sequence”
   7. Each order will be highlighted individually. Verify the result against the instrument printout. Click “Post All” for each order to be verified.
   8. If any Result Comments, i.e. phone reports need to be added:
      1. Do not Post result
      2. Go to “Lab Result” tab.
      3. Open “Comment” box for line HBPCR and add comment, i.e. @CALT, to the box. OK and Save
      4. Go back to “Instrument” tab and “Post” result.
   9. Check Results
      1. Go to “Resulting Worklist” by Tasklist
      2. Choose Tasklist
      3. Enter Tasklist ID
      4. Review Worklist to verify that all results have posted. They should all have “\*” next to them.
      5. Print a new pending worklist and check on any outstanding orders.