Aptima™ HBV Quant Assay

For *in vitro* diagnostic use.

For US export only

General Information

Intended Use

The Aptima HBV Quant assay is an *in vitro* nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) DNA in human plasma and serum on the fully automated Panther™ system.

Plasma may be prepared in ethylenediaminetetraacetic acid (EDTA), anticoagulant citrate dextrose (ACD) solution, and plasma preparation tubes (PPTs). Serum may be prepared in serum tubes and serum separator tubes (SSTs). Specimens are tested using the fully automated Panther® system for sample processing, amplification, and quantitation. Specimens containing HBV genotypes A, B, C, D, E, F, G, and H are validated for quantitation in the assay.

The Aptima HBV Quant assay is intended for use as an aid in the management of patients with chronic HBV infections undergoing HBV antiviral drug therapy. The assay can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing viral response to treatment. The results from the Aptima HBV Quant assay must be interpreted within the context of all relevant clinical and laboratory findings.

The Aptima HBV Quant assay is not intended for use as a screening test in blood or blood products for HBV or as a diagnostic test to confirm the presence of HBV infection.

Summary and Explanation of the Test

Hepatitis B virus (HBV), one of several viruses known to cause hepatitis, has been attributed to lifelong HBV infection, cirrhosis of the liver, liver cancer, liver failure, and, potentially, death. The World Health Organization (WHO) lists HBV as one of the world's most common infectious diseases. The prevalence of HBV infection and method of transmission varies greatly around the world. About one third of the world's population has serological evidence of past or present HBV infection with chronic HBV infection occurring in more than 350 million people worldwide.[1,2,3] HBV infection results in increased risk of hepatic decompensation, cirrhosis, and hepatocellular carcinoma (HCC) with a mortality of 0.5 to 1.2 million deaths and 5-10% of cases of liver transplantation worldwide annually.[4,5] Without appropriate treatment, intervention, and monitoring after diagnosis, the 5 year cumulative incidence of cirrhosis ranges from 8-20%. Once cirrhosis has developed, the annual risk of hepatocellular carcinoma (HCC) is 2-5%.[6]

HBV contains a circular, partially double-stranded DNA genome of approximately 3200 base pairs, which encode four partially overlapping open reading frames (ORF) expressing the polymerase, surface, precore/core, and X proteins. The polymerase ORF overlaps the other 3 ORFs and encodes a key viral replication protein, polymerase. The surface ORF expresses three proteins, which are essential for viral morphogenesis, viral entry into hepatocytes, and provoking the host's immune response.[7] There are 8 HBV genotypes (A-H), and these are typically found in distinct geographical locations. Currently, quantitation of HBV DNA is used to determine which patients with chronic infection should be treated, to monitor response to therapy, and to assess rebounds in viral load that may indicate drug resistance.[5]

The Aptima HBV Quant assay is an *in vitro* nucleic acid amplification test that uses real-time transcription-mediated amplification (TMA) technology on the Panther system to quantify HBV DNA, genotypes A, B, C, D, E, F, G, and H. The Aptima HBV Quant assay targets two highly conserved regions in the polymerase and surface genes (for increased tolerance to potential mutations). The assay is standardized to the 3rd WHO International Standard for Hepatitis B Virus (NIBSC code: 10/264).

Principles of the Procedure

The Aptima HBV Quant assay involves three main steps, which all take place in a single tube on the Panther system: target capture, target amplification by TMA, and detection of the amplification products (amplicon) by the fluorescent labeled probes (torches).

During target capture, viral DNA is isolated from specimens. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins, and release viral genomic DNA. Capture oligonucleotides hybridize to highly conserved regions of HBV DNA, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

Target amplification occurs via TMA, which is a transcription-mediated nucleic acid amplification method that utilizes two enzymes, Moloney murine leukemia virus (MMLV) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Aptima HBV Quant assay utilizes the TMA method to amplify two regions of the HBV genome (polymerase gene and surface gene). Amplification of those regions is achieved using specific primers designed to amplify HBV genotypes A, B, C, D, E, F, G, and H. The dual target region approach with primer design targeting the highly conserved regions ensure accurate quantitation of the HBV DNA.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target and that hybridize specifically to the amplicon in real time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and will emit a signal at a specific wavelength when excited by a light source. As more torches hybridize to amplicon, a higher fluorescent signal is generated. The time taken for the fluorescent signal to reach a specified threshold is proportional to the starting HBV concentration. Each reaction has an internal calibrator/internal control (IC) that controls for variations in specimen processing, amplification, and detection. The concentration of a sample is determined by the Panther system software using the HBV and IC signals for each reaction and comparing them to calibration information.

Warnings and Precautions

A. For *in vitro* diagnostic use only.

B. To reduce the risk of invalid results, carefully read the entire package insert and the *Panther System Operator's Manual* prior to performing this assay.

C. qHBV Target Enhancer Reagent (TER) is corrosive.

- H302 - Harmful if swallowed.
- H314 - Causes severe skin burns and eye damage.

Laboratory Related

D. CAUTION: The controls for this assay contain human plasma. The plasma is negative for hepatitis B surface antigen (HBsAg), antibodies to HCV, antibodies to HIV-1 and HIV-2, and HIV antigen when tested with US Food and Drug Administration licensed procedures.

In addition, the plasma is nonreactive for HBV DNA, HCV RNA, and HIV-1 RNA when tested with licensed nucleic acid tests using pooled samples. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions.[8,9,10]

E. Only personnel adequately trained in the use of the Aptima HBV Quant assay and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.

F. Use only supplied or specified disposable laboratory ware.

G. Use routine laboratory precautions. Do not pipet by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

H. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.

I. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations.[8,9,10,11] Thoroughly clean and disinfect all work surfaces.

J. The controls contain sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing sodium azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

K. Good standard practices for molecular laboratories include environmental monitoring. To monitor a laboratory's environment, the following procedure is suggested:

1. Obtain a cotton-tipped swab and pair with the Aptima Specimen Aliquot Tube (SAT).
2. Label each SAT appropriately.
3. Fill each SAT with 1 mL of Aptima Specimen Diluent.
4. To collect the surface samples, lightly moisten a swab with nuclease-free deionized water.
5. Swab the surface of interest using a top to bottom vertical motion. Rotate the swab approximately one-half turn while swabbing the location.
6. Immediately place the swab sample into the tube and gently swirl the swab in the diluent to extract potential swabbed materials. Press the swab on the side of the transport tube to extract as much liquid as possible. Discard the swab and cap the tube.
7. Repeat steps for remaining swab samples.
8. Test swab with molecular assay.

Specimen Related

L. Specimens may be infectious. Use Universal Precautions[8,9,10] when performing this assay. Proper handling and disposal methods should be established according to local regulations.[11] Only personnel adequately trained in the use of the Aptima HBV Quant assay and trained in handling infectious materials should perform this procedure.

M. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

N. Avoid cross-contamination during the specimen handling steps. Be especially careful to avoid contamination by the spread of aerosols when loosening or uncapping specimens. Specimens can contain extremely high levels of organisms. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

Assay Related

O. Do not use the reagent kit, the calibrator, or the controls after the expiration date.

P. Do not interchange, mix, or combine assay reagents from kits with different master lot numbers. Assay fluids can be from different lot numbers. Controls and the calibrator can be from different lot numbers.

Q. Avoid microbial and nuclease contamination of reagents.

R. Cap and store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See *Reagent Storage and Handling Requirements* and *Panther System Test Procedure* for more information.

S. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagents or fluids. The Panther system verifies reagent levels.

T. Avoid contact of target enhancer reagent with skin, eyes, and mucous membranes. Wash with water if contact with this reagent occurs. If spills of this reagent occurs, dilute with water and follow appropriate site procedures.

Reagent Storage and Handling Requirements

A. The following table shows the storage conditions and stability for reagents, controls, and calibrator.

Reagent	Unopened Storage	Open Kit (Reconstituted)	
		Storage	Stability
qHBV Amplification Reagent	2°C to 8°C		
qHBV Amplification Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHBV Enzyme Reagent	2°C to 8°C		
qHBV Enzyme Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHBV Promoter Reagent	2°C to 8°C		
qHBV Promoter Reconstitution Solution	2°C to 8°C	2°C to 8°C	30 days[a]
qHBV Target Capture Reagent	2°C to 8°C	2°C to 8°C	30 days[a]
qHBV PCAL (Positive Calibrator)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qHBV NC CONTROL – (Negative Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qHBV LPC CONTROL + (Low Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qHBV HPC CONTROL + (High Positive Control)	-15°C to -35°C	15°C to 30°C	Single use vial Use within 24 hours
qHBV Target Enhancer Reagent	15°C to 30°C	15°C to 30°C	30 days[a]

[a] When reagents are removed from the Panther system, they should be immediately returned to their appropriate storage temperatures.

B. Discard any unused, reconstituted reagents, target capture reagent (TCR), and target enhancer reagent (TER) after 30 days or after the Master Lot expiration date, whichever comes first.

C. Reagents stored onboard the Panther system have 72 hours of onboard stability. Reagents can be loaded onto the Panther system up to 5 times. The Panther system logs each time the reagents are loaded.

D. After thawing the calibrator, the solution must be clear, i.e., not cloudy or have precipitates.

⚠ E. The promoter reagent and reconstituted promoter reagent are photosensitive. Protect these reagents from light during storage and preparation for use.

F. The qHBV Target Enhancer Reagent must be at 15°C to 30°C before use.

Specimen Collection and Storage

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during sample handling steps. For example, discard used material without passing over open tubes.*

Whole blood specimens collected in the following glass or plastic tubes may be used:

- Tubes containing EDTA or ACD anticoagulants
- Plasma preparation tubes (PPTs)
- Serum tubes
- Serum separator tubes (SSTs)

For serum, allow the clot to form before further processing.

A. Specimen Collection

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Separate the plasma or serum from the pelleted red blood cells following the manufacturer's instructions for the tube used. Plasma or serum can be tested on the Panther system in the primary tube or transferred to the secondary Aptima Specimen Aliquot Tube (SAT). The minimum volume of serum or plasma for primary collection tubes is 1200 µL and for SATs the minimum volume is 700 µL to obtain the 500 µL reaction volume.

If not tested immediately, plasma and serum can be stored in accordance with the specifications below. If transferred to the SAT, plasma or serum may be frozen at -20°C. Do not exceed 3 freeze–thaw cycles. Do not freeze specimens in EDTA, ACD, or serum primary collection tubes.

B. Specimen Storage Conditions

1. EDTA and ACD Plasma Specimens

Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Plasma may then be stored under one of the following conditions:

- In the primary collection tube or SAT at 2°C to 30°C for up to 24 hours,
- In the primary collection tube or SAT at 2°C to 8°C for up to 5 days or
- In the SAT at -20°C for up to 60 days.

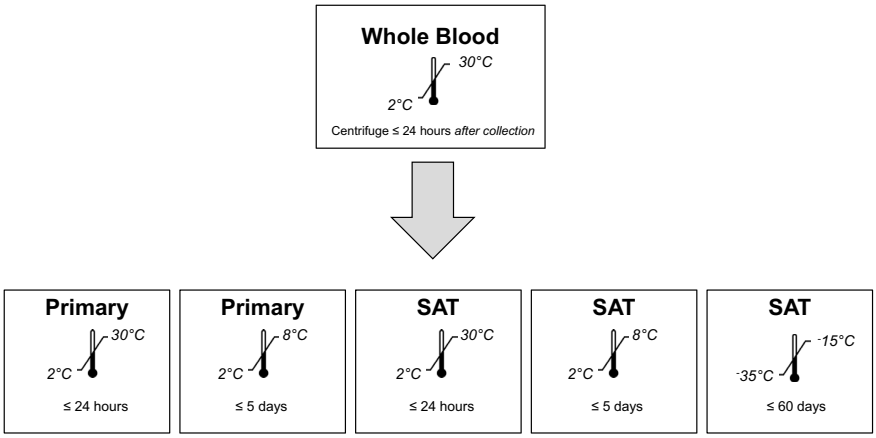

Figure 1. Storage Conditions for EDTA/ACD Tubes

2. PPT Specimens

   Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Plasma may then be stored under one of the following conditions:

   - In the PPT or SAT at 2°C to 30°C for up to 24 hours,
   - In the PPT or SAT at 2°C to 8°C for up to 5 days or
   - In the PPT or SAT at -20°C for up to 60 days.

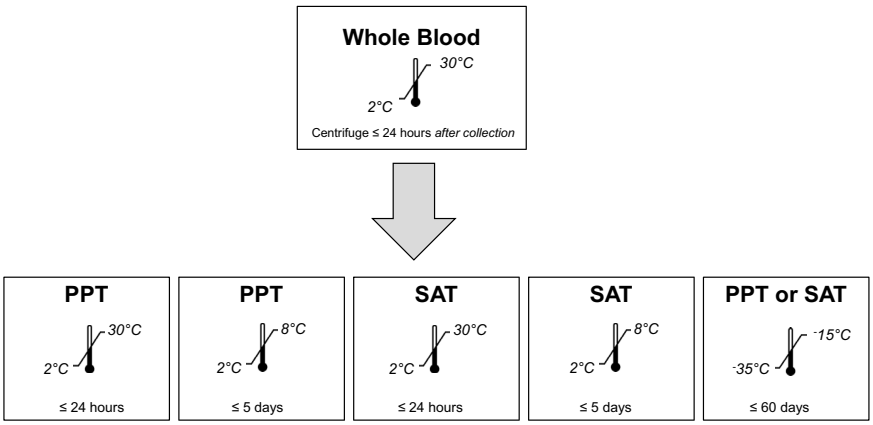

Figure 2. Storage Conditions for PPTs

3. Serum Tube Specimens

   Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Serum may then be stored under one of the following conditions:

   - In the serum tube or SAT at 2°C to 30°C for up to 24 hours,
   - In the serum tube or SAT at 2°C to 8°C for up to 5 days, or
   - In the SAT at -20°C for up to 60 days.

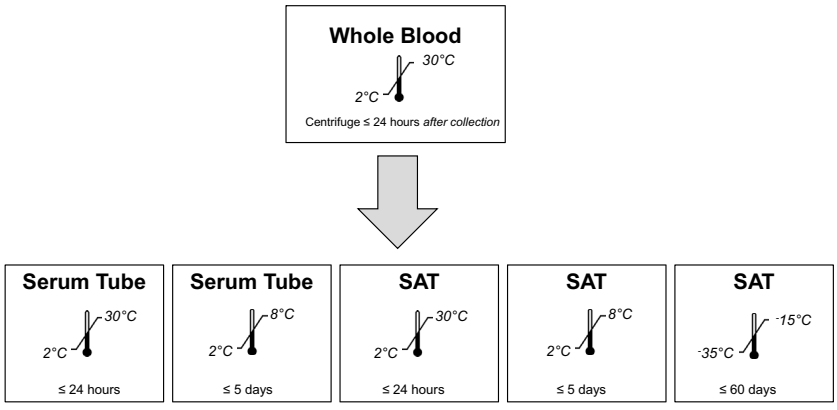

Figure 3. Storage Conditions for Serum Tubes

4. SST Specimens

   Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Serum may then be stored under one of the following conditions:

   - In the SST or SAT at 2°C to 30°C for up to 24 hours,
   - In the SST or SAT at 2°C to 8°C for up to 5 days or
   - In the SST or SAT at -20°C for up to 60 days.

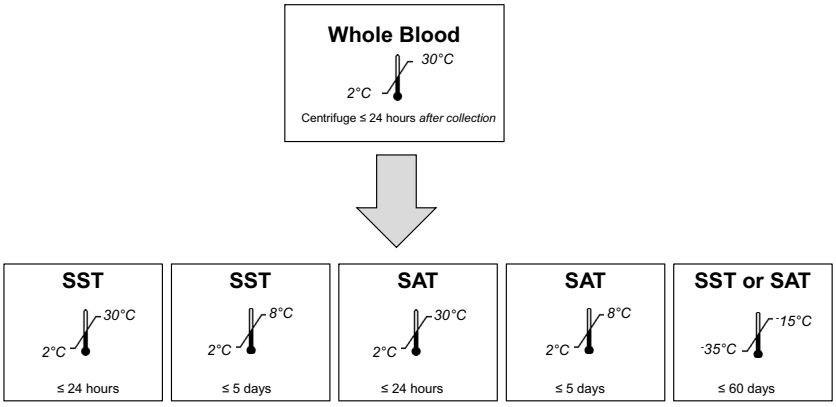

Figure 4. Storage Conditions for SSTs

C. Long Term Frozen Storage

   Plasma or serum samples may be stored at -65°C to -85°C for up to 60 days in SATs.

D. Dilution of Plasma and Serum Specimens

   Plasma and serum specimens may be diluted in the SAT for testing on the Panther system. See *Panther System Test Procedure*, paragraph E “Specimen Handling,” step 6 for more information.

   Note: *If a specimen is diluted, it should be tested immediately after dilution. Do not freeze a diluted specimen.*

Samples Onboard the Panther System

Samples may be left on the Panther system uncapped for up to 8 hours. Samples may be removed from the Panther system and tested as long as the total time onboard does not exceed 8 hours prior to the pipetting of the sample by the Panther system.

Specimen Transport

Maintain sample storage conditions as described in *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther System

Reagents for the Aptima HBV Quant assay are listed below for the Panther system. Reagent identification symbols are also listed next to the reagent name.

Reagents and Materials Provided

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Aptima HBV Quant Assay Kit, 100 tests (Cat. No. PRD-03424)
(1 assay box, 1 calibrator kit, 1 controls kit, and 1 Target Enhancer Reagent box)

Additional calibrators and controls can be ordered separately. See the individual catalog numbers below.

Aptima HBV Quant Assay Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**qHBV Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
E	**qHBV Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution.*	1 vial
PRO	**qHBV Promoter Reagent** *Non-infectious nucleic acids dried in buffered solution.*	1 vial
AR	**qHBV Amplification Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 7.2 mL
ER	**qHBV Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 5.8 mL
PROR	**qHBV Promoter Reconstitution Solution** *Aqueous solution containing glycerol and preservatives.*	1 x 4.5 mL
TCR	**qHBV Target Capture Reagent** *Nucleic acids in a buffered salt solution containing solid phase, non-infectious nucleic acids, and Internal Calibrator.*	1 x 72.0 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Aptima HBV Quant Calibrator Kit (Cat. No. PRD-03425)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
PCAL	**qHBV Positive Calibrator** *Plasmid DNA in buffered solution*	5 x 2.5 mL
	Calibrator Barcode Label	—

Aptima HBV Quant Controls Kit (Cat. No. PRD-03426)
(store at -15°C to -35°C upon receipt)

Symbol	Component	Quantity
NC	**qHBV Negative Control** *HBV negative defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
LPC	**qHBV Low Positive Control** *Inactivated HBV positive plasma in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
HPC	**qHBV High Positive Control** *Inactivated HBV positive plasma in defibrinated human plasma containing gentamicin and 0.2% sodium azide as preservatives.*	5 x 0.8 mL
	Control Barcode Label	—

Aptima HBV Quant Target Enhancer Reagent Box
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
TER	**qHBV Target Enhancer Reagent** *A concentrated solution of lithium hydroxide solution*	1 x 46.0 mL

Materials Required but Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	—
Panther Run Kit for Real Time Assays (for real time assays only)	PRD-03455 (5000 tests)
Aptima Assay Fluids Kit (also known as Universal Fluids Kit) contains Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or, Panther System Run Kit *(when running non-real time-TMA assays in parallel with real time-TMA assays) contains MTUs, waste bags, waste bin covers, auto detect, and assay fluids*	303096 (5000 tests)
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—
Reagent replacement caps *Amplification, Enzyme, and Promoter reagent reconstitution bottles CL0041 (100 caps)*	
TCR bottle	CL0040 (100 caps)
TER bottle	501604 (100 caps)
Plastic-backed laboratory bench covers	—
Lint-free wipes	—
Pipettor	—
Tips	—
Primary collection tubes of the following dimensions may be used: *13 mm x 100 mm* *13 mm x 75 mm* *16 mm x 100 mm*	—
Centrifuge	—
Vortex mixer	—

Optional Materials

Material	Cat. No.
Aptima Specimen Aliquot Tubes (SATs) (100 pack)	503762
Transport tube cap (100 pack) *cap for SAT*	504415
Aptima Specimen Diluent	PRD-03003
Aptima Specimen Diluent Kit *contains specimen diluent, 100 SATs and 100 caps*	PRD-03478
Transfer pipets	—
Commercially available panels, for example: *HBV panels from Quality Control for Molecular Diagnostics (QCMD)*	—
Cotton-tipped swabs	—
Tube rocker	—

Panther System Test Procedure

Note: *See the Panther System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Clean work surfaces where reagents will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared. Use the procedure described above (step A.1).
3. Clean any pipettors. Use the cleaning procedure described above (step A.1).

B. Calibrator and Controls Preparation

Allow the calibrator and controls to reach 15°C to 30°C prior to processing as follows:

1. Remove the calibrator and controls from storage (-15°C to -35°C) and place at 15°C to 30°C. Throughout the thawing process, gently invert each tube to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Option. Calibrator and control tubes may be placed on a tube rocker to mix thoroughly. Ensure tube contents are fully thawed prior to use.

   Note: *Avoid creating excessive foam when inverting the calibrator and controls. Foam compromises the level-sensing by the Panther system.*
2. When the tube contents have thawed, dry the outside of the tube with a clean, dry disposable wipe.
3. To prevent contamination, do not open the tubes at this time.

C. Reagent Reconstitution/Preparation of a New Kit

Note: *Reconstitution of reagents should be performed prior to beginning any work on the Panther system.*

1. To prepare Target Capture Reagent (TCR), perform the following:
   a. Remove the TCR from storage (2°C to 8°C). Check the lot number on the TCR bottle to make sure that it matches the lot number on the Master Lot Barcode Sheet.
   b. Immediately shake the TCR bottle vigorously 10 times. Allow the TCR bottle to remain at 15°C to 30°C to warm for at least 45 minutes. During this period, swirl and invert the TCR bottle at least every 10 minutes.

      Option. The TCR bottle may be prepared on a tube rocker by following these instructions: Remove the TCR from storage (2°C to 8°C) and immediately shake vigorously 10 times. Place the TCR bottle on a tube rocker and leave the TCR at 15°C to 30°C to warm for at least 45 minutes.
   c. Ensure all precipitate is in solution and the magnetic particles are suspended before use.
2. To reconstitute Amplification, Enzyme, and Promoter Reagents, perform the following:
   a. Remove the lyophilized reagents and corresponding reconstitution solutions from storage (2°C to 8°C). Pair each reconstitution solution with its lyophilized reagent.
   b. Ensure that the reconstitution solution and lyophilized reagent have matching label colors. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
      i. Open the lyophilized reagent vial by removing the metallic seal and rubber stopper.
      ii. Firmly insert the notched end of the reconstitution collar (black) onto the vial (Figure 5, Step 1).
      iii. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
      iv. Place the reconstitution solution bottle on a stable surface (i.e., bench). Then, invert the lyophilized reagent vial over the reconstitution solution bottle and firmly attach the collar to the reconstitution solution bottle (Figure 5, Step 2).
      v. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial (Figure 5, Step 3).
      vi. Pick up the assembled bottles, and swirl the assembled bottles for at least 10 seconds (Figure 5, Step 4).
      vii. Wait for at least 30 minutes for the lyophilized reagent to go into solution.
      viii. After the lyophilized reagent has gone into solution, swirl the assembled bottles for at least 10 seconds and then slightly rock the solution within the glass vial back and forth to mix thoroughly.
   c. Slowly tilt the assembled bottles again to allow all of the solution to drain back into the reconstitution solution bottle (Figure 5, Step 5).
   d. Carefully remove the reconstitution collar and glass vial (Figure 5, Step 6).
   e. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 5, Step 7).

f. Discard the reconstitution collar and glass vial (Figure 5, Step 8).

Warning: *Avoid creating excessive foam when reconstituting reagents. Foam compromises the level-sensing by the Panther system.*

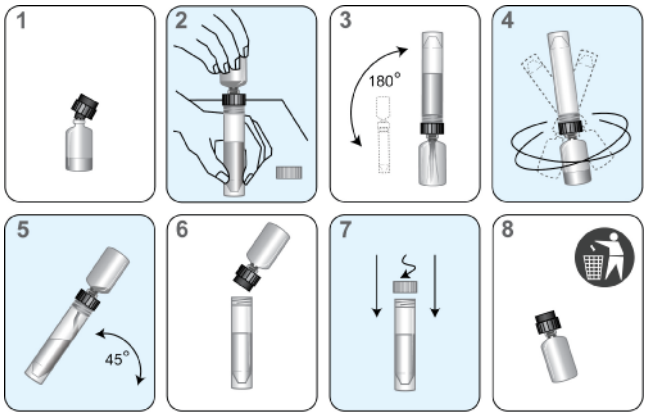

Figure 5. Reagent Reconstitution Process

3. Remove the qHBV Target Enhancer Reagent from storage (15°C to 30°C). Record operator initials and open date on the label. Check the lot number on the TER bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.

D. Reagent Preparation for Previously Prepared Reagents

1. Remove the previously prepared reagents from storage (2°C to 8°C). Previously prepared Amplification, Enzyme and Promoter reagents, and TCR must reach 15°C to 30°C prior to the start of the assay.
2. Remove TER from storage (15°C to 30°C).
3. For previously prepared TCR, perform step C.1 above prior to loading on the system.
4. Swirl and invert the Amplification, Enzyme, and Promoter reagents to mix thoroughly prior to loading on the system. Avoid creating excessive foam when inverting reagents.
5. Do not top off reagent bottles. The Panther system will recognize and reject bottles that have been topped off.

E. Specimen Handling

1. Ensure frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
2. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther System* for additional onboard information.
3. Ensure each primary collection tube contains at least 1200 µL of specimen. Ensure each Aptima Specimen Aliquot Tube (SAT) contains at least 700 µL of specimen. If specimen dilution is necessary, see step E.6 below for additional information.
4. Vortex specimens in SATs for 3 to 5 seconds to mix thoroughly.
5. Just prior to loading specimens into a Sample Rack, centrifuge each specimen at 1000 to 3000*g* for 10 minutes. Do not remove caps. Bubbles in the tube compromise the level-sensing by the Panther system.

   See *System Preparation*, step F.2 below, for information about loading the rack and removing the caps.

6. Diluting a specimen in the SAT

   A specimen may be diluted in the SAT for testing on the Panther system.

   Note: *If a specimen is diluted, it must be tested immediately after dilution.*

   a. Dilution of low-volume specimens

      The volume of specimens may be increased to the minimum volume required (700 µL) using Aptima Specimen Diluent. Specimens with at least 240 µL may be diluted with two parts specimen diluent (1:3) as follows:

      i. Place 240 µL of specimen in the SAT.

      ii. Add 480 µL of specimen diluent.

      iii. Cap the tube.

      iv. Gently invert 5 times to mix.

      Specimens diluted 1:3 can be tested using the 1:3 option on the Panther system (see the *Panther System Operator's Manual* for more information). The software will automatically report the neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

   b. Dilution of high-titer specimens

      If a specimen's result is above the upper limit of quantitation (ULoQ), it may be diluted with 99 parts of Aptima Specimen Diluent (1:100) as follows:

      i. Place 30 µL of specimen in the SAT.

      ii. Add 2970 µL of specimen diluent.

      iii. Cap the tube.

      iv. Gently invert 5 times to mix.

      Specimens diluted 1:100 can be tested using the 1:100 option on the Panther system (see *Panther System Operator's Manual* for more information). The software will automatically report the neat result by applying the dilution factor. These specimens will be flagged as diluted specimens.

      Note: *For diluted specimens with neat concentrations greater than the ULoQ, results will be reported using scientific notation.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther System Operator's Manual* and *Procedural Notes.* Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples into the Sample Rack. Perform the following steps for each sample tube (specimen, and, when necessary, calibrator and controls):
   a. Loosen one sample tube cap, but do not remove it yet.

      Note: *Be especially careful to avoid contamination by the spread of aerosols. Gently loosen caps on samples.*

   b. Load the sample tube into the Sample Rack.

   c. Repeat steps 2.a and 2.b for each remaining sample.

   d. After the samples have been loaded into the Sample Rack, remove and discard each sample tube cap in one Sample Rack. To avoid contamination, do not pass a cap over any other Sample Racks or sample tubes.

e. If necessary, use a new, disposable transfer pipet to remove any bubbles or foam.

f. When the last cap has been removed, load the Sample Rack into the Sample Bay.

Note: *If running other assays and sample types at the same time, secure the Sample Retainer prior to loading the Sample Rack into the Sample Bay.*

g. Repeat steps 2.a to 2.f for the next Sample Rack.

Procedural Notes

A. Calibrator and Controls

1. The qHBV positive calibrator, qHBV low positive control, qHBV high positive control, and qHBV negative control tubes can be loaded in any position in the Sample Rack and in any Sample Bay Lane on the Panther system. Specimen pipetting will begin when one of the following two conditions has been met:
   a. The calibrator and controls are currently being processed by the system.
   b. Valid results for the calibrator and controls are registered on the system.
2. Once the calibrator and control tubes have been pipetted and are processing for the Aptima HBV Quant assay reagent kit, specimens can be tested with the associated reconstituted kit for up to 24 hours **unless:**
   a. The calibrator or control results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. The calibrator and each control tube can be used once. Attempts to use the tube more than once can lead to processing errors.

B. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

Quality Control

A run or specimen result may be invalidated by an operator if technical, operator, or instrument difficulties are observed while performing the assay and are documented. In this case, specimens must be retested.

Assay Calibration

To generate valid results, an assay calibration must be completed. A single positive calibrator is run in triplicate each time a reagent kit is loaded on the Panther system. Once established, the calibration is valid for up to 24 hours. Software on the Panther system alerts the operator when a calibration is required. The operator scans a calibration coefficient found on the Master Lot Barcode Sheet provided with each reagent kit.

During processing, criteria for acceptance of the calibrator are automatically verified by the software on the Panther system. If less than two of the calibrator replicates is valid, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative control, the low positive control, and the high positive control must be tested each time a reagent kit is loaded on the Panther system. Once established, the controls are valid for up to 24 hours. Software on the Panther system alerts the operator when controls are required.

During processing, criteria for acceptance of controls are automatically verified by software on the Panther system. To generate valid results, the negative control must give a result of “Not Detected” and the positive controls must give results within predefined parameters (LPC Nominal Target: 2.7 Log_{10} IU/mL, HPC Nominal Target: 4.6 Log_{10} IU/mL). If any one of the controls has an invalid result, the software automatically invalidates the run. Samples in an invalidated run must be retested using a freshly prepared calibrator and freshly prepared controls.

Internal Calibrator/Internal Control

Each sample contains an internal calibrator/internal control (IC). During processing, IC acceptance criteria are automatically verified by the Panther system software. If an IC result is invalid, the sample result is invalidated. Every sample with an invalid IC result must be retested to obtain a valid result.

The Panther system software is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther System Operator's Manual.*

Interpretation of Results

The Panther system automatically determines the concentration of HBV DNA for specimens and controls by comparing the results to a calibration curve. HBV DNA concentrations are reported in IU/mL and $\log_{10}$ IU/mL. The interpretation of results is provided in Table 1. If the dilution option is used for diluted specimens, the Panther system automatically calculates the HBV concentration for the neat specimen by multiplying the diluted concentration by the dilution factor and diluted samples will be flagged as diluted.

Note: *For diluted specimens, results listed as "Not Detected" or "<10 detected" may be generated by diluting a specimen with a concentration above, but close to the LoD (limit of detection) or LLoQ (lower limit of quantification). It is recommended to collect and test another neat specimen if a quantitative result is not obtained.*

Table 1: Result Interpretation

Reported Aptima HBV Quant Assay Result		Interpretation
IU/mL	**$\log_{10}$ Value**[a]	
Not Detected	Not Detected	HBV DNA not detected.
<10 detected	<1.0	HBV DNA is detected but at a level below the LLoQ
10 to 1,000,000,000	1.0 to 9.0	HBV DNA concentration is within the linear range of 10 to 1,000,000,000 IU/mL
> 1,000,000,000	> 9.0	HBV DNA concentration is above the ULoQ
Invalid[b]	Invalid[b]	There was an error in the generation of the result. Specimen should be retested

[a] Value is truncated to two decimal places.
[b] Invalid results are displayed in blue colored font.

Note: *For diluted specimens with neat concentrations greater than the ULoQ, results will be reported using scientific notation.*

Limitations

A. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions given in this package insert may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Though rare, mutations within the highly conserved regions of the viral genome covered by the primers and/or probes in the Aptima HBV Quant assay may result in underquantification of or failure to detect the virus.

Performance

Limit of Detection Using the 3rd WHO International Standard

The limit of detection (LoD) of the assay is defined as the concentration of HBV DNA that is detected at 95% or greater probability according to CLSI EP17-A2.[12]

The LoD was determined by testing panels of the 3rd WHO International Standard for Hepatitis B Virus DNA (NIBSC 10/264) diluted in HBV negative human plasma and serum. A minimum of 36 replicates of each dilution were tested with each of three reagent lots for a minimum of 108 replicates per dilution. Probit analysis was performed to generate the predicted detection limits. The LoD values shown in Table 2 are the results from the reagent lot with the highest predicted detection limit. The LoD for the Aptima HBV Quant assay using the 3rd WHO International Standard is 5.58 IU/mL for plasma and 4.29 IU/mL for serum.

Table 2: Limit of Detection Using the 3rd WHO International Standard for HBV

Predicted Detection Limit	Concentration (IU/mL)	
	Plasma	Serum
10%	0.16	0.19
20%	0.27	0.30
30%	0.39	0.42
40%	0.55	0.56
50%	0.75	0.73
60%	1.02	0.96
70%	1.42	1.29
80%	2.09	1.81
90%	3.58	2.91
95%	5.58	4.29

Limit of Detection Across HBV Genotypes

The LoD was determined by testing dilutions of HBV positive clinical specimens for genotypes A, B, C, D, E, F, G, and H in HBV negative human plasma and serum. Concentrations were determined using a CE-marked and a Health Canada licensed comparator assay. A minimum of 24 replicates of each panel member were tested with each of two reagent lots for a minimum of 48 replicates per panel member. Probit analysis was performed to generate 50% and 95% predicted detection limits. The LoD values shown in Table 3 are the results from the reagent lot with the highest predicted detection limit.

Table 3: Limit of Detection Across HBV Genotypes Using Clinical Specimens

Genotype	Predicted Detection Limit	Concentration (IU/mL)	
		Plasma	Serum
A	50%	0.48	0.88
	95%	3.05	3.95
B	50%	0.59	0.69
	95%	3.00	4.97
C	50%	0.79	0.93
	95%	5.32	4.78
D	50%	0.82	1.37
	95%	4.61	7.29
E	50%	0.93	1.01
	95%	4.80	4.90
F	50%	0.75	0.69
	95%	3.13	3.30
G	50%	0.52	0.62
	95%	2.86	3.05
H	50%	1.05	1.36
	95%	6.44	6.31

Linear Range

The linear range was established by testing panels of HBV DNA diluted in HBV negative human plasma and serum according to CLSI EP06-A.[13] Panels ranged in concentration from 0.86 log IU/mL to 9.26 log IU/mL. The Aptima HBV Quant assay demonstrated linearity across the range tested, with an upper limit of quantitation (ULoQ) of 9 log IU/mL as shown in Figure 6.

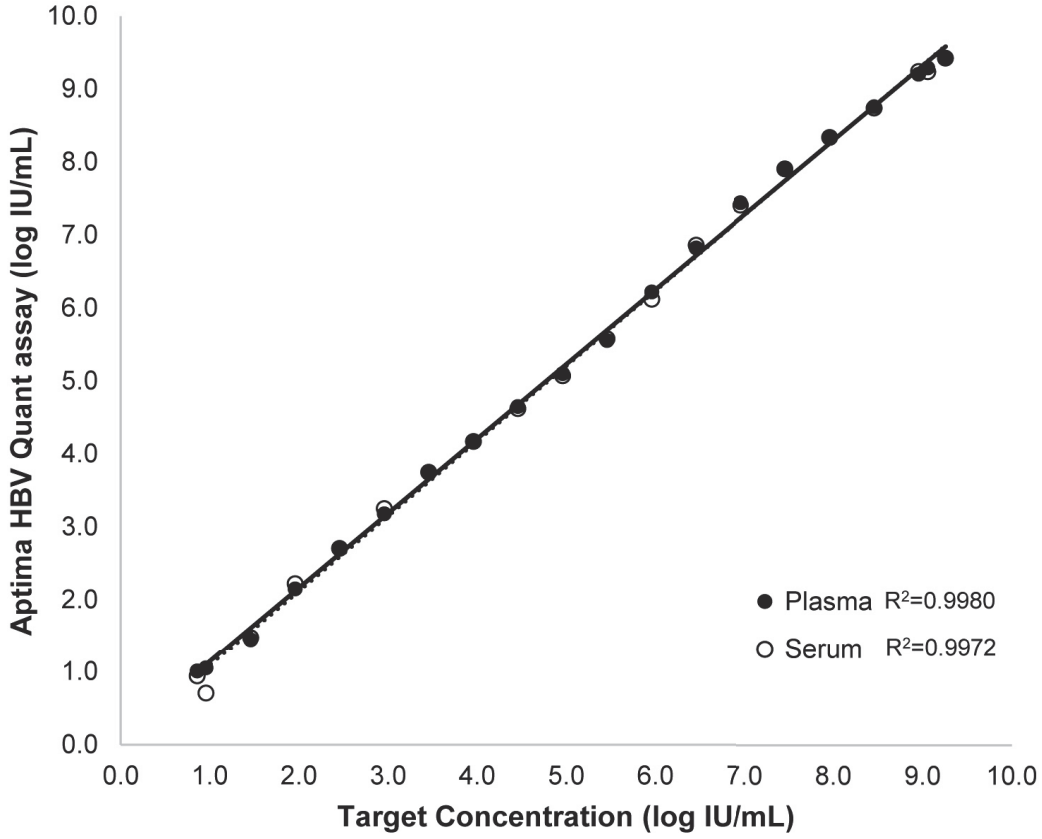

Figure 6. Linearity in Plasma and Serum

Linearity Across HBV Genotypes

The linear response for genotypes A, B, C, D, E, F, G, and H was confirmed by testing panels of HBV DNA diluted in buffer at concentrations ranging from 1.44 log IU/mL to 8.44 log IU/mL. Linearity was demonstrated across the range tested for all genotypes tested as shown in Figure 7.

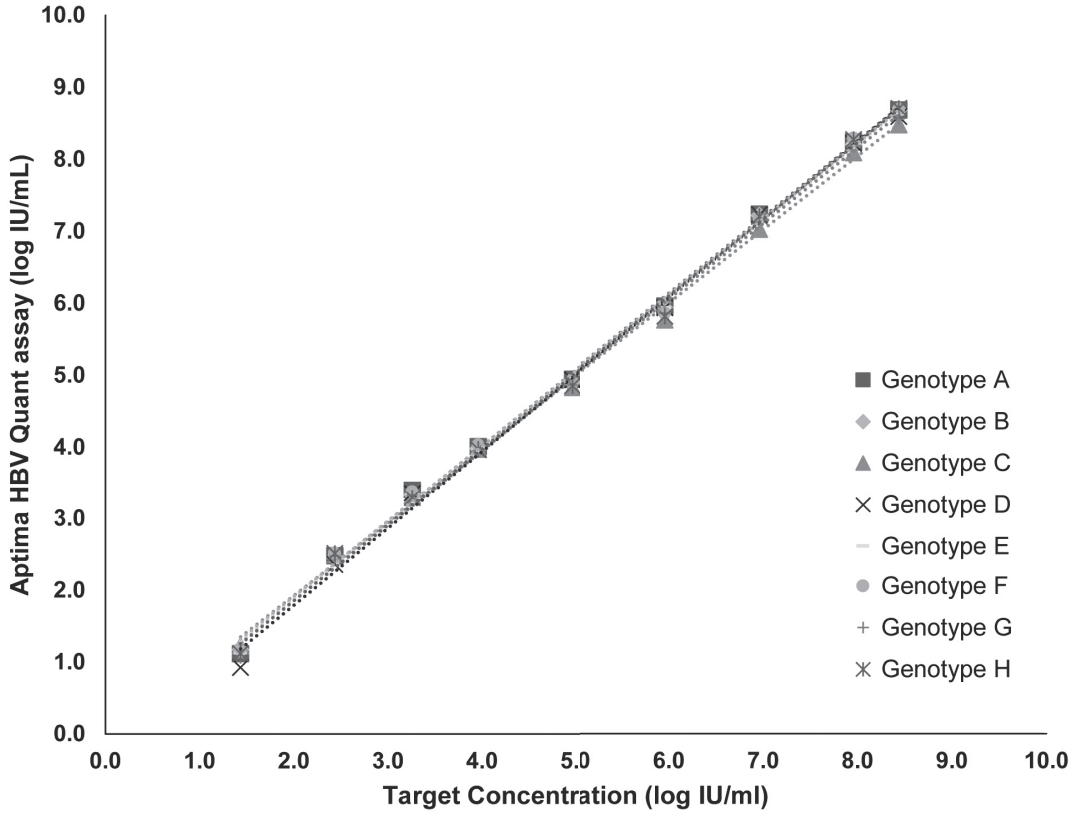

Figure 7. Linearity Across HBV Genotypes A to H

Lower Limit of Quantitation Using the 3rd WHO International Standard

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which HBV DNA is reliably quantitated within a total error, according to CLSI EP17-A2.[12] Total error was estimated by two methods: Total Analytical Error (TAE) = |bias| + 2SD, and Total Error (TE) = SQRT(2) x 2SD. To ensure accuracy and precision of measurements, the total error of the Aptima HBV Quant assay was set at 1 log IU/mL (i.e., at the LLoQ, the difference between two measurements of more than 1 log IU/mL is statistically significant).

The LLoQ was determined by testing panels of the 3rd WHO International Standard for Hepatitis B Virus RNA (NIBSC 10/264) diluted in HBV negative human plasma and serum. A minimum of 45 replicates of each dilution were tested with each of three reagent lots for a minimum of 135 replicates per dilution. The results from the reagent lot with the highest concentration meeting the TE and TAE requirements are shown in Table 6. The calculated LLoQ for the 3rd WHO International Standard for Hepatitis B Virus is 4.80 IU/mL for plasma and 6.34 IU/mL for serum.

Table 4: Determination of LLoQ Using the 3rd WHO International Standard for HBV Diluted in Plasma

Reagent Lot	Target Concentration		Aptima HBV Quant	SD	|Bias|	Calculated TE	Calculated TAE
	(IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)
1	7	0.85	0.63	0.27	0.22	0.75	0.75
	8	0.90	0.68	0.28	0.22	0.78	0.77
	9	0.95	0.79	0.25	0.17	0.70	0.66
2	7	0.85	0.48	0.20	0.37	0.57	0.77
	8	0.90	0.47	0.18	0.44	0.51	0.79
	9	0.95	0.61	0.19	0.34	0.54	0.73
3	7	0.85	0.53	0.21	0.32	0.59	0.74
	8	0.90	0.52	0.21	0.38	0.61	0.81
	9	0.95	0.70	0.23	0.25	0.65	0.71

SD=standard deviation

Table 5: Determination of LLoQ Using the 3rd WHO International Standard for HBV Diluted in Serum

Reagent Lot	Target Concentration		Aptima HBV Quant	SD	|Bias|	Calculated TE	Calculated TAE
	(IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)	(log IU/mL)
1	7	0.85	0.77	0.38	0.08	1.06	0.83
	8	0.90	0.80	0.31	0.10	0.88	0.72
	9	0.95	0.91	0.27	0.05	0.77	0.59
2	7	0.85	0.57	0.20	0.28	0.57	0.68
	8	0.90	0.70	0.22	0.20	0.63	0.64
	9	0.95	0.69	0.23	0.27	0.66	0.73
3	7	0.85	0.65	0.24	0.20	0.67	0.67
	8	0.90	0.65	0.24	0.25	0.68	0.73
	9	0.95	0.67	0.22	0.28	0.63	0.73

SD=standard deviation

Table 6: Summary of the Calculated LLoQ Using the 3rd WHO International Standard for HBV

Reagent Lot	Plasma LLoQ		Serum LLoQ	
	(log IU/mL)	(IU/mL)	(log IU/mL)	(IU/mL)
1	0.68	4.80	0.80	6.34
2	0.61	4.09	0.57	3.72
3	0.52	3.34	0.65	4.51

SD=standard deviation

Determination of the Lower Limit of Quantitation Across HBV Genotypes

The LLoQ was determined by testing dilutions of HBV positive clinical specimens for genotypes A, B, C, D, E, F, G, and H in HBV negative human plasma and serum. A minimum of 36 replicates of each panel member were tested with each of two reagent lots for a minimum of 72 replicates per panel member. The results from the reagent lot with the highest concentration meeting the TE and TAE requirements are shown in Table 7 for plasma and Table 8 for serum. The calculated LLoQ for genotypes A, B, C, D, E, F, G, and H in plasma and serum are summarized in Table 9. This established the overall LLoQ for the assay as 10 IU/mL.

Table 7: Determination of LLoQ Across Genotypes in Plasma

Genotype	Target Concentration		Aptima HBV Quant	SD	|Bias|	Calculated TE	Calculated TAE
	(IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)
A	7	0.85	0.86	0.71	0.02	2.01	1.44
	8	0.90	0.76	0.34	0.14	0.96	0.82
	9	0.95	0.95	0.27	0.01	0.76	0.55
B	7	0.85	0.85	0.36	0.01	1.01	0.72
	8	0.90	0.93	0.24	0.03	0.67	0.51
	9	0.95	0.94	0.23	0.01	0.64	0.46
C	7	0.85	0.62	0.22	0.23	0.62	0.67
	8	0.90	0.65	0.25	0.25	0.70	0.75
	9	0.95	0.70	0.21	0.26	0.59	0.67
D	8	0.90	0.47	0.19	0.43	0.53	0.81
	9	0.95	0.58	0.17	0.38	0.48	0.72
	10	1.00	0.64	0.24	0.36	0.69	0.85
E	7	0.85	0.59	0.19	0.25	0.53	0.63
	8	0.90	0.59	0.23	0.31	0.66	0.78
	9	0.95	0.68	0.28	0.27	0.80	0.83
F	7	0.85	0.92	0.26	0.07	0.74	0.59
	8	0.90	1.09	0.29	0.18	0.83	0.77
	9	0.95	1.04	0.31	0.09	0.89	0.72
G	7	0.85	0.81	0.27	0.04	0.75	0.57
	8	0.90	0.91	0.26	0.01	0.72	0.52
	9	0.95	0.83	0.26	0.12	0.74	0.64
H	7	0.85	0.65	0.25	0.19	0.71	0.69
	8	0.90	0.67	0.24	0.23	0.68	0.71
	9	0.95	0.62	0.21	0.33	0.59	0.75

SD=standard deviation

Table 8: Determination of LLoQ Across Genotypes in Serum

Genotype	Target Concentration		Aptima HBV Quant	SD	|Bias|	Calculated TE	Calculated TAE
	(IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)	(log IU/ml)
A	7	0.85	0.67	0.28	0.18	0.79	0.74
	8	0.90	0.80	0.34	0.10	0.96	0.78
	9	0.95	0.83	0.26	0.12	0.74	0.65
B	7	0.85	0.56	0.19	0.29	0.54	0.67
	8	0.90	0.71	0.22	0.20	0.62	0.63
	9	0.95	0.59	0.21	0.36	0.59	0.78
C	7	0.85	0.64	0.27	0.20	0.77	0.75
	8	0.90	0.75	0.25	0.15	0.70	0.64
	9	0.95	0.79	0.21	0.16	0.58	0.57
D	8	0.90	0.42	0.14	0.48	0.40	0.76
	9	0.95	0.47	0.18	0.49	0.51	0.84
	10	1.00	0.60	0.23	0.40	0.64	0.85
E	7	0.85	0.56	0.24	0.29	0.69	0.78
	8	0.90	0.63	0.18	0.27	0.50	0.63
	9	0.95	0.67	0.25	0.28	0.70	0.78
F	7	0.85	0.88	0.21	0.03	0.60	0.46
	8	0.90	0.90	0.29	0.01	0.81	0.58
	9	0.95	0.98	0.24	0.02	0.67	0.49
G	7	0.85	0.82	0.24	0.03	0.67	0.50
	8	0.90	0.90	0.25	0.01	0.70	0.50
	9	0.95	1.01	0.23	0.05	0.64	0.51
H	8	0.90	0.69	0.29	0.21	0.81	0.78
	9	0.95	0.77	0.26	0.19	0.74	0.71
	10	1.00	0.78	0.28	0.22	0.80	0.79

SD=standard deviation

Table 9: Summary of LLoQ Across Genotypes in Plasma and Serum

Genotype	Plasma LLoQ		Serum LLoQ	
	(log IU/mL)	(IU/mL)	(log IU/mL)	(IU/mL)
A	0.95	8.90	0.83	6.79
B	0.93	8.60	0.56	3.59
C	0.62	4.14	0.64	4.38
D	0.64	4.32	0.60	4.01
E	0.59	3.91	0.56	3.60
F	0.92	8.25	0.88	7.56
G	0.81	6.42	0.82	6.55
H	0.62	4.20	0.78	6.01

Reproducibility

To assess reproducibility, a 28 member panel was made by diluting HBV positive clinical specimens (genotype A and C) or spiking HBV DNA (genotype A and C) into HBV negative plasma and serum. The panel was tested by three operators using three reagents lots on three Panther systems over 20 or more test days.

Table 10 and Table 11 shows the reproducibility of assay results (in log IU/mL) between instruments, between operators, between lots, between runs, within runs, and overall. Total variability was primarily due to within-run variability (i.e., random error).

Table 10: Reproducibility of the Aptima HBV Quant Assay for Genotype A

Matrix	N	Mean Concentration (log IU/mL)	Inter-Operator		Inter-Instrument		Inter-Lot		Inter-Run		Intra-Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV(%)	SD	CV (%)	SD	CV(%)
Plasma	161	1.24	0.02	1.96	0.02	1.75	0.10	7.88	0.02	1.50	0.21	16.80	0.23	18.80
Plasma	162	1.97	0.01	0.75	0.01	0.71	0.08	3.84	0.02	0.76	0.14	7.36	0.17	8.40
Plasma	162	3.23	0.01	0.29	0.01	0.24	0.04	1.39	0.01	0.22	0.08	2.47	0.09	2.87
Plasma	162	4.14	0.03	0.76	0.01	0.28	0.04	0.90	0.01	0.15	0.06	1.38	0.08	1.84
Plasma	162	5.75	0.02	0.39	0.01	0.20	0.06	0.97	0.01	0.13	0.09	1.51	0.11	1.85
Plasma	162	6.98	0.05	0.79	0.03	0.48	0.06	0.91	0.01	0.11	0.09	1.35	0.13	1.87
Plasma	162	7.69	0.03	0.38	0.02	0.23	0.01	0.15	0.01	0.12	0.10	1.30	0.11	1.39
Serum	160	1.17	0.02	1.93	0.02	1.71	0.06	5.54	0.02	1.64	0.20	17.07	0.21	18.21
Serum	162	1.82	0.02	1.15	0.01	0.79	0.10	5.43	0.01	0.72	0.15	8.13	0.18	9.90
Serum	162	3.16	0.01	0.26	0.02	0.62	0.09	2.78	0.02	0.59	0.11	3.38	0.14	4.47
Serum	162	4.06	0.01	0.19	0.01	0.22	0.04	0.99	0.01	0.15	0.07	1.68	0.08	1.98
Serum	162	5.60	0.02	0.36	0.01	0.24	0.06	1.15	0.01	0.22	0.13	2.40	0.15	2.70
Serum	162	6.30	0.03	0.49	0.03	0.42	0.01	0.17	0.01	0.16	0.11	1.77	0.12	1.90
Serum	162	7.48	0.05	0.62	0.03	0.36	0.02	0.25	0.02	0.23	0.08	1.09	0.10	1.35

CV=coefficient of variation, SD=standard deviation

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD and CV are shown as 0.

Table 11: Reproducibility of the Aptima HBV Quant Assay for Genotype C

Matrix	N	Mean Concentration (log IU/mL)	Inter-Operator		Inter-Instrument		Inter-Lot		Inter-Run		Intra-Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV(%)	SD	CV (%)	SD	CV(%)
Plasma	161	1.28	0.02	1.73	0.02	1.40	0.07	5.39	0.02	1.28	0.18	14.32	0.20	15.52
Plasma	162	1.98	0.02	0.79	0.01	0.68	0.08	4.02	0.01	0.61	0.14	6.91	0.16	8.08
Plasma	162	3.23	0.01	0.31	0.01	0.39	0.05	1.49	0.01	0.18	0.06	1.97	0.08	2.52
Plasma	162	4.13	0.01	0.18	0.01	0.29	0.04	0.90	0.01	0.15	0.07	1.69	0.08	1.95
Plasma	162	5.78	0.01	0.14	0.02	0.41	0.06	1.04	0.01	0.16	0.10	1.70	0.12	2.05
Plasma	162	6.83	0.01	0.20	0.03	0.42	0.03	0.42	0.01	0.08	0.06	0.93	0.08	1.13
Plasma	162	7.75	0.03	0.33	0.02	0.19	0.02	0.24	0.01	0.08	0.07	0.94	0.08	1.04
Serum	160	1.20	0.02	1.54	0.02	1.55	0.05	4.24	0.02	1.78	0.18	14.74	0.19	15.59
Serum	162	1.90	0.02	1.20	0.02	0.87	0.05	2.73	0.01	0.72	0.15	8.10	0.17	8.71
Serum	162	3.19	0.03	0.93	0.03	0.92	0.05	1.68	0.00	0.05	0.07	2.34	0.10	3.16
Serum	162	4.04	0.01	0.14	0.01	0.31	0.04	0.94	0.00	0.12	0.05	1.30	0.07	1.64
Serum	162	5.69	0.01	0.16	0.02	0.36	0.05	0.88	0.01	0.13	0.09	1.50	0.10	1.78
Serum	162	6.32	0.04	0.64	0.03	0.45	0.04	0.66	0.01	0.14	0.10	1.57	0.12	1.87
Serum	162	7.23	0.04	0.55	0.02	0.25	0.05	0.68	0.01	0.13	0.10	1.42	0.12	1.69

CV=coefficient of variation, SD=standard deviation

Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD and CV are shown as 0.

Potentially Interfering Substances

The susceptibility of the Aptima HBV Quant assay to interference by elevated levels of endogenous substances or by drugs commonly prescribed to HBV infected individuals was evaluated. HBV negative plasma samples and samples spiked with HBV to a concentration 4.3 log IU/mL of HBV DNA were tested.

No interference in the performance of the assay was observed in the presence of albumin (90 mg/mL), hemoglobin (5 mg/mL), triglycerides (30 mg/mL), or unconjugated bilirubin (0.2 mg/mL).

Clinical plasma specimens from patients with elevated levels of defined substances or from patients with the diseases listed in Table 12 were tested with the Aptima HBV Quant assay. No interference in the performance of the assay was observed.

Table 12: Tested Clinical Specimen Types

	Clinical Specimen Types
1	Antinuclear antibody (ANA)
2	Rheumatoid factor (RF)
3	Alcoholic cirrhosis (AC)
4	Alcoholic hepatitis
5	Non-alcoholic hepatitis
6	Autoimmune hepatitis
7	Elevated alanine aminotransferase (ALT)
8	Hepatocellular carcinoma (HCC)
9	Multiple sclerosis (MS)
10	Systemic lupus erythematosus (SLE)
11	Hyperglobulinemia
12	Rheumatoid arthritis (RA)
13	Anti-Jo1 antibody (JO-1)
14	Multiple myeloma (MM)
15	Hemolyzed (elevated hemoglobin)
16	Icteric (elevated bilirubin)
17	Lipemic (elevated lipid)
18	Elevated protein
19	HBV antibodies (vaccinated)
20	HCV antibodies
21	HIV-1 and HIV-2 antibodies

No interference in the performance of the assay was observed in the presence of the exogenous substances listed in Table 13 at concentrations at least three times the C_{max} (human plasma).

Table 13: Exogenous Substances

Exogenous Substance Pool	Exogenous Substances Tested
1	Saquinavir, ritonavir, amprenavir, indinavir, lopinavir, nelfinavir mesylate
2	Clarithromycin, valganciclovir hydrochloride, efavirenz, nevirapine
3	Paroxetine HCl, enfuvirtide, zidovudine, didanosine, abacavir sulfate
4	Ribavirin, entecavir, adefovir dipivoxil, tenofovir disoproxil fumarate, lamivudine, ganciclovir, acyclovir
5	Stavudine, ciprofloxacin, fluoxetine, azithromycin, valacyclovir, sertraline, zalcitabine
6	Interferon alpha -2a, interferon alpha -2b, pegylated interferon alpha-2b

Specificity

Specificity was determined using 292 fresh and 747 frozen HBV negative clinical specimens. A total of 521 plasma and 518 serum specimens were tested. Specificity was calculated as the percentage of HBV negative samples with results of "Not Detected." HBV DNA was not detected in 1038 samples. Specificity was 99.9% (1038/1039, 95% CI: 99.5 -100%).

Table 14: Specificity in Plasma and Serum Clinical Specimens

	Fresh Plasma	Frozen Plasma	Plasma Total	Fresh Serum	Frozen Serum	Serum Total	Combined
Valid replicates (n)	145	376	521	147	371	518	1,039
Not Detected	145	376	521	147	370	517	1,038
Specificity (95% CI)	100% (97.4-100)	100% (99.0-100)	100% (99.3-100)	100% (97.5-100)	99.7% (98.5-100)	99.8% (98.9-100)	99.9% (99.5-100)

CI=confidence interval

Analytical Specificity

Potential cross-reactivity to the pathogens listed in Table 15 was evaluated in HBV negative human plasma in the presence or absence of 4.3 log IU/mL HBV DNA. No cross-reactivity or interference was observed in bacterially contaminated plasma or in specimens from subjects infected with other blood-borne pathogens or those that had received HBV and flu vaccines.

Table 15: Pathogens Tested for Analytical Specificity

Microorganism/Pathogen	Source	Microorganism/Pathogen	Source
Hepatitis C virus	Clinical specimen	Human herpesvirus type 8	Culture Fluid
Hepatitis A virus	Clinical specimen	Japanese encephalitis virus	Ascitic Fluid
HBV vaccinated	Clinical specimen	Murray Valley encephalitis virus	Cell lysate
HIV-1 and -2	Clinical specimen	St. Louis encephalitis virus	Culture Fluid
Human T-cell lymphotropic virus type 1 and 2	Clinical specimen	Vaccinia virus	Cell lysate
Parvovirus B19	Clinical specimen	Yellow fever virus	Culture Fluid
Cytomegalovirus	Clinical specimen	*Candida albicans*	Culture
Dengue virus type1-4	Clinical specimen	*Chlamydia trachomatis*	Culture
Epstein-Barr virus	Clinical specimen	*Corynebacterium diphtheriae*	Culture
Flu vaccinated	Clinical specimen	*Mycobacterium gordonae*	Culture
Human papillomavirus	Clinical specimen	*Mycobacterium smegmatis*	Culture
Herpes simplex virus 1 and 2	Clinical specimen	*Neisseria gonorrhoeae*	Culture
Rubella virus	Clinical specimen	*Propionibacterium acnes*	Culture
Varicella zoster virus	Clinical specimen	*Staphylococcus aureus*	Culture
West Nile virus	Clinical specimen	*Staphylococcus epidermidis*	Culture
BK human polyomavirus	Cell lysate	*Streptococcus pneumoniae*	Culture
Human herpesvirus 6B	Culture Fluid	*Trichomonas vaginalis*	Culture

Repeatability of Clinical Specimens

Repeatability was evaluated by testing three replicates of naturally infected HBV positive plasma and serum clinical specimens. The average concentration and standard deviation for the plasma and serum samples tested are shown in Tables 16 and 17, respectively.

Table 16: Repeatability of Clinical Plasma Specimens

Plasma Specimen	Average Concentration (log IU/mL)	SD
1	2.08	0.09
2	2.98	0.01
3	2.45	0.09
4	2.32	0.06
5	2.58	0.08
6	3.60	0.06
7	3.45	0.04
8	3.95	0.04
9	3.81	0.05
10	4.26	0.03
11	5.65	0.07
12	6.32	0.06
13	6.79	0.06
14	7.40	0.05
15	8.17	0.02

SD=standard deviation

Table 17: Repeatability of Clinical Serum Specimens

Serum Specimen	Average Concentration (log IU/mL)	SD
1	1.93	0.17
2	2.29	0.09
3	2.78	0.05
4	1.98	0.06
5	2.53	0.07
6	3.53	0.06
7	3.38	0.03
8	3.77	0.02
9	3.45	0.17
10	4.26	0.04
11	6.31	0.02
12	5.45	0.04
13	5.74	0.08
14	7.44	0.04
15	8.47	0.03

SD=standard deviation

Sample Dilution Using Specimen Diluent

To assess recovery of HBV DNA in samples diluted with Aptima Specimen Diluent, plasma and serum samples that spanned the linear range were diluted 1:3 with Aptima Specimen Diluent. In addition, high-titer naturally infected clinical specimens and HBV DNA spiked samples with concentrations above the ULoQ were diluted 1:100 with the Aptima Specimen Diluent. Each sample was tested neat and diluted (1:3 or 1:100) in triplicate. The differences between the average reported concentration (dilution factor applied to the diluted sample result) and the average neat concentration are shown in Table 18 for plasma and Table 19 for serum. The sample concentrations were accurately recovered in the diluted samples.

Table 18: Sample Dilution With Aptima Specimen Diluent in Plasma

Dilution	Average Neat Concentration (log IU/mL)	Average Reported Concentration[a] (log IU/mL)	Difference (log IU/mL)
1:3	2.08	1.71	-0.37
	2.98	3.02	0.04
	2.45	2.30	-0.15
	2.32	2.06	-0.26
	2.58	2.46	-0.12
	3.60	3.62	0.02
	3.45	3.36	-0.09
	3.95	3.91	-0.04
	3.81	3.72	-0.09
	4.26	4.24	-0.02
	5.65	5.50	-0.15
	6.32	6.08	-0.24
	6.79	6.40	-0.39
	7.40	7.06	-0.34
	8.17	8.05	-0.12
1:100	8.17	7.82	-0.35
	>9.00[b] (10.20[c])	10.40	0.20

[a]Reported concentration is the value calculated after the dilution factor has been applied.

[b]Spiked specimen.

[c] Target concentration value, which is above ULoQ.

Table 19: Sample Dilution With Aptima Specimen Diluent in Serum

Dilution	Average Neat Concentration (log IU/mL)	Average Reported Concentration? (log IU/mL)	Difference (log IU/mL)
1:3	1.93	1.50	-0.43
	2.29	2.00	-0.29
	2.78	2.45	-0.33
	1.98	1.50	-0.48
	2.53	2.23	-0.30
	3.53	3.59	0.06
	3.38	3.21	-0.17
	3.77	3.68	-0.09
	3.45	3.35	-0.10
	4.26	4.16	-0.10
	6.31	5.98	-0.33
	5.45	5.24	-0.21
	5.74	5.62	-0.12
	7.44	7.19	-0.25
	8.47	8.31	-0.16
1:100	8.47	8.19	-0.28
	>9.00[b] (10.20[c])	10.43	0.23

[a]Reported concentration is the value calculated after the dilution factor has been applied.

[b]Spiked specimen.

[c] Target concentration value, which is above ULoQ.

Method Correlation

The performance of the Aptima HBV Quant assay was assessed against a CE-marked and a Health Canada licensed comparator assay by testing undiluted clinical specimens from HBV infected patients. A total of 614 clinical specimens within the linear range common to both assays were used for the linear regression as shown in Figure 8.

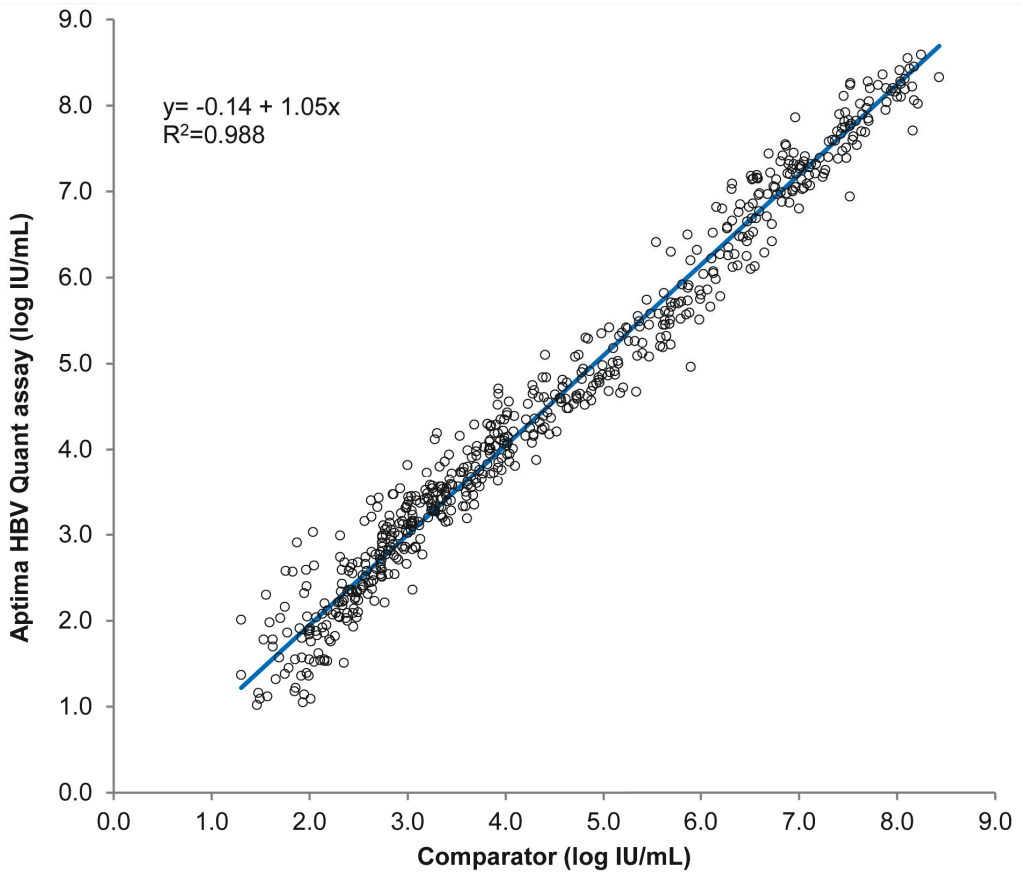

Figure 8. Correlation between the Aptima HBV Quant Assay and Comparator Assay

Carryover

To establish that the Panther system minimizes the risk of false positive results arising from carryover contamination, a study was conducted using spiked panels on three Panther systems. Carryover was assessed using high titer HBV DNA spiked plasma samples (8 log IU/mL) interspersed between HBV negative samples in a checkerboard pattern. Testing was carried out over fifteen runs. The overall carryover rate was 0.0% (0/705).

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