Xpert® HBV Viral Load*

Frequently Asked Questions: *Sales Relevant Information*

Q: Is the Xpert HBV Viral Load assay a diagnostic or a monitoring test?

A: This assay is used for quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma (EDTA) from chronically HBV-infected individuals using the automated GeneXpert Systems. The assay is intended for use in conjunction with clinical presentation and other laboratory markers as an indicator of disease prognosis and for use as an aid in assessing viral response to antiviral treatment as measured by changes in plasma or serum HBV DNA levels. The test is not intended to be used as a diagnostic test to confirm the presence of HBV infection.

Q: Is the Xpert HBV Viral Load assay a laboratory or a Point of Care (POC) test?

A: The Xpert HBV Viral Load assay requires EDTA whole blood to be spun down to separate the plasma and this step is normally done in a lab environment. So Xpert HBV Viral Load is a lab-based test.

Q: Will the Xpert HBV Viral Load assay work for blood donor/transplant screening?

A: The test is not intended to be used as a blood donor nor for organ donation/ transplant screening test for HBV.

Q: Will Xpert HBV Viral Load be available on Omni system?

A: Xpert HBV Viral Load is not planned for the Omni system as yet.

Q: My customer has a GeneXpert System already running Xpert HBV Viral Load. Will my customer have to buy new system or modules?

A: No, there is no change in the instrument needed. Please simply check whether your customer has the software version GX DX 4.7b or higher / INF Xpertise 6.4b or higher installed. For running the assay on an existing instrument, your customer should make sure that the Xpert HBV Viral Load assay definition file is installed.

* CE-IVD. *In Vitro* Diagnostic Medical Device. Not available in all countries. Not available in the United States.

Frequently Asked Questions: *Technical Information*

Q: Is a software upgrade required to run Xpert HBV Viral Load on the GeneXpert®/Infinity Systems?

A: Customers will need to upgrade to GeneXpert Dx software version 4.7b and for Infinity to Xpertise version 6.4b. Customers using Cepheid Link will need to upgrade to GeneXpert software version 4.8 and/or Xpertise version 6.5. If customers are connected to a Laboratory Information System (LIS), please note they will have to contact their LIS provider to implement the test settings as the result reporting will be different from that of the current Xpert HBV assay.

Q: What genotypes are detected by the Xpert HBV Viral Load assay?

A: The assay detects genotypes A to H

Q: Which are the validated collection devices to use with Xpert HBV Viral Load?

A: The validated collection devices to use with Xpert HBV Viral Load are K2-EDTA tubes, PPT-EDTA or serum collection tubes.

Q: What is the smallest sample size I can use with the Xpert HBV Viral Load assay?

A: To ensure correct quantification customers must use at least 600µL using a precision pipette; an insufficient sample volume (≤600µL) will cause an error 2097 due to the non-acceptance of the criteria to pass the Sample Volume Adequacy control (SVA).

Q: Does the assay have an Early Assay Termination (EAT) feature?

A: No, the assay does not have an Early Assay Termination feature.

Q: What are the quality controls included in the Xpert HBV Viral Load assay?

A: Each test includes a Sample Volume Adequacy (SVA) control, Internal Quantitative Standard High and Low (IQS-H and IQS-L), which is also a sample processing control (SPC), a Probe Check Control (PCC) and Lot Specific Parameters (LSP) for quantification.

Q: What is the purpose of the Lot Specific Parameters (LSP) control?

A: Each kit lot has built-in LSP generated from a HBV calibration panel, traceable to the 4th WHO International Standard for HBV (NIBSC code: 10/266), and the Internal Quantitative Standard High and Low (IQS-H and IQS-L). The LSP are unique for the reagent lot and are used to ensure correct quantification.

Q: What are the recommended storage conditions for the kits?

A: Customer will have to store the Xpert HBV Viral Load cartridges at 2–35 °C until the expiration date provided on the label.

Q: How do I have to store samples?

A: Whole blood may be held at 2-35°C for up to 24 hours or at 2–8°C for up to 3 days prior to plasma/serum preparation. Centrifugation should be performed according to the manufacturer instructions.

- After centrifugation and separation, plasma and serum may be held at 2–35°C for up to 24 hours or at 2–8°C for up to 7 days prior to testing.
- Plasma and serum specimens are stable frozen (-80 to -20°C) for 6 weeks.
- Plasma and serum specimens are stable for up to three freeze/thaw cycles.
- Plasma and serum specimens must be thawed and equilibrated to room temperature prior to transfer to the cartridge.

FAQs: *Technical Information (continued)*

Q: Why do I need to collect 600µL with a precision pipette and 1mL with the plastic pipette provided in the kit?

A: 600µl sample has to be added to the cartridge for correct sample processing. The transfer pipette provided in the kit has a low accuracy and will transfer less volume than the mark indicates (partly because the viscosity of plasma/serum). Transferring the volume up to the 1 mL mark ensures that ≥ 600 µl sample has been added.

Q: How long can samples be held in the cartridge before starting the test?

A: The samples may be kept in the cartridge prior to testing for up to 4 h at 5–35°C.

Frequently Asked Questions: *Operational Information*

Q: Will there be any external control material for validation?

A: External control material provided by Acrometrics and Qnostics has been tested with the Xpert HBV Viral Load assay and work well. Equivalent controls from Exact Diagnostics do not perform well. Please contact technical support for further information

Q: Does the test have potentially interfering substances?

A: The susceptibility of the Xpert HBV Viral Load assay to interference by elevated levels of endogenous substances, by autoimmune disease markers, and by drugs prescribed to HBV infected patients was evaluated. The inhibitory effects were evaluated both in the presence and absence of approximately 30 IU/mL HBV DNA reference material (4th WHO International Standard for HBV, NIBSC code: 10/266).

Elevated levels of the endogenous substances listed in the table below were shown not to interfere with the quantification of the Xpert HBV Viral Load assay with the mean log10 titer of each of the positive HBV samples containing potentially interfering substances within ± 0.10 log10 IU/mL of the positive control. Negative results were obtained for all samples without HBV target demonstrating there was no impact on the assay specificity.

Substance	Tested Concentration
Albumin	9 g/dL
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Human DNA	0.4 mg/dL
Triglycerides	3000 mg/dL

The drug components as presented in the table below were shown to not interfere with the quantification of the Xpert HBV Viral Load assay or impact the assay specificity when tested at three times peak plasma level concentration (Cmax) in the presence and absence of HBV DNA.

Pool	Drugs
1	Zidovudine, Saquinavir, Clarithromycin, Interferon-alfa-2b, Ritonavir, Ombitasvir, Paritaprevir, Dasabuvir, Didanosine
2	Abacavir Sulfate, Fosamprenavir, Peginterferon-alfa-2a, Ribavirin, Entecavir, Adefovir Dipivoxil
3	Tenofovir disoproxil fumarate, Lamivudine, Indinavir sulfate, Ganciclovir, Valganciclovir HCl, Acyclovir, Paroxetine, Telbivudine
4	Stavudine, Efavirenz, Lopinavir, Enfuvirtide, Ciprofloxacin Fluoxetin
5	Nevirapine, Nelfinavir, Azithromycin, Valacyclovir, Sertraline, Tenofovir, Alafenamide

Testing of K_2EDTA plasma specimens from five individuals positive for each of the autoimmune disease markers systemic lupus erythematosus (SLE), anti-nuclear antibody (ANA), or rheumatoid factor (RF) showed no interference with the performance of the Xpert HBV Viral Load assay. The mean $\log_{10}$ concentrations of samples spiked with Xpert HBV Viral Load DNA were within ± 0.10 $\log_{10}$ IU/mL of the positive control. Negative results were obtained for all samples without HBV target demonstrating there was no impact on the assay specificity.

Q: Does the assay have any limitations?

A: Rare mutations within the target region of the Xpert HBV Viral Load assay may affect primer or probe binding resulting in under quantitation or failure to detect the virus. The assay has been validated only for use with serum and EDTA plasma. Good laboratory practices and changing gloves between the handling of specimens are recommended to avoid contamination of specimens or reagents.

FAQs: *Operational Information (continued)*

Q: Can we get HBV Viral Load results in copies/mL also?

A: The Xpert HBV Viral Load assay does not provide an answer in copies/mL. No conversion factor has been established for the Xpert HBV Viral Load assay.

Q: Why is HBV Viral ideal over HBsAg?

A: There are Hepatitis B viruses that are HBsAg negative providing a HBsAg negative result despite a present HBV infection. The Xpert HBV Viral Load assay detects both HBsAg positive and negative Hepatitis B viruses.

Q: What does a test result "HBV detected less than 10 IU/mL" imply?

A: The quantitative range of the Xpert HBV Viral Load assay is 10 - 1 000 000 000 IU/mL. A result ≤ 10IU/mL indicates that the patient has an HBV infection but the virus titer is too low to be determined with the Xpert HBV Viral Load assay.

Q: Does the result "HBV not detected" indicate infection is cured?

A: No. This result only indicates that HBV virus was not detected at the time of evaluation. This may be because the patient is on therapy or is a patient who does not have viremia.

Q: What are the recommended storage and transport conditions for the samples?

A: Whole blood may be held at 2-35°C for up to 24 hours or at 2–8°C for up to 3 days prior to plasma/serum preparation.

After centrifugation and separation, plasma and serum may be held at 2–35 °C for up to 24 hours or at 2–8 °C for up to 7 days prior to testing.

- Plasma and serum specimens are stable frozen (-80 to -20°C) for 6 weeks.
- Plasma and serum specimens are stable for up to three freeze/thaw cycles.
- Plasma and serum specimens must be thawed and equilibrated to room temperature prior to transfer to the cartridge.

Transportation of whole blood, plasma, or serum specimens must comply with country, federal, state, and local regulations for the transportation of etiologic agents.

Q: What will be the Test run Time (TRT) for this new test?

A: The TRT for the Xpert HBV Viral Load assay is approximately 59 minutes.