GeneXpert®
Powered By CEPHEID INNOVATION

Xpert® HBV Viral Load

REF **GXHBV-VL-CE-10**

Instructions for Use

CE 2797 **IVD**

Cepheid®

In Vitro Diagnostic Medical Device

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Xpert® HBV Viral Load

For *In Vitro* Diagnostic Use.

1 Proprietary Name

Xpert® HBV Viral Load

2 Common or Usual Name

Xpert HBV VL

3 Intended Use

The Cepheid Xpert® HBV Viral Load (VL) test is an *in vitro* nucleic acid amplification test designed for the quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma (EDTA) from chronically HBV-infected individuals using the automated GeneXpert® Systems.

The test 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 donor screening test for HBV, or to be used as a diagnostic test to confirm the presence of HBV infection.

4 Summary and Explanation

Hepatitis B Virus (HBV) is a small, enveloped DNA virus in the family Hepadnaviridae and is responsible for acute and chronic HBV hepatitis. The virus has a small circular DNA genome that is partially double-stranded, partially single-stranded, and is 42nm in diameter. HBV contains numerous antigenic components which include hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg). HBV is transmitted by percutaneous or mucosal exposure to the blood or body fluids of an infected person, from an infected mother to her newborn, through close contact within households, through unscreened blood transfusion or unsafe injections in healthcare settings, through injection drug use, and from sexual contact with an infected person.

Chronic Hepatitis B (CHB) may present either as hepatitis B e antigen (HBeAg)-positive or HBeAg-negative CHB. Age-specific HBsAg seroprevalence varies markedly by geographical region, with the highest prevalence (>5%) in sub-Saharan Africa, East Asia, some parts of the Balkan regions, the Pacific Islands and the Amazon Basin of South America. Prevalence below 2% is seen in regions such as Central Latin America, North America and Western Europe. Overall, almost half of the global population lives in areas of high endemicity.[1] Morbidity and mortality of CHB are linked to persistence of viral replication and evolution to cirrhosis and/or hepatocellular carcinoma (HCC).[2] Mortality from viral hepatitis has increased over time and will continue to rise unless people are diagnosed and treated.[3]

HBV vaccine is available for infants and has considerably reduced the number of new chronic infections but coverage is only 39%.[3] In 2015, 3.5% of the world's population was living with chronic HBV infection with the Western Pacific and African regions being the most heavily affected areas.[3] Only 9% of those with HBV knew their diagnosis, and of those diagnosed, only 8% were receiving therapy.[3] Nucleoside and nucleotide analogues, such as tenofovir and entecavir, are recommended for those eligible for therapy as these antiviral agents are effective at suppressing HBV replication, preventing progression to cirrhosis, and reducing liver-related deaths.[1] Therapy for HBV is continued lifelong.[1]

5 Principle of the Procedure

The Xpert® HBV VL test is an automated test for quantitative detection of the Hepatitis B virus. The test is performed on the Cepheid GeneXpert and GeneXpert Infinity Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR. The systems consist of an instrument, a personal computer and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the purification and PCR processes. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx Operator Manual* or *GeneXpert Infinity Operator Manual*.

The Xpert® HBV VL test includes reagents for the detection of HBV DNA in specimens as well as two internal controls used for quantitation of HBV DNA. The internal controls are also used for adequate processing of the target and to monitor the presence of inhibitor(s) in the PCR reactions. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The test is standardized against 4th World Health Organization (WHO) International Standard for HBV DNA for Nucleic Acid Amplification Technologies (NIBSC code: 10/266).[4]

6 Reagents and Instruments

6.1 Materials Provided

The HBV VL test kit contains sufficient reagents to process 10 specimens and/or quality control samples. The kit contains the following:

HBV VL Cartridges with Integrated Reaction Tubes	**10**
● Bead 1, Bead 2 and Bead 3 (freeze-dried)	1 of each per cartridge
● Lysis Reagent (Guanidinium Thiocyanate)	1.7 mL per cartridge
● Rinse Reagent	0.5 mL per cartridge
● Elution Reagent	1.5 mL per cartridge
● Binding Reagent	1.5 mL per cartridge
● Proteinase-K Reagent	0.48 mL per cartridge
Disposable 1 mL Transfer Pipettes	**10 per kit**
CD	**1 per kit**
● Assay Definition File (ADF)	
● Instructions to Import ADF into GeneXpert and Infinity Software	
● Instructions for Use (Package Insert)	

Note Safety Data Sheets (SDS) are available are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post- mortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert® HBV VL cartridges at 2–35 °C until the expiration date provided on the label.
- Bring the cartridges to room temperature prior to use if they have been stored cold.
- Do not use cartridges that have passed the expiration date.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that has leaked.

8 Materials Required but Not Provided

- GeneXpert® Dx Instrument System or GeneXpert® Infinity Instrument System (catalog number varies by configuration): GeneXpert instrument, computer with proprietary GeneXpert Software Version 4.7b or higher (GeneXpert Dx systems) or Xpertise 6.4b or higher (Infinity-80/Infinity-48s), barcode scanner, and appropriate GeneXpert Instrument System operator manual.
- Printer: If a printer is needed, contact Cepheid Technical Support to arrange for the purchase of a recommended printer.
- Bleach or sodium hypochlorite
- Denatured ethanol

9 Warnings and Precautions

9.1 General

- For *in vitro* diagnostic use.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be treated with standard precautions. Guidelines for sample handling are available from the U.S. Centers for Disease Control and Prevention[5] and the Clinical and Laboratory Standards Institute.[6]
- Good laboratory practices, including changing gloves between handling samples, are recommended to avoid contamination of samples or reagents.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not substitute Xpert HBV VL test reagents with other reagents.
- Do not open the Xpert HBV VL test cartridge lid until ready to add the sample.
- Do not use a cartridge that has been dropped after removing from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the lid may yield invalid results.
- Do not use a cartridge that has a damaged reaction tube.
- Do not cover the barcode label on the cartridge.
- Use either a transfer pipette or precision pipette to add the sample to the cartridge. Do not pour the sample directly from the collection device into the cartridge.
- Each single-use Xpert HBV VL cartridge is used to process one test. Do not reuse cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse spent disposable pipettes.
- Wear clean lab coats and gloves. Change gloves between processing each sample.
- In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with a freshly prepared solution of 0.5% sodium hypochlorite (or a 1:10 dilution of household chlorine bleach). Follow by wiping the surface with 70% ethanol. Let the work surfaces dry completely before proceeding.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution’s environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific national or regional disposal procedures. If national or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.[7]

10 Chemical Hazards[8,9]

Lysis Reagent (Guanidinium Thiocyanate)

- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - Causes mild skin irritation
  - Causes eye irritation
- **UN GHS Precautionary Statements**
  - **Prevention**

- Wash thoroughly after handling.

- **Response**
  - If skin irritation occurs: Get medical advice/attention.
  - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - If eye irritation persists: Get medical advice/attention.
  - Call a POISON CENTER or doctor/physician if you feel unwell.

11 Specimen Collection, Transport and Storage

Whole blood should be collected in K_2-EDTA tubes, PPT-EDTA or serum collection tubes and centrifuged to separate the plasma/serum and red blood cells per the manufacturer's instructions.

- A minimum of 0.6 mL plasma or serum is required for the Xpert HBV VL test. If using the transfer pipette included in the kit, the pipette must be filled to the fourth mark (1.0 mL) with plasma or serum. Alternatively, if using a precision pipette, 0.6 mL plasma or serum is required. See the instructions in Section 12.2, Option 1 and Option 2, respectively.
- 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.
- Transportation of whole blood, plasma or serum specimens must comply with country, federal, state and local regulations for the transportation of etiologic agents.

12 Procedure

12.1 Preparing the Specimen

Note Start the test within 4 hours of adding the sample to the cartridge.

1. Following centrifugation of whole blood specimens, plasma may be pipetted directly into the cartridge. Sufficient volume is critical to obtaining valid test results (see instructions in Section 12.2. Preparing the Cartridge).
2. If using frozen specimens, place the specimens at room temperature (20–35°C) until completely thawed and equilibrated to room temperature before use.
3. Plasma and serum specimens stored at 2–8°C should be removed from the refrigerator and equilibrated to room temperature before use.
4. Plasma specimens stored at 2–8°C or frozen and thawed should be vortexed for 10 seconds before use. If the specimen is cloudy, clarify by a quick centrifugation.

12.2 Preparing the Cartridge

1. Wear protective disposable gloves.
2. Bring the cartridges to room temperature prior to use if they have been stored cold.
3. Inspect the cartridge for damage. If damaged, do not use it.
4. Label the cartridge with the sample identification.
5. Open the cartridge lid.
6. Add the sample to the cartridge.

   - **Option 1:** If using the transfer pipette included in the kit (see Figure 1), fill the pipette to the fourth mark (1.0 mL) or slightly above with plasma or serum from the collection tube. Empty the contents of the pipette into the sample chamber of the cartridge (see Figure 2).

- **Option 2:** If using a precision pipette, transfer 0.6 mL plasma or serum from the collection tube into the sample chamber of the cartridge (see Figure 2).

Note Do not remove the thin plastic film that covers the inner ring of 13 ports of the cartridge.

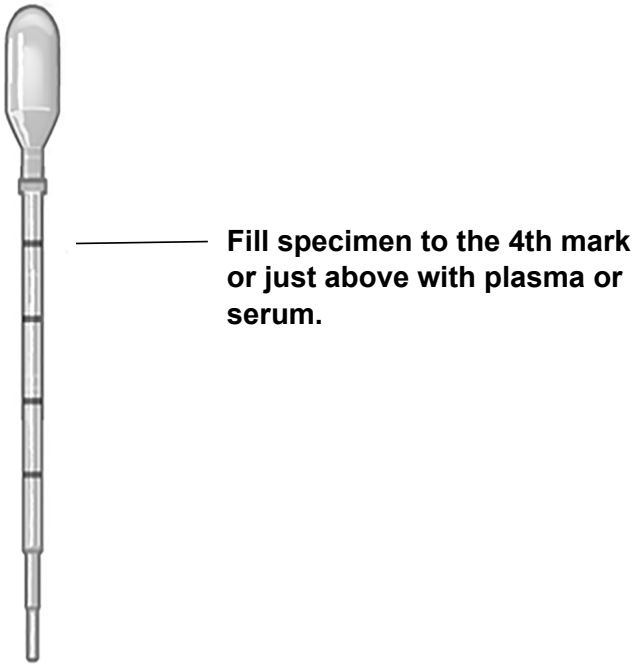

Figure 1. Xpert HBV VL Test Transfer Pipette

7. Close the cartridge lid. Ensure the lid snaps firmly into place.

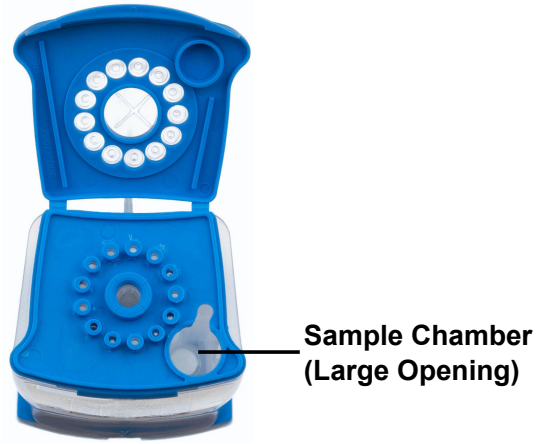

Figure 2. Xpert HBV VL Cartridge (Top View)

12.3 Starting the Test

Important **Before starting the test, make sure that the Xpert HBV VL Assay Definition File is imported into the software.**

This section lists the basic steps for running the test. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model of instrument that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert instrument system:
   - If using the GeneXpert Dx instrument, first turn on the GeneXpert Dx instrument and then turn on the computer. The GeneXpert Dx software will launch automatically or may require double-clicking the GeneXpert Dx software shortcut icon on the Windows® desktop.

     or
   - If using the GeneXpert Infinity instrument, power up the instrument. The GeneXpert software will launch automatically or may require double clicking the Xpertise software shortcut icon on the Windows® desktop.
2. Log on to the GeneXpert Instrument System software using your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or click **Orders** and **Order Test** (Infinity). The **Create Test** window opens.
4. Scan in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test results.
5. Scan in Sample ID or type the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test results.
6. Scan the barcode on the Xpert HBV VL test cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.

Note If the barcode on the Xpert HBV VL cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity). Type your password in the dialog box that appears.
8. For the GeneXpert Infinity System, place the cartridge on the conveyor belt. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed into the waste container.

   or

   For the GeneXpert Dx Instrument:

   a) Open the instrument module door with the blinking green light and load the cartridge.
   b) Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
   c) Wait until the system releases the door lock before opening the module door and removing the cartridge.
   d) Dispose of used cartridges in the appropriate specimen waste container according to your institution's standard practices.

13 Viewing and Printing Results

This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual* depending upon the instrument used.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the **View Results** window to view and/or generate a PDF report file.

14 Quality Control

Each test includes a Sample Volume Adequacy (SVA), Internal Quantitative Standard High and Low (IQS-H and IQS-L), Lot Specific Parameters (LSP) and a Probe Check Control (PCC).

- **Sample Volume Adequacy (SVA)** – Ensures the sample has been correctly added to the cartridge. The SVA verifies that the correct volume of sample has been added in the sample chamber. The SVA passes if it meets the validated acceptance criteria. If the SVA does not pass, either **Error 2096** will be displayed if no sample has been added to the cartridge or **ERROR 2097** if an insufficient quantity of sample has been added to the cartridge. The system will prevent the user from resuming the test.
- **Internal Quantitative Standard High and Low (IQS-H and IQS-L)** – IQS-H and IQS-L are two linearized plasmids with a sequence unrelated to HBV that are included in each cartridge and go through the entire test process. These are standards used to calculate the HBV DNA concentration in the sample. In addition, the IQS-H and IQS-L detect sample-

associated inhibition of the real-time PCR assay, thereby acting as sample processing controls. IQS-H and IQS-L pass if they meet the validated acceptance criteria.

- **Lot Specific Parameters (LSP) for Quantification** – 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)[4], and the IQS-H and IQS-L. The LSP are unique for the reagent lot and are used to ensure correct quantification.
- **Probe Check Control (PCC)** – Before the start of the PCR reaction, the GeneXpert Instrument System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity and dye stability. The PCC passes if the fluorescence signals meet the validated acceptance criteria.
- **External Controls** – Following good laboratory practice, external controls, not provided in the kit, should be used in accordance with the requirements of local and state accrediting organizations, as applicable.

15 Interpretation of Results

The results are interpreted automatically by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms, and are shown in the View Results window (see Figure 3 through Figure 8). The possible results are shown in Table 1.

Table 1. Xpert HBV VL Test Results and Interpretation

Result	Interpretation
HBV DETECTED IU/mL (log X.XX) See Figure 3.	The HBV DNA is detected at XX IU/mL (log X.XX). ● The HBV DNA has a titer within the quantitative range of the test (10-1.00E09 IU/mL. ● IQS-H and IQS- L: PASS. ● Probe Check – PASS; all probe check results pass.
HBV DETECTED >1.00E09 IU/mL See Figure 4.	The HBV DNA is detected above the quantitative range of the test. ● IQS-H and IQS- L: PASS. ● Probe Check – PASS; all probe check results pass.
HBV DETECTED <10 IU/mL See Figure 5.	The HBV DNA is detected below the quantitative range of the test. ● IQS-H and IQS- L: PASS. ● Probe Check – PASS; all probe check results pass.
HBV NOT DETECTED See Figure 6.	HBV DNA is not detected. ● IQS-H and IQS- L: PASS. ● Probe Check – PASS; all probe check results pass.
INVALID See Figure 7.	Presence or absence of the HBV DNA cannot be determined. Repeat test according to the instructions in Section 16.2. Retest Procedure. ● IQS-H and/or IQS- L: FAIL; Cycle threshold (Ct) values are not within the valid range. ● Probe Check – PASS; all probe check results pass.
ERROR See Figure 8.	Presence or absence of the HBV DNA cannot be determined. Repeat test according to the instructions in Section 16.2. Retest Procedure. ● Probe Check – FAIL *; all or one of the probe check results fail. * If the probe check passed, the error is caused by the maximum pressure limit exceeding the valid range or by a system component failure.
NO RESULT	Presence or absence of HBV DNA cannot be determined. Repeat test according to the instructions in Section 16.2. Retest Procedure. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Note Test screenshots are for example only. The version number may vary from the screenshots shown in these instructions for use.

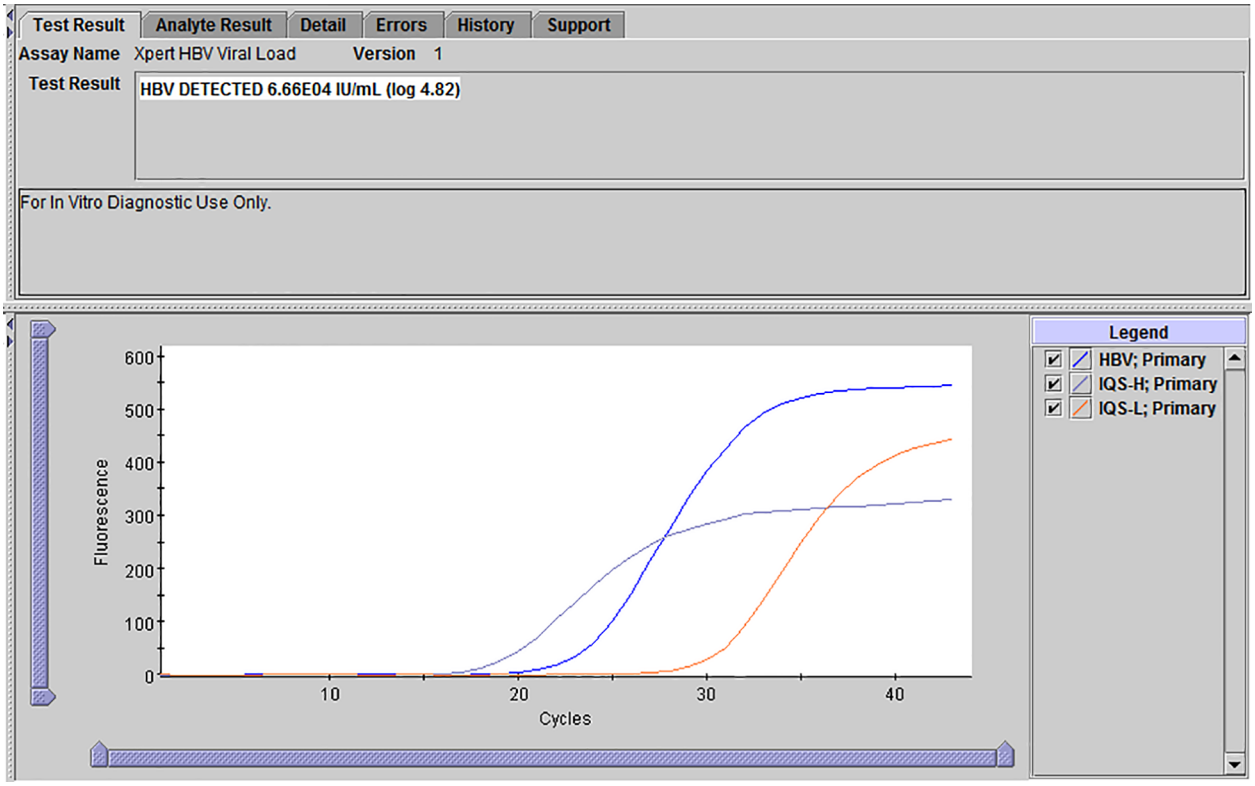

Figure 3. Result: HBV Detected and Quantified

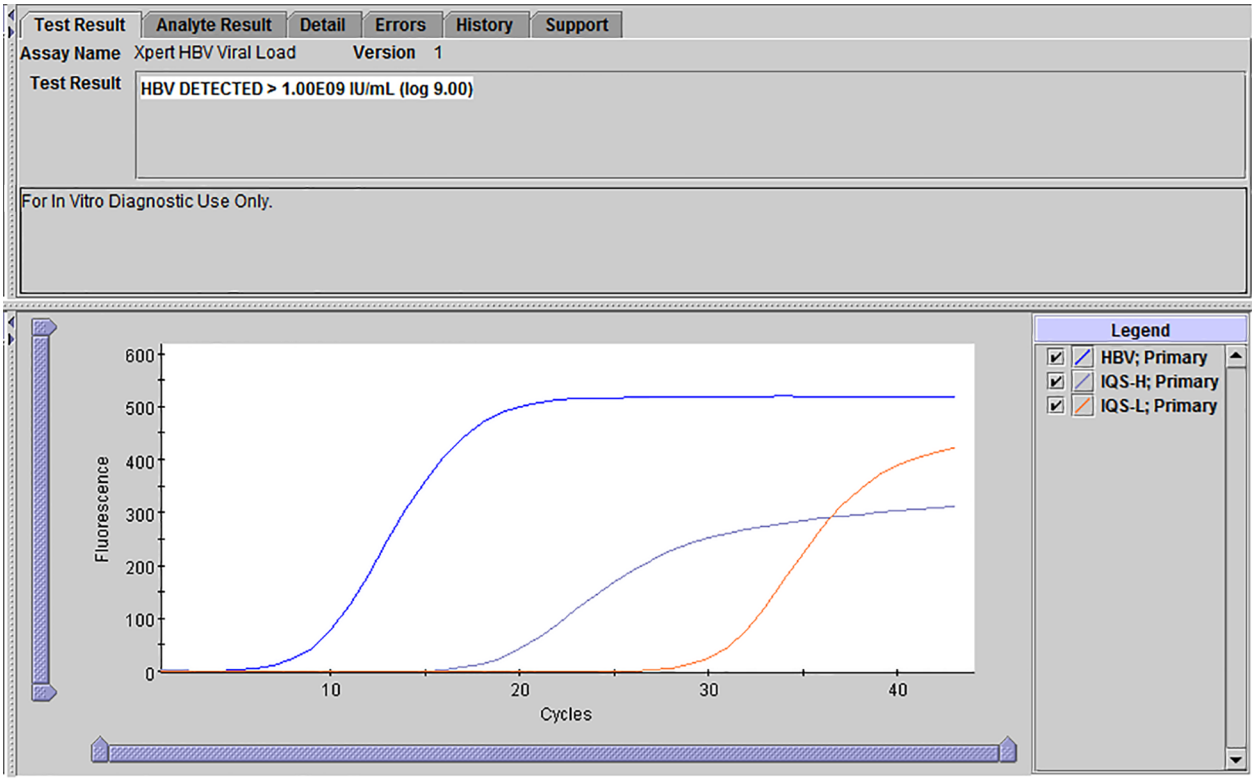

Figure 4. Result: HBV Detected but with Titer Above the Quantitative Range of the Test

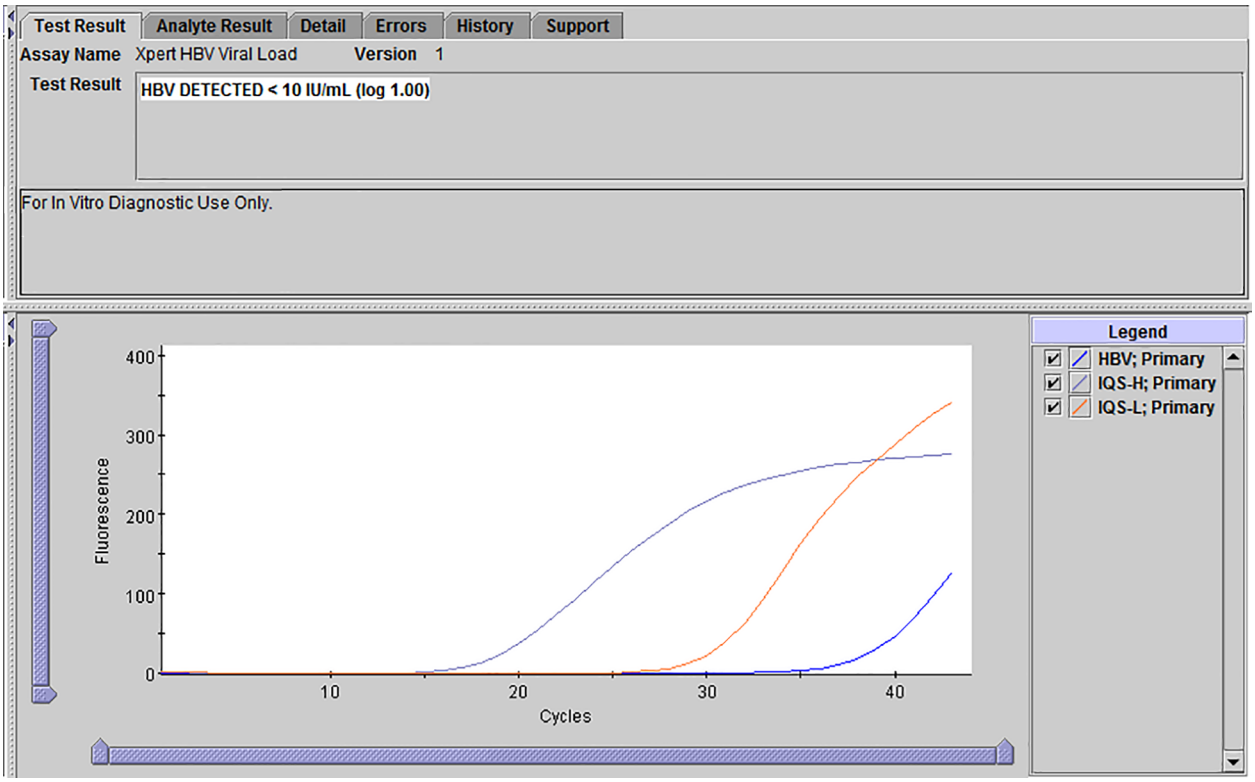

Figure 5. Result: HBV Detected but with Titer Below the Quantitative Range of the Test

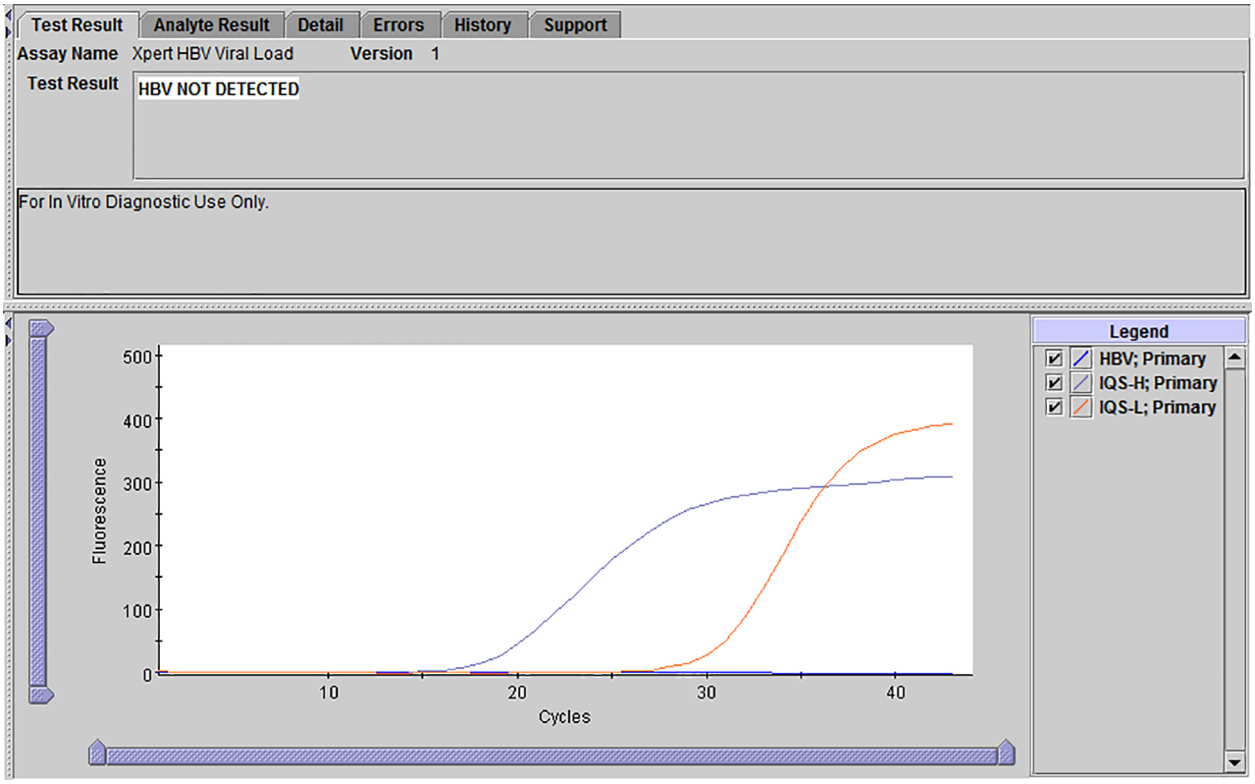

Figure 6. Result: HBV Not Detected

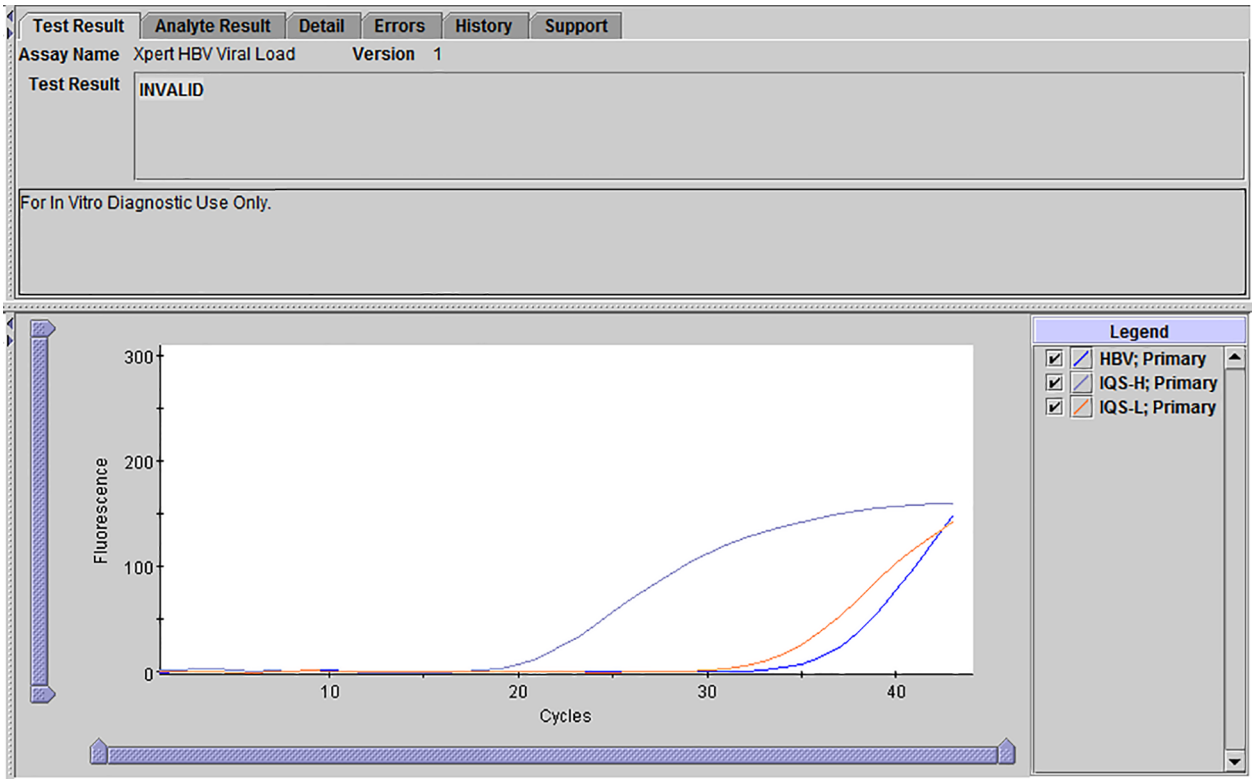

Figure 7. Result: Invalid Result

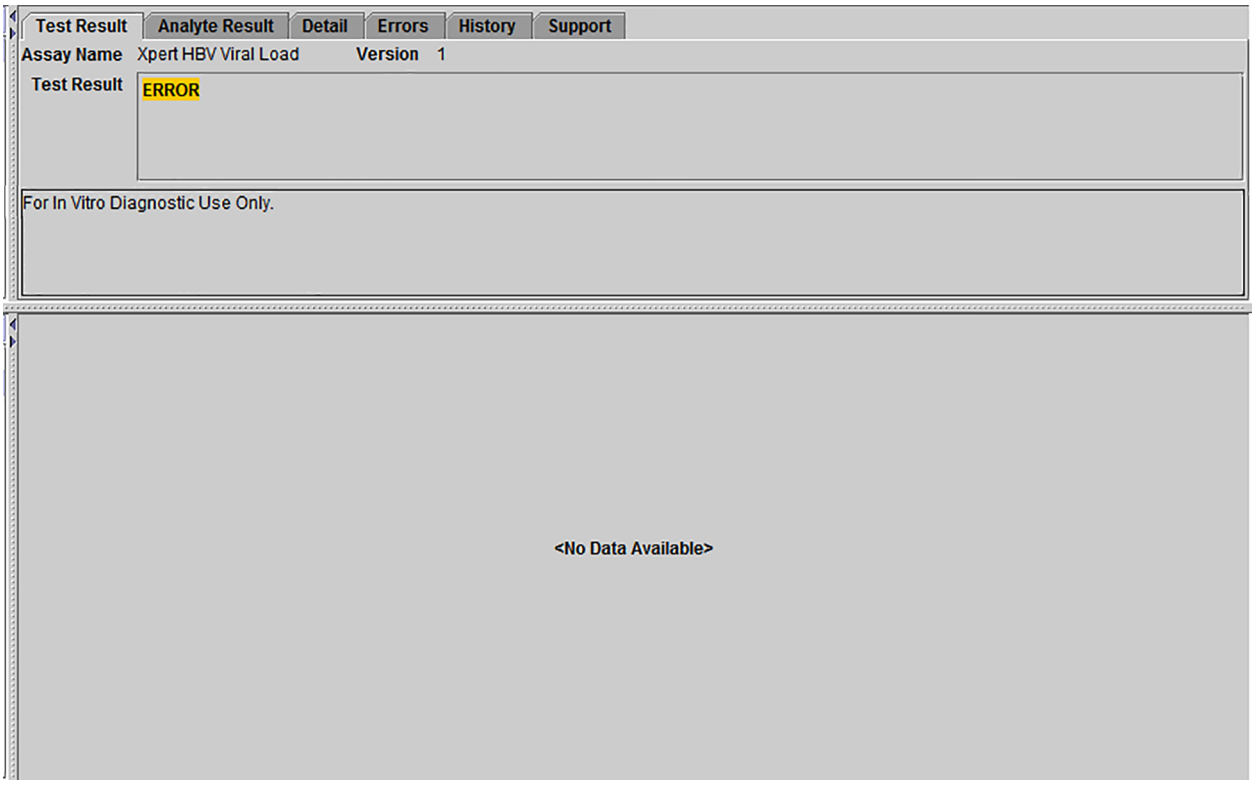

Figure 8. Result: Error

16 Retests

16.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test according to instructions in the Section 16.2. Retest Procedure.

- An **INVALID** result indicates one or more of the following:
  - The IQS-H and/or IQS-L Ct values are not within the valid range.
  - The sample was not properly processed or PCR was inhibited.
- An **ERROR** result indicates that the test was aborted. Possible causes include: insufficient volume of sample was added, the reaction tube was filled improperly, a reagent probe integrity problem was detected, or the maximum pressure limit was exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress or a power failure occurred.

16.2 Retest Procedure

If the result of a test is either **INVALID**, **ERROR**, or **NO RESULT**, use a new cartridge to retest the affected specimen (do not reuse the cartridge).

1. Remove a new cartridge from the kit.
2. Follow the procedures in Section 12. Procedure, including Section 12.2. Preparing the Cartridge and Section 12.3. Starting the Test.

17 Limitations

- Good laboratory practices and changing gloves between the handling of specimens are recommended to avoid contamination of specimens or reagents.
- Rare mutations within the target region of the Xpert HBV VL test may affect primer or probe binding resulting in under-quantitation or failure to detect the virus.
- This test has been validated only for use with serum and EDTA plasma. Testing of other sample types may result in inaccurate results.
- A negative test result does not preclude HBV infection. Therefore, the Xpert HBV VL test should not be used as a diagnostic test to confirm the presence of HBV infection.

18 Performance Characteristics

18.1 Limit of Detection

The limit of detection (LOD) of the HBV VL assay was determined for HBV genotype A by testing serial dilutions of the 4th WHO International Standard for HBV DNA (NIBSC code 10/266)[4] diluted in HBV-negative EDTA plasma and serum. Panels of six concentration levels and a negative were tested using either four or three reagent lots for EDTA plasma and serum panels, respectively. Each panel member was tested across three days with 24 replicates per reagent lot. In total 96 replicates per plasma panel member and 72 replicates per serum panel member were tested.

The results for EDTA plasma and serum are shown in Table 2. The study demonstrated that the HBV VL assay detected HBV DNA for the WHO International Standard at a concentration of 3.20 IU/mL in EDTA plasma and a concentration of 5.99 IU/mL in serum with a positivity rate of 95% as determined by PROBIT regression.

Table 2. Limit of Detection for the Xpert HBV VL Assay using the 4th WHO International Standard for HBV

Genotype	Matrix	Nominal HBV Concentration (IU/mL)	Number of Valid Replicates	Number of Positives	Positivity Rate (%)	95% LOD by PROBIT (95% Confidence Interval)
A	Plasma	10	95	95	100	3.20 IU/mL (2.79 – 3.60 IU/mL)
		5	96	94	98	
		2.5	96	82	85	
		1.25	96	62	65	
		0.625	96	41	43	
		0	96	0	0	
A	Serum	10	72	70	97	5.99 IU/mL (5.13 – 6.86 IU/mL)
		5	72	63	88	
		2.5	72	58	81	
		1.25	72	37	51	
		0.625	71	15	21	
		0	72	0	0	

The limit of detection for HBV genotypes B through H was determined by testing six- or seven-member panels prepared by spiking HBV positive specimens representing each genotype (genotypes B through G from the WHO International Reference Panel, PEI code: 5086/08, and a genotype H clinical specimen) into HBV-negative EDTA plasma. Each panel member was tested over three days using three reagent lots for a total of 24 replicates per member. The results are presented in Table 3.

Table 3. Limit of Detection for HBV Genotypes B through H in EDTA Plasma

Genotype	95% LOD by PROBIT (IU/mL)	95% Confidence Interval (IU/mL)
B	1.34	0.98 – 1.69
C	1.63	1.23 – 2.03
D	3.96	3.01 – 4.92
E	3.77	2.76 – 4.78
F	2.39	1.82 – 2.96
G	1.21	0.95 – 1.47
H	3.84	2.91 – 4.77

The limit of detection for HBV genotypes B through H was verified in serum in accordance with CLSI EP17-A2[10] using 24 replicates. A higher concentration was tested if a positivity rate of > 85% was not achieved. See Table 4 for results.

Table 4. Verification of LOD for Genotypes B through H in Serum

Genotype	Nominal HBV Concentration (IU/mL)	Positivity Rate (%)
B	1.34	88
C	3.25	96
D	3.96	96
E	3.77	96

Genotype	Nominal HBV Concentration (IU/mL)	Positivity Rate (%)
F	2.39	92
G	1.21	88
H	3.84	100

The performance of the HBV VL assay was also evaluated with a precore mutant by testing a sequenced HBV clinical specimen including the two precore mutations (C1858T and G1896A) and the two basal core promoter mutations (A1762T and G1764A), diluted to a concentration of 10 IU/mL in EDTA plasma and serum with one reagent lot. A positivity rate of 100% was achieved for each of the 24 replicates tested in each matrix.

18.2 Lower Limit of Quantitation (LLOQ)

The lower limit of quantitation (LLOQ) is defined as the lowest concentration of HBV DNA that is quantified with acceptable precision and trueness and is determined using the total analytical error (TAE) and an approach based upon the difference between two measurements. The LLOQ was evaluated with four independent samples, representing HBV genotypes A through D, in EDTA plasma near the test limit of detection. Each sample was tested using four reagent lots with 8-24 replicates per lot. TAE was estimated with the Westgard model according to the CLSI guideline EP17-A2[10] with the criterion, [(Absolute Bias) + 2 SDs ≤ 1 $\log_{10}$ IU/mL]. The difference between two measurements approach was evaluated with the criterion, [(2 x SQRT(2) x SD) ≤ 1 $\log_{10}$ IU/mL].

The LLoQ analyses for each sample are presented in Table 5.

Table 5. Determination of the LLOQ for the Xpert HBV VL Test

HBV Genotype	Lot	N	HBV Concentration ($\log_{10}$ IU/mL)		Bias	Total SD	Total Analytical Error[a]	Two Measurement Approach[b]
			Expected	Observed				
A	1	24	1.00	1.02	0.02	0.20	0.42	0.57
	2	24	1.00	1.05	0.05	0.16	0.37	0.45
	3	24	1.00	0.94	-0.06	0.20	0.46	0.57
	4	23	1.00	1.02	0.02	0.14	0.30	0.40
B	1	16	1.00	1.18	0.18	0.11	0.39	0.30
	2	24	1.00	1.18	0.18	0.17	0.53	0.49
	3	8	1.00	1.17	0.17	0.19	0.54	0.53
	4	8	1.00	1.25	0.25	0.19	0.64	0.55
C	1	16	1.00	1.10	0.10	0.17	0.44	0.47
	2	24	1.00	1.11	0.11	0.22	0.55	0.61
	3	8	1.00	0.83	-0.17	0.24	0.65	0.68
	4	8	1.00	1.01	0.01	0.18	0.36	0.50
D	1	16	1.00	0.81	-0.19	0.28	0.74	0.78
	2	24	1.00	0.79	-0.21	0.27	0.75	0.76
	3	8	1.00	0.83	-0.14	0.14	0.42	0.39
	4	8	1.00	0.91	-0.09	0.11	0.31	0.32

[a] TAE calculated according to the Westgard model where [TAE = | Bias | + (2×SD) ≤ 1 $\log_{10}$ IU/mL] ensuring there is a 95% probability that the measurement will be less than 1 $\log_{10}$ IU/mL from the true value.

[b] Two measurements approach [2 × (SQRT(2) × SD) ≤ 1 $\log_{10}$ IU/mL] indicates that a difference of less than 1 $\log_{10}$ IU/mL can be explained by a random measurement error.

The results demonstrate that the Xpert HBV VL test can quantify 10 IU/mL of HBV DNA with an acceptable trueness and precision.

18.3 Precision/Reproducibility

The precision/reproducibility of the Xpert HBV VL test was evaluated in K_2EDTA plasma using an analysis of variance (ANOVA) to estimate total variance.

This study was a multi-center (3 sites; 2 external and 1 internal) blinded study to estimate the major components of variance of the Xpert HBV VL test using an eight-member panel consisting of eight HBV positive members. The HBV positive members were prepared by diluting either a well characterized HBV plasmid or a HBV positive clinical specimen into human EDTA plasma. Two operators, one with prior PCR experience and one without, at each of the three study sites, tested one panel in duplicate, two times per day (equivalent to eight replicates per day) over six testing days for a total of 144 replicates per panel member. Three lots of the Xpert HBV VL test were used, with each lot representing two days of testing. Precision and reproducibility were evaluated in accordance with CLSI EP05-A3[11] and CLSI EP15-A3.[12]

The precision and reproducibility of the Xpert HBV VL test was evaluated by using nested ANOVA with terms for Site/Instrument, Lot, Day, Operator/Run and Within-Run. The standard deviation and the percentage of variability due to each component of the $\log_{10}$ HBV transformed concentrations were calculated as shown in Table 6.

Table 6. Precision/Reproducibility of the Xpert HBV VL Test

HBV DNA Concentration (Log_{10}IU/mL)			Contribution to Total Variance SD (CV%)										Total Precision	
			Site/ Instrument		Lot		Day		Operator/ Run		Within-run			
Expected	Observed	N	SD	(%)[a]	SD	(%) [a]	SD	(%) [a]	SD	(%) [a]	SD	(%) [a]	SD	CV (%)[b]
9.00	9.13[c]	144	<0.01	<0.01	0.04	23.4	<0.01	<0.01	0.02	4.9	0.07	71.7	0.08	19.7
8.00	8.17	144	<0.01	<0.01	0.04	26.7	<0.01	<0.01	0.02	5.4	0.06	67.9	0.07	16.9
7.00	7.15	144	0.01	2.2	0.03	12.2	0.01	3.9	<0.01	<0.01	0.07	81.8	0.07	16.8
6.00	6.18	144	<0.01	<0.01	0.04	32.1	0.01	4.3	<0.01	<0.01	0.05	63.6	0.06	14.7
4.70	4.87	144	0.02	4.5	0.03	15.3	<0.01	<0.01	<0.01	<0.01	0.07	80.2	0.07	17.1
3.00	3.19	144	<0.01	<0.01	0.03	28.8	<0.01	<0.01	0.02	11.5	0.04	59.7	0.06	13.2
2.00	2.17	144	<0.01	<0.01	0.02	8.6	<0.01	<0.01	0.01	1.0	0.08	90.5	0.08	19.0
1.00	1.13	144	<0.01	<0.01	<0.01	<0.01	0.05	11.0	0.01	0.3	0.15	88.8	0.16	37.7

[a] (%) is contribution of variance component to overall variance

[b] "CV" is lognormal CV, as obtained using the formula: $Lognormal\ CV(\%) = 100 * \sqrt{10^{\left(\ln(10)*\sigma^2_{log10\_data}\right)} - 1}$

[c] Observed value is above the quantitative range of the Xpert HBV VL test.

18.4 Linear Range

Genotype A

The linear range of the Xpert HBV VL test was determined by analysis of an eight member panel covering a HBV concentration range from 1.00 – 9.00 $\log_{10}$ IU/mL. Panels were prepared by spiking an HBV genotype A clinical specimen or a high titer HBV plasmid DNA stock in HBV-negative EDTA plasma and serum. Each panel member was analyzed in replicates of eight per reagent lot, except for the lowest dilutions which were analyzed in replicates of sixteen per reagent lot, using two reagent lots. The results are presented in Figure 9 and Figure 10.

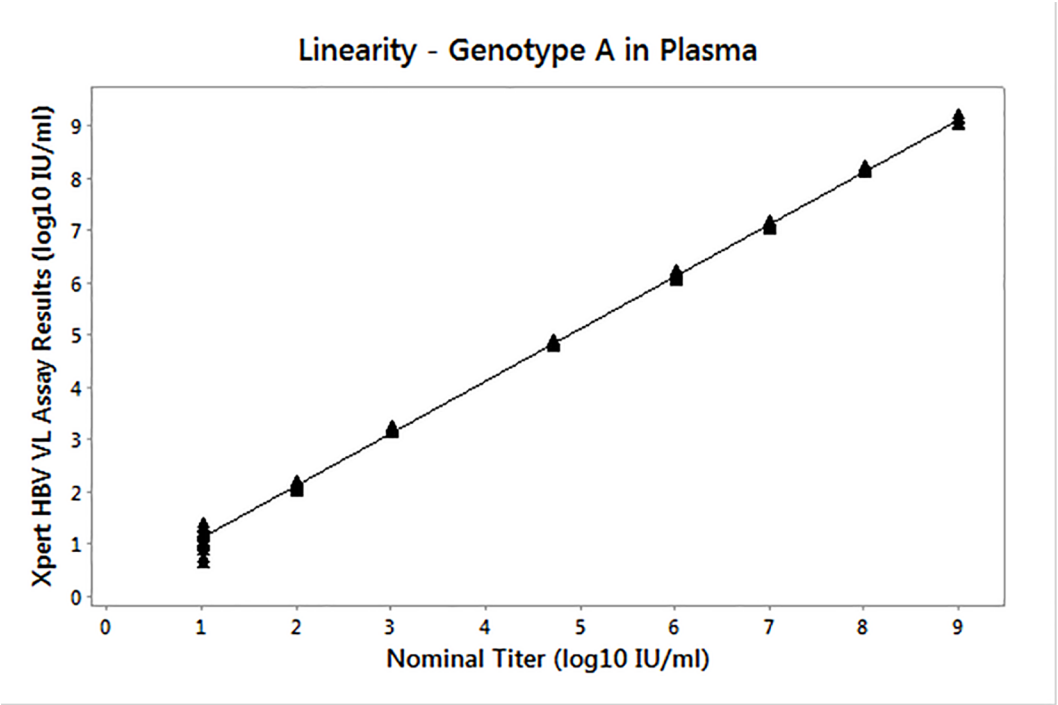

Figure 9. Linearity for the Xpert HBV VL Test in EDTA Plasma

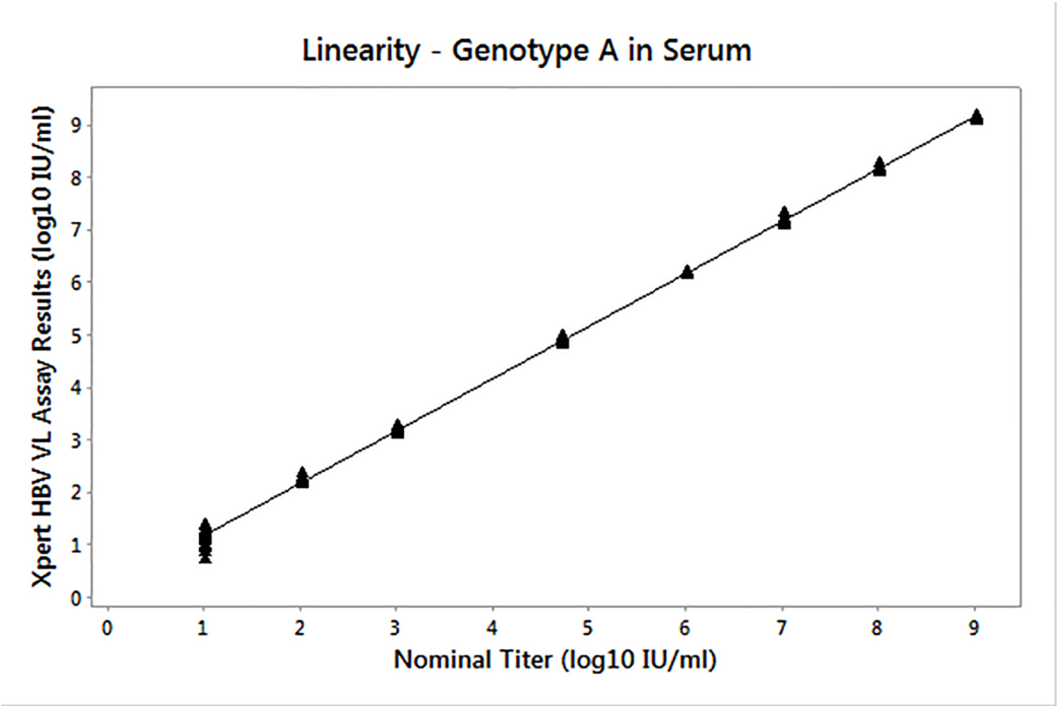

Figure 10. Linearity for the Xpert HBV VL Test in EDTA Serum

Genotypes B-H

To confirm the linearity, dilution panels representing HBV genotypes B through H were prepared to cover a measuring range as wide as possible by diluting a clinical specimen representing each genotype in HBV-negative EDTA plasma. Panel members were analyzed with the same number of replicates as for HBV genotype A using one reagent lot.

Linearity was demonstrated according to CLSI guideline EP06-A[13] for genotype A-H with an R^2 >0.99. The Xpert HBV VL test is linear across a range of 1.00 – 9.00 log_{10} IU/mL for genotype A and across the range tested for genotypes B through H (see Table 7).

Table 7. Linearity of the Xpert HBV VL Test by Genotype

Genotype	Linear Regression Equation	R^2	Tested Titer Range (Log_{10}IU/mL)
A (Plasma)	y = 1.005x + 0.093	0.999	1.00 – 9.00
A (Serum)	y = 1.000x + 0.167	0.999	1.00 – 9.00
B	y = 0.998x – 0.027	0.995	1.00 – 6.83
C	y = 0.998x – 0.119	0.998	1.00 – 7.69
D	y = 0.993x + 0.101	0.998	1.00 – 7.41
E	y = 1.010x – 0.149	0.999	1.00 – 8.14
F	y = 0.994x – 0.068	0.999	1.00 – 7.96
G	y = 0.990x + 0.538	0.999	1.00 – 8.61
H	y = 0.991x + 0.122	0.999	1.00 – 6.35

18.5 Analytical Specificity (Exclusivity)

The analytical specificity of the Xpert HBV VL test was evaluated by adding potentially cross-reacting organisms at a concentration of 1×10^6 CFU/mL for microorganisms, or 1×10^5 copies/mL or $TCID_{50}$/mL for viruses into HBV-negative EDTA plasma and EDTA plasma containing approximately 30 IU/mL of HBV reference material (4th WHO International Standard for HBV, NIBSC code: 10/266)[4]. Tested organisms are listed in Table 8. None of the tested organisms showed cross reactivity or interfered with the quantification of the Xpert HBV VL test.

Table 8. Analytical Specificity Organisms

Viruses		Bacteria	Yeast
BK Human polyoma virus	Human Immunodeficiency virus 1	*Staphylococcus epidermidis*	*Candida albicans*
Cytomegalovirus	Human Immunodeficiency virus 2	*Staphylococcus aureus*	
Epstein-Barr virus	Human papilloma virus 16		
Hepatitis A virus	Human papilloma virus 18		
Hepatitis C virus	Human T-cell lymphotropic virus type 1		
Herpes simplex virus 1	Human T-cell lymphotropic virus type 2		
Herpes simplex virus 2	Varicella Zoster virus		
Human herpes virus 6	Vaccina virus		
Human herpes virus 8			

18.6 Potentially Interfering Substances

The susceptibility of the Xpert HBV VL test 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).[4]

Elevated levels of the endogenous substances listed in Table 9 were shown not to interfere with the quantification of the Xpert HBV VL test with the mean $\log_{10}$ titer of each of the positive HBV samples containing potentially interfering substances 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.

Endogenous Substances

Table 9. Endogenous Substances and Concentration Tested

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

Drugs

The drug components as presented in Table 10 were shown to not interfere with the quantification of the Xpert HBV VL test or impact the test specificity when tested at three times peak plasma level concentration (C_{max}) in the presence and absence of HBV DNA.

Table 10. Drug Pools Tested

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

Autoimmune Disease Markers

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 VL test. The mean $\log_{10}$ concentrations of samples spiked with HBV 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.

18.7 Matrix Equivalency (K_2EDTA Plasma, PPT-EDTA and Serum)

Matrix equivalency for the Xpert HBV VL test was conducted with 32 matched HBV positive clinical specimens and 23 matched HBV negative clinical specimens collected in K_2EDTA plasma, PPT-EDTA plasma and serum collection tubes. The 23 matched HBV negative clinical specimens were spiked with HBV positive material, from clinical specimens representing HBV genotypes B through G and a DNA plasmid expressing the HBV genotype A target sequence, with titers across the entire linear range.

Matrix equivalency was demonstrated in the tested samples as shown in Figure 11 and Figure 12.

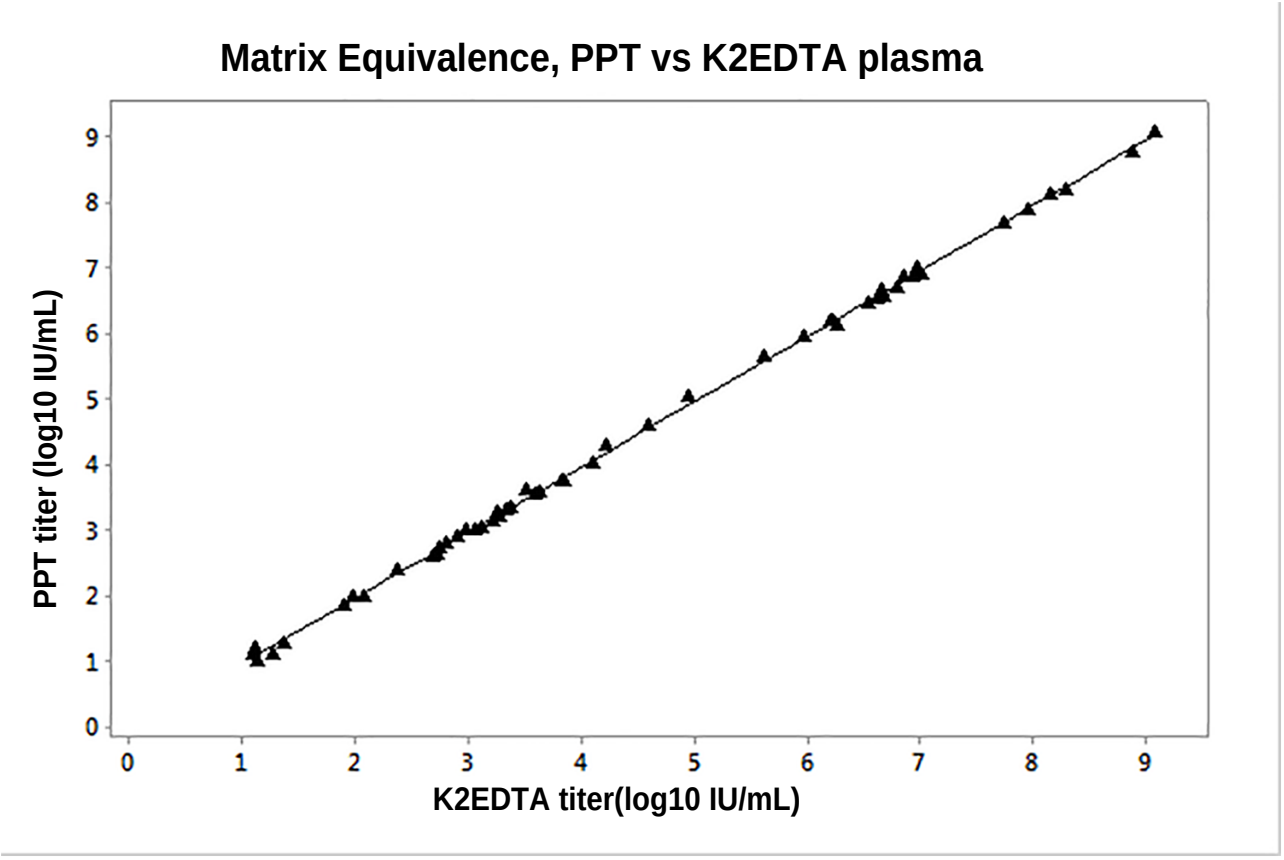

Figure 11. Linear Regression Plot of PPT-EDTA Plasma versus K_2EDTA Plasma

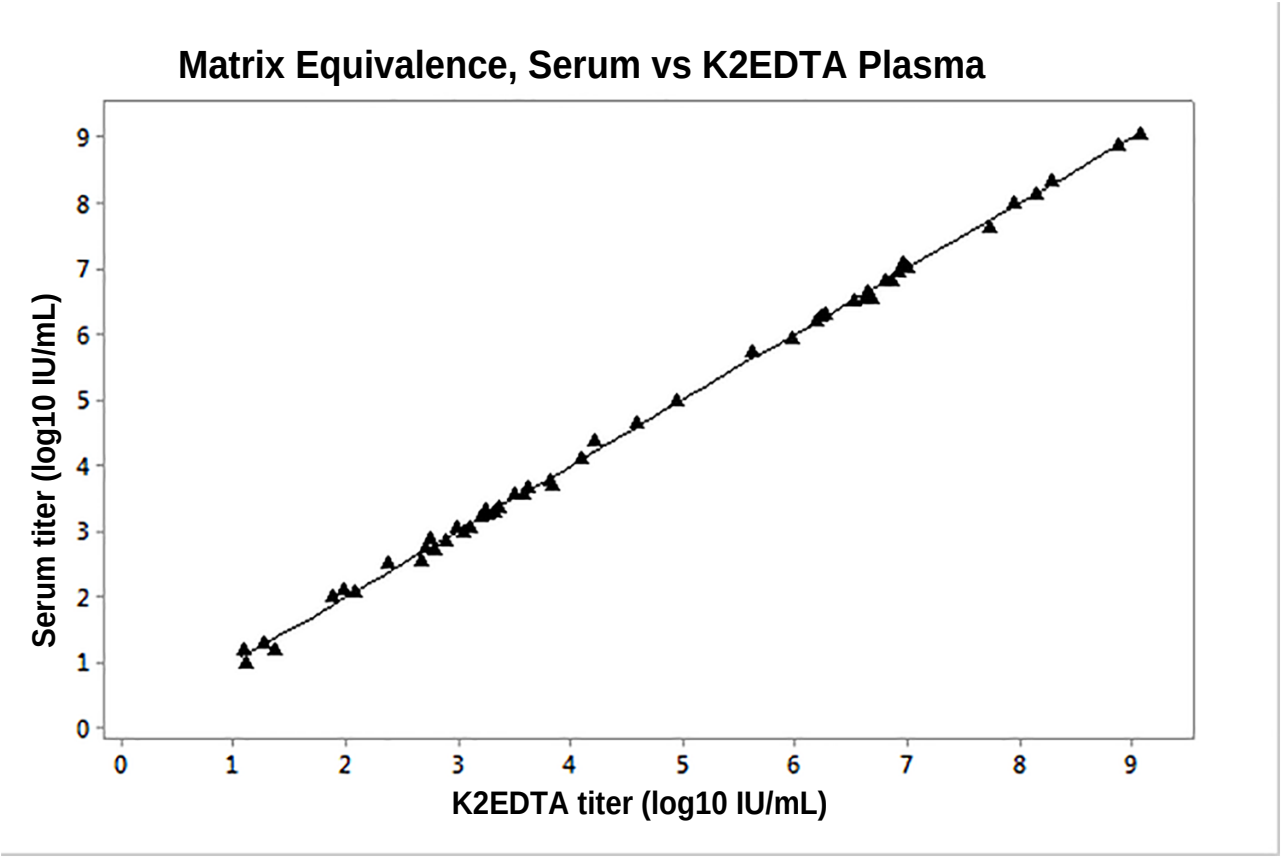

Figure 12. Linear Regression Plot of Serum versus K_2EDTA Plasma

The results show that the Xpert HBV VL test performs equivalently in K_2-EDTA plasma, PPT-EDTA plasma and serum for specimens in the range of approximately 1.0-9.0 log10 IU/mL.

18.8 Whole System Failure

The whole system failure rate for the HBV VL assay was determined by testing 100 replicates of EDTA plasma spiked with the 4th WHO International Standard for HBV DNA (NIBSC code 10/266)[4], a genotype A sample. The spiked samples were tested at a target concentration of approximately 3 x LLOQ (30 IU/mL).

The results of this study determined that all replicates were valid and positive for the HBV target, resulting in a whole system failure rate of 0.0%

18.9 Carryover Contamination

A high titer HBV positive sample (>1 x 10^7 IU/mL) was tested, immediately followed by testing a HBV negative sample in the same GeneXpert instrument module. The procedure was repeated twenty (20) times in two modules. The carryover rate for the Xpert HBV VL test was 0%.

19 Clinical Performance

19.1 Specificity in Normal Healthy Blood Donors

The specificity of the Xpert HBV VL test was evaluated using 99 serum and 100 EDTA plasma specimens from HBV negative blood donors. The specificity of the Xpert HBV VL test was 100.0% [95% CI: 98.1-100.0 (199/199)].

19.2 Method Correlation

A multi-site study was conducted to evaluate the performance of the Xpert HBV VL test compared to a HBV DNA quantitative comparator method using leftover standard of care serum and EDTA plasma specimens from individuals known to be infected with HBV.

Of the 876 eligible subjects, 351 (40.1%) were female and 489 (55.8%) were male. The average age was 47.2 ± 15.9 years, with a range of 18 to 89 years. Of these 876 specimens, 560 were within the quantitation range of the both the HBV VL assay and the comparator assay. The result of the Deming regression and simple linear regression analyses are shown in Figure 13.

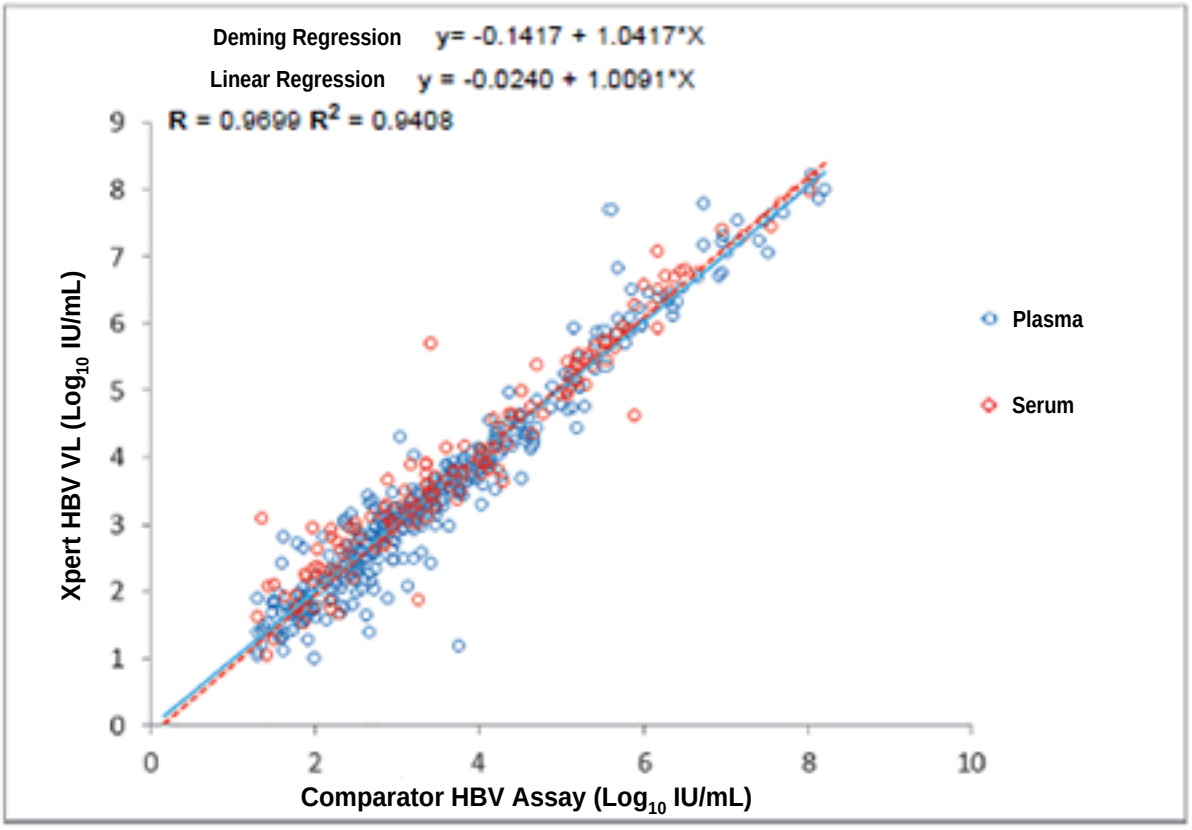

Figure 13. Correlation of the Xpert HBV VL Test vs. the Comparator Method using Serum and EDTA Plasma Specimens

20 References

1. World Health Organization (WHO). Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. March 2015. Accessed March 14, 2018 at: http://www.who.int/hiv/pub/hepatitis/hepatitis-b-guidelines/en/.
2. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of chronic hepatitis B virus infection. J. Hepat. 2012; 57:167-185. Available at: http://dxdoi.org/10.1016/j.jhep.2012.02.010.
3. World Health Organisation. Global hepatitis report, 2017. WHO. April 2017.
4. The 4th WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Amplification Techniques (NIBSC code: 10/266). National Institute for Biological Standards and Control; 2016.
5. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical Laboratories* (5th edition), accessed March 5, 2018 at http://www.cdc.gov/biosafety/publications/.
6. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections, Approved Guideline*. Document M29 (refer to latest edition).
7. World Health Organization. Safe management of wastes from health-care activities. 2nd Edition. WHO, 2014. Accessed April 20, 2018 at http://www.who.int/water_sanitation_health/publications/wastemanag/en/.
8. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.
9. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).
10. Clinical and Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*; Approved Guideline – Second Edition. CLSI document EP17-A2. Clinical and Laboratory Standards Institute, Wayne, PA, 2012.
11. Clinical and Laboratory Standards Institute. *Evaluation of Precision of Quantitative Measurement Procedures*; Approved Guideline – Third Edition. CLSI document EP05-A3. Clinical and Laboratory Standards Institute, Wayne, PA, 2014.
12. Clinical and Laboratory Standards Institute. *User Verification of Precision and Estimation of Bias*; Approved Guideline – Third Edition. CLSI document EP15-A3. Clinical and Laboratory Standards Institute, Wayne, PA, 2014.
13. Clinical and Laboratory Standards Institute. *Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach*. Approved Guideline. CLSI document EP06-A. Clinical and Laboratory Standards Institute, Wayne, PA, 2003.

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22 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

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Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/CustomerSupport.

23 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse
LOT	Batch code
	Consult instructions for use
	Manufacturer
	Country of manufacture
	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
CE	CE marking – European Conformity
	Temperature limitation
	Biological risks
	Caution
	Warning
CH REP	Authorized Representative in Switzerland
	Importer

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