Aptima® HBV Quant Assay

Instructions for Use
For *in vitro* diagnostic use
For U.S. 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 for the presence of HBV DNA in blood or blood products or as a diagnostic test to confirm the presence of HBV infection.

Summary and Explanation of the Test

Hepatitis B virus, 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. In 2019, an estimated 296 million people were living with chronic HBV infection worldwide.[1] 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.[2,3] 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 is 2-5%.[4]

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 three 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.[5] There are 8 HBV genotypes (A-H), and these are typically found in distinct geographical locations.

Due to the dynamic nature of chronic hepatitis B infection, continuous monitoring of HBV DNA and alanine aminotransferase (ALT) levels is important.[6] For the majority of HBV infected individuals who are undergoing antiviral treatment, the goal is HBV DNA suppression. Quantitative nucleic acid tests with a broad linear range are effective tools to monitor HBV DNA viral load during the course of treatment.

Principles of the Procedure

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 quantitate 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).

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 ensures accurate quantitation of the HBV DNA.

Detection is achieved using single-stranded nucleic acid torches that are present during the amplification of the target, which 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.

Summary of Safety and Performance

The SSP (Summary of Safety and Performance) is available in the European database on medical devices (Eudamed), where it is linked to the device identifiers (Basic UDI-DI). To locate the SSP for Aptima HBV Quant assay, refer to the Basic Unique Device Identifier (BUDI): 54200455DIAGAPTHBVAF.

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

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

D. Target Enhancer Reagent (TER) is corrosive. See Safety Data Sheet information at the end of this section.

Laboratory Related

E. CAUTION: The controls for this assay contain human plasma. The plasma is non-reactive 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. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions.[7,8,9]

F. 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.

G. Use only supplied or specified disposable laboratory ware.

H. 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.

I. 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.

J. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations.[7,8,9,10] Thoroughly clean and disinfect all work surfaces.

K. 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.

L. 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

M. Specimens may be infectious. Use Universal Precautions[7,8,9] when performing this assay. Proper handling and disposal methods should be established in accordance with local regulations.[10] Only personnel adequately trained in the use of the Aptima HBV Quant assay and trained in handling infectious materials should perform this procedure.

N. 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.

O. 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

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

Q. 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.

R. Avoid microbial and nuclease contamination of reagents.

S. 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.

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

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

V. Some reagents of this kit are labeled with hazard information.

Note: Hazard communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on https://www.hologic.com/package-inserts.

North America Hazard Information	
	**HBV VL Kit Controls** ***Human Serum / Human Plasma 95-100%*** ***Sodium azide <1%*** — —
	**Target Enhancer Reagent** ***Lithium Hydroxide, Monohydrate 5-10%*** — **DANGER** H302 - Harmful if swallowed H314 - Causes severe skin burns and eye damage P264 - Wash face, hands and any exposed skin thoroughly after handling P270 - Do not eat, drink or smoke when using this product P330 - Rinse mouth P501 - Dispose of contents/ container to an approved waste disposal plant P260 - Do not breathe dusts or mists P280 - Wear protective gloves/protective clothing/eye protection/face protection P301 + P330 + P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower P304 + P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P321 - Specific treatment (see supplemental first aid instructions in the SDS) P363 - Wash contaminated clothing before reuse P405 - Store locked up P301 + P317 - IF SWALLOWED: Get medical help. P316 - Get emergency medical help immediately.
EU Hazard Information	
—	**Amplification Reagent** ***Magnesium Chloride 60-65%*** — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
—	**Enzyme Reagent** ***HEPES 1-5%*** ***Triton X-100 1-5%*** — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.
—	**Enzyme Reconstitution Solution** ***Glycerol 20-25%*** ***Triton X-100 5-10%*** ***HEPES 1-5%*** — H412 – Harmful to aquatic life with long lasting effects. P273 – Avoid release to the environment. P501 - Dispose of contents/ container to an approved waste disposal plant.

Promoter Reagent

Magnesium Chloride 60-65%

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—

H412 – Harmful to aquatic life with long lasting effects.
P273 – Avoid release to the environment.
P501 - Dispose of contents/ container to an approved waste disposal plant.

Target Capture Reagent

HEPES 15-20%
Lauryl Sulfate Lithium Salt 5-10%
Succinic Acid 1-5%
Lithium Hydroxide, Monohydrate 1-5%

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—

H412 – Harmful to aquatic life with long lasting effects.
P273 – Avoid release to the environment.
P501 - Dispose of contents/ container to an approved waste disposal plant.

HBV VL Kit Calibrators

HEPES 15-20%
Lauryl Sulfate Lithium Salt 5-10%
Succinic Acid 1-5%
Lithium Hydroxide, Monohydrate 1-5%

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H412 – Harmful to aquatic life with long lasting effects.
P273 – Avoid release to the environment.
P501 – Dispose of contents/container to an approved waste disposal plant.

HBV VL Kit Controls

Human Serum / Human Plasma 95-100%
Sodium azide <1%

—

DANGER

H300 - Fatal if swallowed.
H410 - Very toxic to aquatic life with long lasting effects.
P264 - Wash face, hands, and any exposed skin thoroughly after handling.
P273 - Avoid release to the environment.
P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P321 - Specific treatment (see supplemental first aid instructions on this label).
P330 - Rinse mouth.
P391 - Collect spillage.

Target Enhancer Reagent

Lithium Hydroxide, Monohydrate 5-10%

—

DANGER

H302 – Harmful if swallowed.
H314 – Causes severe skin burns and eye damage.
P264 - Wash face, hands and any exposed skin thoroughly after handling.
P270 - Do not eat, drink or smoke when using this product.
P330 - Rinse mouth.
P501 - Dispose of contents/ container to an approved waste disposal plant.
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P301 + P330 + P331 - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P304 + P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 - Immediately call a POISON CENTER or doctor.
P321 - Specific treatment (see supplemental first aid instructions on this label).
P363 - Wash contaminated clothing before reuse.
P405 - Store locked up.

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 8 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. Ensure that precipitates are dissolved. Do not use the calibrator if gelling, precipitation, or cloudiness is present.

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.*

Note: *Only plastic secondary tubes are recommended for storage.*

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

- Tubes containing ethylenediaminetetraacetic acid (EDTA) or acid citrate dextrose (ACD) anticoagulants
- Plasma preparation tubes
- Serum tubes
- Serum separator tubes

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

A. Specimen Collection

Whole blood can be stored at 2°C to 30°C for up to 24 hours, and the plasma must be separated via centrifugation in a primary tube prior to processing. Separate the plasma or serum from the red blood cells following the manufacturer's instructions for the tube used. Plasma or serum can be tested on the Panther system in a primary tube or transferred to a secondary tube such as the Aptima® Specimen Aliquot Tube. To obtain the 500 µL reaction volume, the minimum volume of plasma or serum for primary collection tubes is up to 1200 µL and for secondary tubes, the minimum volume is 700 µL. The following table identifies dead volume requirements for each primary and secondary tube type.

Tube (Size and Type)	Dead Volume on Panther
Aptima Sample Aliquot Tube (SAT)	0.2 mL
12x75 mm	0.5 mL
13x100 mm	0.5 mL
13x100 mm with Gel	0.3 mL
16x100 mm with Gel	0.7 mL

If not tested immediately, plasma and serum can be stored in accordance with the specifications below. If transferred to the SAT or a secondary tube, 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 secondary tube at 2°C to 30°C for up to 24 hours,
- In the primary collection tube or secondary tube at 2°C to 8°C for up to 5 days, or
- In the secondary tube at -20°C for up to 60 days.

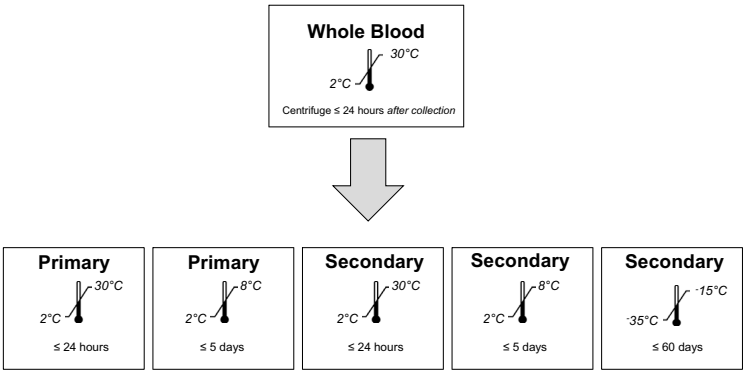

Figure 1. Storage Conditions for EDTA/ACD Tubes

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

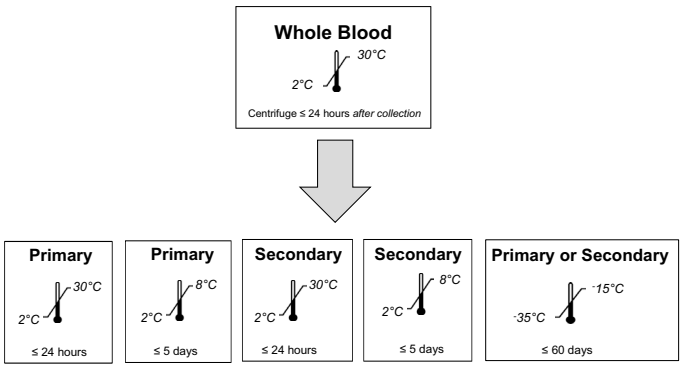

Figure 2. Storage Conditions for PPTs

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

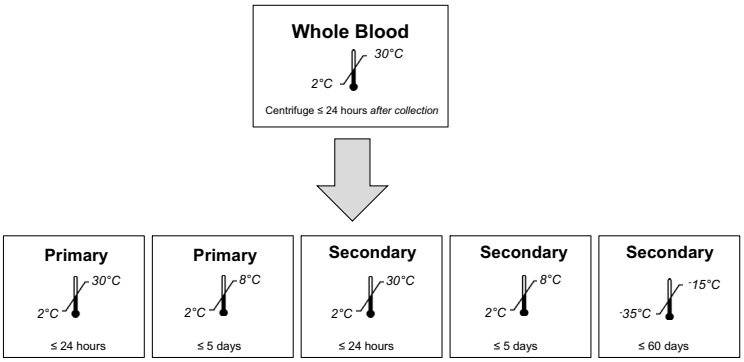

Figure 3. Storage Conditions for Serum Tubes

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

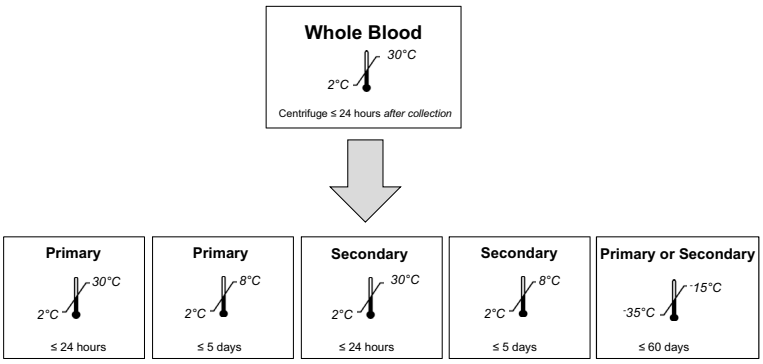

Figure 4. Storage Conditions for SSTs

C. Long Term Frozen Storage

   Plasma or serum samples may be stored at -70°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 or a secondary tube for testing on the Panther system. See *Panther System Test Procedure*, step E.5 below 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

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*	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	303095
Panther Fusion™ System	PRD-04172
Panther™ System Continuous Fluid and Waste (Panther Plus)	PRD-06067
Aptima® HBV Quant Calibrator Kit	PRD-03425
Aptima® HBV Quant Controls Kit	PRD-03426
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)

Material	Cat. No.
Tips, 1000 µL conductive, liquid sensing	901121 (10612513 Tecan) 903031 (10612513 Tecan)
Not all products are available in all regions. Contact your representative for region-specific information.	MME-04134 (30180117 Tecan) MME-04128
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	—
Disposable, powderless gloves	—
Replacement non-penetrable caps	103036A
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 tube options:	
13 mm x 100 mm	—
13 mm x 75 mm	—
16 mm x 100 mm	—
Centrifuge	—
Vortex mixer	—

Optional Materials

Material	Cat. No.
Secondary tube options:	
12 mm x 75 mm	—
13 mm x 100 mm	—
16 mm x 100 mm	—
Aptima® Specimen Aliquot Tubes (SAT) (100 pack)	FAB-18184
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	—
Cotton-tipped swabs	—
Tube rocker	PRD-03488

Panther System Test Procedure

Note: *See the Panther/Panther Fusion 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 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 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.*

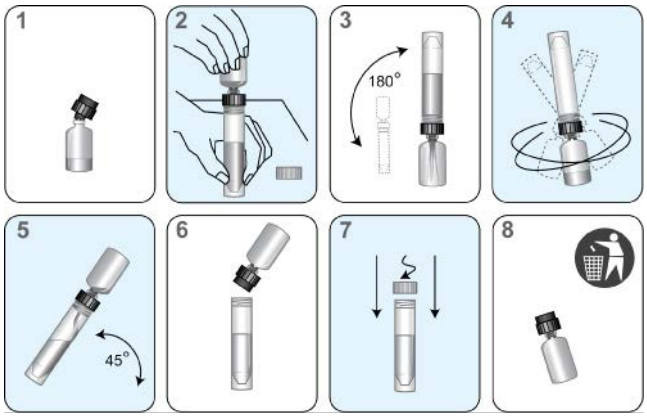

Figure 5. Reagent Reconstitution Process

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.

   Option: The previously prepared reagents may be prepared on a tube rocker by following these instructions: Remove the reagents from storage (2°C to 8°C). Place the reagents on a tube rocker and leave at 15°C to 30°C to warm for at least 30 minutes.
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 that processed specimens in primary tubes or undiluted plasma specimens in secondary tubes have been stored properly per *Specimen Collection*.
2. Ensure frozen specimens are thoroughly thawed. Vortex the thawed specimens for 3 to 5 seconds to mix thoroughly.
3. Allow the specimens to reach 15°C to 30°C prior to processing. See *Samples Onboard the Panther System* for additional onboard information.
4. Ensure each primary collection tube contains up to 1200 µL of specimen or each SAT contains at least 700 µL of specimen. Refer to the table provided in *Specimen Collection* to identify dead volume requirements for each primary and secondary tube type. If specimen dilution is necessary, see step E.5 below for additional information.
5. Dilute a plasma or serum specimen 1:3 in a SAT or 1:100 in a secondary tube.

   A specimen may be diluted in a secondary tube 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 Aptima 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/Panther Fusion 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 or a secondary tube.

      ii. Add 2970 µL of Aptima 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/Panther Fusion 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.*

6. Just prior to loading specimens into a Sample Rack, centrifuge each specimen at 1000 to 3000g for 10 minutes. Do not remove caps. Bubbles in the tube can 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.

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion 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 are 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/ Panther Fusion 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, the Panther system automatically calculates the HBV DNA concentration for the neat specimen by multiplying the diluted concentration by the dilution factor, and diluted samples will be flagged as diluted.

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 quantitation). 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	$\log_{10}$ IU/mL[a]	
Not Detected	Not Detected	HBV DNA not detected.
<10 detected	<1.00	HBV DNA is detected but at a level below the LLoQ.
10 to 1,000,000,000	1.00 to 9.00	HBV DNA concentration is within the linear range of 10 to 1,000,000,000 IU/mL.
> 1,000,000,000	> 9.00	HBV DNA concentration is above the ULoQ.
Invalid[b]	Invalid[b]	Error indicated 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.
For diluted specimens with neat concentrations greater than the ULoQ, results will be reported using scientific notation.

The acceptance criteria for each of the Aptima HBV Quant assay controls are outlined in Table 2 below.

Note: *The recovery range listed below shifts based on the assigned value of each specific lot. Refer to the assigned concentration listed on the Control Barcode Sheet insert provided with each Control box.*

Table 2: Acceptance Criteria for Recovery Range for Aptima HBV Quant Assay Controls

Component	Recovery Range for Valid Runs
Negative Control	n/a
Low Positive Control	+/- 0.55 $\log_{10}$ IU/mL
High Positive Control	+/- 0.5 $\log_{10}$ IU/mL

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.

Analytical 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.[11]

The LoD was determined by testing panels of the 3rd WHO International Standard for Hepatitis B Virus DNA (NIBSC 10/264, genotype A) diluted in HBV negative human EDTA 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 95% predicted detection limits. The LoD values 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 4.8 IU/mL for plasma and 5.9 IU/mL for serum.

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 an approved 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 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 (95% Predicted Detection Limit) Across HBV Genotypes Using Clinical Specimens

	Concentration (IU/mL)	
Genotype	**Plasma**	**Serum**
A	3.3	4.1
B	2.9	3.9
C	4.9	5.2
D	5.7	5.4
E	5.8	5.8
F	3.0	4.0
G	2.8	7.4
H	5.5	6.3

Linear Range

The linear range was established by testing panels of HBV genotype A virus (0.78 log_{10} IU/mL to 7.30 log_{10} IU/mL) and plasmid DNA (5.30 log_{10} IU/mL to 9.18 log_{10} IU/mL) diluted in HBV negative human plasma and serum according to CLSI EP06-A.[12] The Aptima HBV Quant assay demonstrated linearity across the range tested, with an upper limit of quantitation (ULoQ) of 9 log_{10} IU/mL as shown in Figure 6.

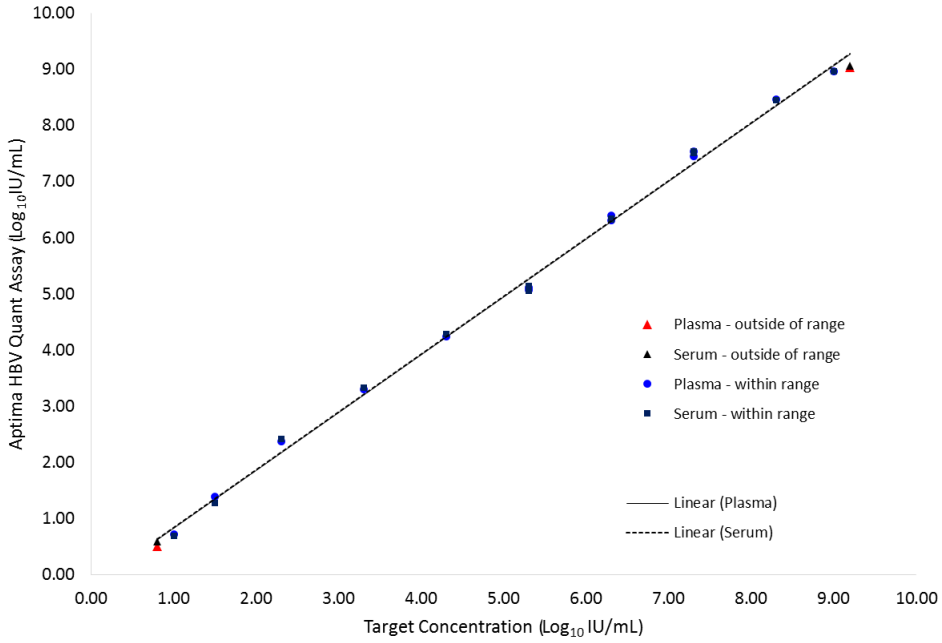

Figure 6. Linearity in Plasma and Serum

Linearity Across HBV Genotypes

The linearity of HBV genotypes was established by testing individual clinical positive samples for genotypes A, E, F, G, and H, and PEI 1st WHO Reference panels (PEI 5086/08) for genotypes B, C, and D. Virus was used for the lower range of the assay (4 $\log_{10}$ IU/mL and below for genotypes A-G, 3 $\log_{10}$ IU/mL, and below for genotype H). Plasmid DNA was used for the upper range with a 2 $\log_{10}$ overlap. Dilutions in negative human plasma were tested for all genotypes. Linearity was demonstrated across the range tested for all genotypes tested as shown in Figure 7.

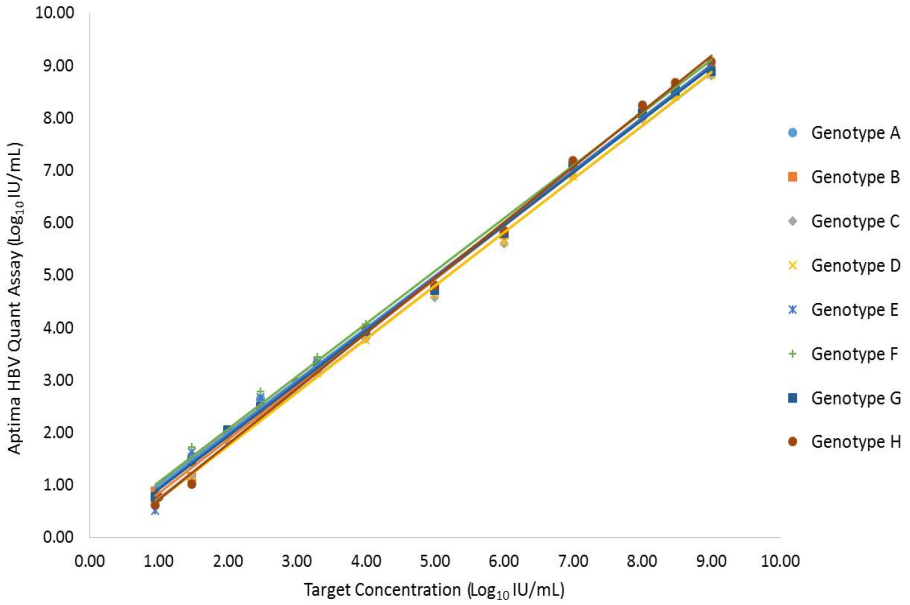

Figure 7. Linear Range and Linearity (Plasma)

Lower Limit of Quantitation Using the 3rd WHO International Standard

The lower limit of quantitation is defined as the lowest concentration at which HBV DNA is reliably quantitated within a total error, according to CLSI EP17-A2.[11] Total error was estimated by the following 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 $\log_{10}$ IU/mL (i.e., at the LLoQ, the difference between two measurements of more than 1 $\log_{10}$ IU/mL is statistically significant).

The LLoQ was determined by testing panels of the 3rd WHO International Standard for Hepatitis B Virus DNA (NIBSC 10/264, genotype A) diluted in HBV negative human plasma and serum. Forty-five (45) replicates of each dilution were tested with each of three reagent lots for a minimum of 135 replicates per dilution. The results for the three reagent lots are shown in Table 4 for plasma and Table 5 for serum. The results for the lowest observed concentration that met the accuracy goal (TE ≤ 1 $\log_{10}$ IU/mL and TE ≤ 1 $\log_{10}$ IU/mL) with >95% detection and greater than or equal to the LoD are shaded in both tables and summarized in Table 6.

The calculated LLoQ for the 3rd WHO International Standard for Hepatitis B Virus is 6 IU/mL (0.79 $\log_{10}$IU/mL) for plasma and 8 IU/mL (0.88 $\log_{10}$IU/mL) for serum, which are based on the highest calculated concentration among the three reagent lots in accordance with CLSI EP17-A2. The LLoQ was established across genotypes (see *Lower Limit of*

Quantitation Across HBV Genotypes). This genotype data establishes the overall LLoQ for the assay as 10 IU/mL.

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

Reagent Lot	Aptima HBV Quant (IU/mL)	Aptima HBV Quant (Log_{10} IU/mL)	SD (Log_{10} IU/mL)	Bias (Log_{10} IU/mL)	Calculated TE (Log_{10} IU/mL)	Calculated TAE (Log_{10} IU/mL)
1	3	0.53	0.21	0.32	0.59	0.74
	3	0.52	0.21	0.38	0.61	0.81
	5	0.70	0.23	0.25	0.65	0.71
2	6	0.76	0.26	0.09	0.73	0.60
	5	0.69	0.22	0.21	0.63	0.65
	6	0.77	0.25	0.18	0.70	0.68
3	6	0.79	0.31	0.05	0.88	0.68
	8	0.88	0.23	0.02	0.66	0.48
	9	0.96	0.23	0.00	0.66	0.47

SD=standard deviation.
Panel members that met the accuracy goal (TE ≤ 1 and TAE ≤ 1), > LoD, and > 95% detection for Reagent Lots 1, 2, and 3 are shaded.

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

Reagent Lot	Aptima HBV Quant (IU/mL)	Aptima HBV Quant (Log_{10} IU/mL)	SD (Log_{10} IU/mL)	Bias (Log_{10} IU/mL)	Calculated TE (Log_{10} IU/mL)	Calculated TAE (Log_{10} IU/mL)
1	4	0.65	0.24	0.20	0.67	0.67
	5	0.65	0.24	0.25	0.68	0.73
	5	0.67	0.22	0.28	0.63	0.73
2	5	0.70	0.29	0.14	0.82	0.72
	5	0.72	0.27	0.18	0.77	0.72
	6	0.75	0.24	0.20	0.68	0.68
3	8	0.88	0.29	0.04	0.83	0.63
	10	0.98	0.23	0.08	0.66	0.55
	11	1.02	0.28	0.07	0.78	0.62

SD=standard deviation.
Panel members that met the accuracy goal (TE ≤ 1 and TAE ≤ 1), > LoD, and > 95% detection for Reagent Lots 1, 2, and 3 are shaded.

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

Reagent Lot	Plasma LLoQ		Serum LLoQ	
	IU/mL	Log_{10} IU/mL	IU/mL	Log_{10} IU/mL
1	5	0.70	4	0.65
2	5	0.69	5	0.72
3	6	0.79	8	0.88

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. Assignment of the concentration for clinical specimens was determined using a comparator assay. A total 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 for the lowest observed concentration meeting the accuracy goal (TE ≤ 1 $\log_{10}$ IU/mL and TAE ≤ 1 $\log_{10}$ IU/mL) with >95% detection for each reagent lot are shown in Table 7 for plasma and Table 8 for serum. The highest observed concentration among the reagent lots for each genotype is summarized in Table 9. Genotype D in serum had the highest LLoQ at 9 IU/mL (0.96 $\log_{10}$ IU/mL). This supported the overall LLoQ for the assay as 10 IU/mL.

Table 7: Determination of LLoQ Across HBV Genotypes in Plasma

HBV Genotype	Reagent Lot	Aptima HBV Quant[a] (IU/ml)	Aptima HBV Quant[a] (Log_{10} IU/ml)	SD (Log_{10} IU/ml)	Bias (Log_{10} IU/ml)	Calculated TE (Log_{10} IU/ml)	Calculated TAE (Log_{10} IU/ml)
A	1	6	0.74	0.24	0.10	0.67	0.58
	2	7	0.85	0.20	0.00	0.57	0.40
B	1	4	0.65	0.24	0.20	0.67	0.67
	2	6	0.75	0.25	0.10	0.70	0.59
C	1	4	0.61	0.21	0.35	0.60	0.77
	2	6	0.75	0.30	0.20	0.84	0.80
D	1	7	0.87	0.28	0.31	0.81	0.88
	2	8	0.91	0.30	0.26	0.86	0.87
E	1	8	0.88	0.32	0.29	0.89	0.92
	2	7	0.82	0.21	0.36	0.60	0.78
F	1	6	0.76	0.27	0.08	0.76	0.62
	2	7	0.86	0.30	0.01	0.84	0.60
G	1	4	0.59	0.21	0.25	0.60	0.68
	2	4	0.65	0.23	0.19	0.64	0.64
H	1	6	0.78	0.28	0.39	0.80	0.96
	2	7	0.83	0.27	0.34	0.78	0.89

SD=standard deviation.

[a] Additional levels were run, but that data is not reported in the table.

Table 8: Determination of LLoQ Across HBV Genotypes in Serum

HBV Genotype	Reagent Lot	Aptima HBV Quant[a] (IU/ml)	Aptima HBV Quant[a] (Log_{10} IU/ml)	SD (Log_{10} IU/ml)	Bias (Log_{10} IU/ml)	Calculated TE (Log_{10} IU/ml)	Calculated TAE (Log_{10} IU/ml)
A	1	4	0.65	0.26	0.25	0.73	0.77
	2	6	0.81	0.26	0.04	0.74	0.56
B	1	5	0.70	0.19	0.26	0.54	0.64
	2	5	0.72	0.25	0.18	0.72	0.69
C	1	5	0.66	0.26	0.30	0.74	0.82
	2	6	0.81	0.26	0.10	0.74	0.62
D	1	7	0.84	0.27	0.42	0.75	0.95
	2	9	0.96	0.27	0.29	0.76	0.83
E	1	6	0.79	0.26	0.38	0.75	0.91
	2	8	0.89	0.28	0.29	0.79	0.84
F	1	6	0.76	0.23	0.08	0.66	0.55
	2	5	0.71	0.26	0.14	0.72	0.65
G	1	8	0.89	0.28	0.29	0.80	0.85
	2	5	0.66	0.24	0.29	0.67	0.77
H	1	6	0.74	0.24	0.43	0.67	0.90
	2	6	0.81	0.29	0.37	0.82	0.95

SD=standard deviation.

[a] Additional levels were run, but that data is not reported in the table.

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

Genotype	Plasma LLoQ		Serum LLoQ	
	IU/mL	Log_{10} IU/mL	IU/mL	Log_{10} IU/mL
A	7	0.85	6	0.81
B	6	0.75	5	0.72
C	6	0.75	6	0.81
D	8	0.91	9	0.96
E	8	0.88	8	0.89
F	7	0.86	6	0.76
G	4	0.65	8	0.89
H	7	0.83	6	0.81

Traceability to the 3rd WHO International Standard

A series of secondary standards with known concentrations was used throughout product development and product manufacturing to establish traceability to the WHO standard. The concentrations tested for the HBV WHO standard were between 2.0 and 4.0 $\log_{10}$ IU/mL, the secondary standards ranged in concentration from 2.4 to 8.4 $\log_{10}$ IU/mL. The Aptima HBV Quant assay controls and calibrators were also tested along with the secondary standards and the WHO standard. All of the panels had similar results, and they were distributed linearly across the assay's linear range, as presented in Figure 8.

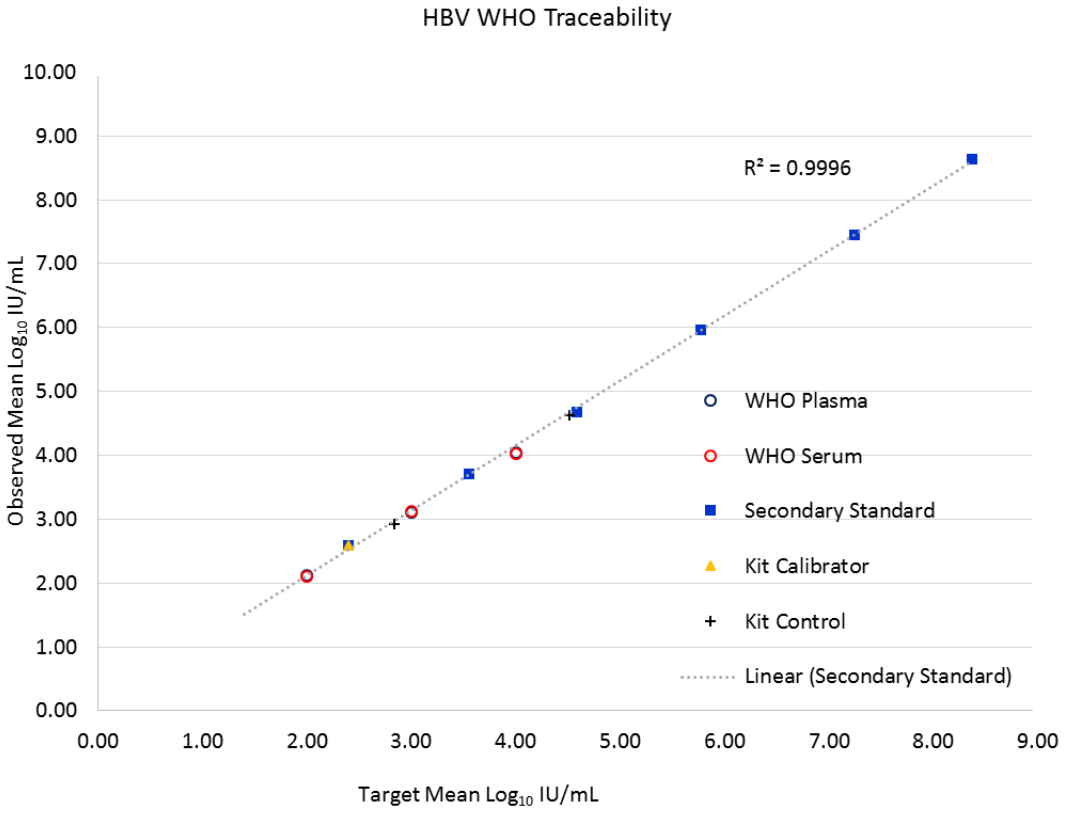

Figure 8. Traceability Between the 3rd HBV WHO Standard Target Concentrations and Observed Concentration From the Aptima HBV Quant Assay

Precision

The Aptima HBV Quant precision panel was built by diluting HBV genotype A virus and HBV plasmid DNA into HBV-negative clinical plasma and HBV-negative clinical serum (the four highest panel members in each matrix were plasmid DNA). Eleven panel members in each matrix spanned the range of the assay (target concentrations of 1.30 $\log_{10}$ IU/mL to 8.90 $\log_{10}$ IU/mL) and were tested in three replicates per run by one operator, using three reagent lots on one Panther system over three days, two runs a day.

Table 10 shows the precision of assay results (in $\log_{10}$ IU/mL) between days, between lots, between runs, within runs, and overall. Total variability was primarily due to the intra-run measurement (i.e., random error).

Table 10: Precision of the Aptima HBV Quant Assay

Matrix	Sample	N	Mean Concentration (IU/mL)	Mean Concentration (log_{10} IU/mL)	Inter-Lot SD	Inter-Lot Log Normal CV (%)	Inter-Day SD	Inter-Day Log Normal CV (%)	Inter-Run SD	Inter-Run Log Normal CV (%)	Intra-Run SD	Intra-Run Log Normal CV (%)	Total SD	Total Log Normal CV (%)
Plasma	Virus	34[a]	32	1.21	0.07	16.2	0.07	16.2	0.05	11.7	0.28	71.8	0.28	78.2
		54	97	1.95	0.05	11.7	0.03	6.8	0.02	5.7	0.15	36.8	0.17	39.9
		54	1,474	3.16	0	1.0	0.02	3.8	0.02	4.8	0.07	15.6	0.07	16.8
		54	10,602	4.02	0.02	4.8	0.02	3.6	0.01	2.4	0.06	14.9	0.07	16.2
		54	429,428	5.63	0.03	6.7	0.01	2.7	0.01	2.4	0.06	14.1	0.07	16.1
	Plasmid DNA	54	652,103	5.8	0.06	14.2	0.01	3.4	0.01	2.0	0.06	13.1	0.09	19.8
	Virus	54	7,617,612	6.88	0.02	5.6	0.00	0.4	0.02	4.2	0.06	13.4	0.07	15.1
	Plasmid DNA	54	10,662,942	7.02	0.02	5.3	0.01	2.3	0.01	2.6	0.06	13.1	0.06	14.5
	Virus	54	89,149,358	7.95	0.01	3.4	0.01	2.7	0.01	1.6	0.04	9.7	0.05	10.8
	Plasmid DNA	54	103,400,000	8.01	0.04	9.4	0.02	3.7	0.01	2.1	0.05	12.0	0.07	15.9
		54	612,200,000	8.78	0.03	7.6	0.01	1.9	0.01	1.5	0.04	9.0	0.05	12.0
Serum	Virus	29[a]	33	1.27	0.13	31.0	0.08	18.6	0.06	13.9	0.29	75.0	0.33	88.4
		54	88	1.92	0.05	11.2	0.02	4.8	0.02	5.5	0.12	29.1	0.14	32.3
		54	1,446	3.15	0.02	3.7	0.01	1.9	0.00	1.1	0.08	17.8	0.08	18.3
		54	7,873	3.89	0.02	4.8	0.01	2.6	0.01	2.1	0.06	13.4	0.06	14.6
		54	313,518	5.49	0.01	3.3	0.01	3.0	0.01	3.2	0.08	17.4	0.08	18.3
	Plasmid DNA	54	599,225	5.77	0.04	8.5	0.01	2.5	0.01	2.5	0.06	14.7	0.08	17.4
	Virus	54	7,011,440	6.84	0.02	3.5	0.01	3.2	0.01	3.3	0.07	16.9	0.08	17.9
	Plasmid DNA	54	8,845,332	6.94	0.05	11.9	0.01	2.2	0.01	2.2	0.05	12.2	0.07	17.4
	Virus	54	70,350,774	7.84	0.03	6.3	0.02	3.5	0.02	4.4	0.06	14.4	0.07	16.7
	Plasmid DNA	53[b]	122,800,000	8.08	0.04	9.9	0.01	2.2	0.01	1.8	0.04	10.4	0.06	14.6
		54	678,700,000	8.83	0.02	3.6	0.01	2.2	0.01	1.8	0.05	11.7	0.05	12.5

SD=standard deviation.

[a]Detected replicates with quantifiable result, total detected replicates = 54.

[b]One replicate had an invalid result.

Log Normal CV(%) = sqrt(10^(SD^2 * ln(10)) - 1) * 100

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 concentrations of approximately 30 IU/mL (1.48 $\log_{10}$ IU/mL) and 20,000 IU/mL (4.30 $\log_{10}$ IU/mL) 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, ten samples for each substance, listed in Table 11 were tested with the Aptima HBV Quant assay. No interference in the performance of the assay was observed.

Table 11: 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

No interference in the performance of the assay was observed in the presence of the exogenous substances listed in Table 12 at concentrations at least three times the C_{max} (human plasma).

Table 12: 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

Analytical Specificity

Potential cross-reactivity to the pathogens listed in Table 13 was evaluated in HBV negative human plasma in the presence or absence of 30 IU/mL (1.5 $\log_{10}$ IU/mL) and 20,000 (4.3 $\log_{10}$ IU/mL) HBV DNA. No cross-reactivity was observed. No interference was observed in the presence of the pathogens.

Table 13: Pathogens Tested for Analytical Specificity

Microorganism/Pathogen	Concentration		Microorganism/Pathogen	Concentration	
Adenovirus 5	100,000	TCID50/mL[a]	*Candida albicans*	1,000,000	CFU/mL[e]
BK human polyomavirus	1,000	TCID50/mL	*Chlamydia trachomatis*	1,000,000	IFU/mL[f]
Cytomegalovirus	100,000	TCID50/mL	*Corynebacterium diphtheriae*	1,000,000	CFU/mL
Dengue virus 1	10,000	TCID50/mL	*Neisseria gonorrhoeae*	1,000,000	CFU/mL
Dengue virus 2	10,000	TCID50/mL	*Propionibacterium acnes*	1,000,000	CFU/mL
Dengue virus 3	10,000	TCID50/mL	*Staphylococcus aureus*	1,000,000	CFU/mL
Dengue virus 4	100,000	TCID50/mL	*Staphylococcus epidermidis*	1,000,000	CFU/mL
Epstein-Barr virus	100,000	copies/mL	*Streptococcus pneumoniae*	1,000,000	CFU/mL
Flu H1N1	100,000	TCID50/mL	*Trichomonas vaginalis*	1,000,000	CFU/mL
Hepatitis A virus	100,000	TCID50/mL			
Hepatitis C virus	100,000	IU/mL[b]			
Hepatitis G virus	100,000	copies/mL			
Human herpes virus 6B	100,000	copies/mL			
Human herpes virus 8	100,000	copies/mL			
HIV-1	100,000	IU/mL			
HIV-2	10,000	TCID50/mL			
Human papillomavirus	100,000	copies/mL			
Herpes simplex virus 1 (HSV-1)	100,000	TCID50/mL			
Herpes simplex virus 2 (HSV-2)	100,000	TCID50/mL			
Human T-cell lymphotropic virus 1 (HTLV-1)	100,000	vp/mL[c]			
Human T-cell lymphotropic virus 2 (HTLV-2)	100,000	vp/mL			
Japanese encephalitis virus	N/A	N/A			
Murray Valley encephalitis virus	2,000	LD50/mL[d]			
Parvovirus B19	100,000	IU/mL			
Rubella virus	10,000	TCID50/mL			
St. Louis encephalitis virus	100,000	TCID50/mL			
Vaccinia virus	1,000	TCID50/mL			
West Nile virus	100,000	TCID50/mL			
Yellow fever virus	100,000	TCID50/mL			

[a] TCID50/mL = Tissue culture Infectious dose units per mL

[b] IU/mL = International units per mL

[c] vp/mL = Viral particles per mL

[d] LD50/mL = Lethal dose per mL

[e] CFU/mL = Colony forming units per mL

[f] IFU/mL = Inclusion forming units per mL

Matrix Equivalency

One hundred eighteen sample sets of matched blood collection tubes (serum tube, ACD, K2 EDTA, K3 EDTA, PPT, SST) were assessed for matrix equivalency. Of these, 44 sets were naturally infected HBV-positive, and 74 sets were HBV-negative spiked with HBV virus. Correlation for each blood collection tube type, as measured using the serum collection tube as a comparator, is shown in Table 14.

Table 14: Matrix Equivalency Study

Blood Collection Tube	Deming Regression	95% CI of Slope		95% CI of Intercept		R^2	Mean Difference (Log_{10})
		Lower Limit	Upper Limit	Lower Limit	Upper Limit		
ACD	y = 1.01x - 0.04	1.00	1.02	-0.10	0.01	0.998	-0.01
K2 EDTA	y = 1.02x - 0.14	1.00	1.03	-0.20	-0.07	0.997	-0.07
K3 EDTA	y = 1.01x - 0.12	1.00	1.03	-0.18	-0.06	0.997	-0.06
PPT	y = 1.02x - 0.14	1.00	1.03	-0.21	-0.07	0.996	-0.06
SST	y = 1.00x - 0.03	0.99	1.01	-0.07	0.03	0.999	-0.01

CI=confidence interval.

Sample Dilution Using Aptima Specimen Diluent (1:3)

To assess detection accuracy of HBV DNA in samples diluted with Aptima Specimen Diluent, samples that spanned the linear range were diluted 1:3 with Aptima Specimen Diluent (such as 240 µL of sample combined with 480 µL of Aptima Specimen Diluent). Each sample was tested neat and diluted (1:3) in triplicate. Testing was performed using one lot of assay reagents on two Panther systems with two Aptima Specimen Diluent lots. The differences between the average reported concentration in native matrix (dilution factor applied to the diluted sample result) and the average concentration in Aptima Specimen Diluent are shown in Table 15 for plasma and Table 16 for serum. The sample concentrations were accurately recovered in the diluted samples.

Table 15: Plasma Specimen 1:3 Dilution Matrix Comparison Summary

Plasma Matrix Average Reported Concentration (Log_{10} IU/mL) n = 9	Diluent Average Reported Concentration (Log_{10} IU/mL) n = 18	Difference Diluent from Plasma Matrix (Log_{10} IU/mL)
1.20[a]	1.11[b]	-0.09
1.56[a]	1.36[b]	-0.20
2.15	2.04	-0.11
3.10	2.97	-0.13
3.92	3.89	-0.03
4.82	4.79	-0.03
5.70	5.70	0.00
7.07	6.98	-0.09
7.74	7.60	-0.14
8.74	8.62	-0.12
9.29	9.19	-0.10
9.39	9.29	-0.10

[a]n=21.
[b]n=42.

Table 16: Serum Specimen 1:3 Dilution Matrix Comparison Summary

Serum Matrix Average Reported Concentration (Log_{10} IU/mL) n = 9	Diluent Average Reported Concentration (Log_{10} IU/mL) n = 18	Difference Diluent from Serum Matrix (Log_{10} IU/mL)
1.21[a]	1.11[b]	-0.10
1.54[a]	1.36[b]	-0.18
2.21	2.03	-0.18
3.06	2.98	-0.08
3.90	3.83	-0.07
4.77	4.76	-0.01
5.77	5.74	-0.03
7.03	7.00	-0.03
7.85	7.71	-0.14
8.87	8.76	-0.11
9.37	9.30	-0.07
9.46	9.36	-0.10

[a]n=21.
[b]n=42.

Sample Dilution Using Aptima Specimen Diluent (1:100)

To assess detection accuracy of HBV DNA in samples diluted with Aptima Specimen Diluent, plasma or serum, eight individual plasma specimens and eight individual serum specimens spiked with HBV virus targeting between 6 to 8 log_{10} IU/mL, along with eight individual plasma specimens and eight individual serum specimens spiked with HBV plasmid DNA targeting 9.16 log_{10} IU/mL, were tested in 5 replicates. A 1:100 dilution was performed with one part sample and 99 parts Aptima Specimen Diluent just prior to testing. Testing was performed using one lot of assay reagents on two Panther systems with two Aptima Specimen Diluent lots. The difference between the average reported concentration in native matrix (dilution factor applied to the diluted sample result) and the average concentration in

Aptima Specimen Diluent was calculated for each sample set as shown in Table 17 for plasma and Table 18 for serum.

Table 17: Plasma Specimen 1:100 Dilution Matrix Comparison Summary

Plasma Matrix Average Reported Concentration (Log_{10} IU/mL) n = 5	Diluent Average Reported Concentration (Log_{10} IU/mL) n = 10	Difference Diluent from Plasma Matrix (Log_{10} IU/mL)
7.86	7.85	-0.01
7.84	7.83	-0.01
7.78	7.75	-0.03
7.80	7.80	0.00
6.58	6.53	-0.05
6.58	6.52	-0.06
6.58	6.53	-0.05
6.58	6.53	-0.05
9.24[a]	9.05[a]	-0.19
9.21[a]	9.05[a]	-0.16
9.25[a]	9.03[a]	-0.22
9.27[a]	9.04[a]	-0.23
9.13[a]	8.82[a]	-0.31
9.12[a]	8.81[a]	-0.31
9.09[a]	8.84[a]	-0.25
9.05[a]	8.84[a]	-0.21

[a]Spiked using plasmid DNA.

Table 18: Serum Specimen 1:100 Dilution Matrix Comparison Summary

Serum Matrix Average Reported Concentration (Log_{10} IU/mL) n = 5	Diluent Average Reported Concentration (Log_{10} IU/mL) n = 10	Difference Diluent from Serum Matrix (Log_{10} IU/mL)
7.70	7.85	0.15
7.84	7.85	0.01
7.79	7.82	0.03
7.75	7.79	0.04
6.77	6.77	0.00
6.75	6.80	0.05
6.75	6.71	-0.04
6.70	6.73	0.03
9.27[a]	9.08[a]	-0.19
9.24[a]	9.06[a]	-0.18
9.29[a]	9.08[a]	-0.21
9.31[a]	9.11[a]	-0.20
9.14[a]	8.91[a]	-0.23
9.18[a]	8.92[a]	-0.26
9.19[a]	8.90[a]	-0.29
9.08[a]	8.84[a]	-0.24

[a]Spiked using plasmid DNA.

Confirmation of the LLoQ in Specimens Diluted in Aptima Specimen Diluent

The LLoQ of the Aptima HBV Quant assay was confirmed with HBV genotype A clinical specimens diluted into Aptima Specimen Diluent. Specimens were prepared in HBV negative human plasma and serum at 21, 30, and 45 IU/mL. Each panel was diluted 1:3 into Aptima Specimen Diluent just prior to testing to give final concentrations of approximately 7, 10, and 15 IU/mL. A total of 36 replicates of each panel member were tested with one reagent lot across three days. An LLoQ ≤ 10 IU/mL for HBV plasma and serum diluted into Aptima Specimen Diluent was confirmed as shown in Table 19.

Table 19: Confirmation of LLoQ - Samples in Aptima Specimen Diluent

Matrix	% Detected	Aptima HBV Quant (IU/ml)	Aptima HBV Quant (Log_{10} IU/ml)	SD (Log_{10} IU/ml)	Bias (Log_{10} IU/ml)	Calculated TE (Log_{10} IU/ml)	Calculated TAE (Log_{10} IU/ml)
Plasma	100%	3	0.50	0.19	0.10	0.54	0.48
Serum	100%	2	0.38	0.12	0.46	0.33	0.70

Precision of Diluted Samples

The Aptima HBV Quant precision panel was built by diluting HBV-positive plasma and HBV plasmid DNA into HBV-negative clinical plasma and serum. Positive panels were diluted into Aptima Specimen Diluent. These were tested in five replicates per run by one operator, using three lots of Aptima Specimen Diluent on one Panther system over three test days, two runs a day.

Table 20 shows the precision of assay results (in SD log_{10} IU/mL) for three lots of Aptima Specimen Diluent. Total variability was ≤ 0.15 SD across all panel members and diluent lots.

Table 20: Precision of Panels Diluted in Aptima Specimen Diluent

Matrix	Target Concentration Log_{10} IU/mL	Dilution	Lot 1 Specimen Diluent (n=10) Average Log_{10} IU/mL	Lot 1 SD	Lot 2 Specimen Diluent (n=10) Average Log_{10} IU/mL	Lot 2 SD	Lot 3 Specimen Diluent (n=10) Average Log_{10} IU/mL	Lot 3 SD	Combined Lots (n=30) Average Log_{10}IU/mL	Combined SD
Plasma	3.30	Neat	3.46	0.07	3.43	0.08	3.46	0.06	3.45	0.07
		1:3	3.36	0.09	3.35	0.07	3.39	0.09	3.37	0.08
	4.30	Neat	4.33	0.06	4.27	0.03	4.41	0.05	4.34	0.08
		1:3	4.34	0.05	4.35	0.05	4.38	0.10	4.35	0.07
	9.18	Neat	9.13	0.05	9.10	0.03	9.26[a]	0.15	9.16[a]	0.11
		1:100	9.18	0.03	9.14	0.04	9.33	0.10	9.21	0.10
Serum	3.30	Neat	3.52	0.05	3.48	0.06	3.50	0.07	3.50	0.06
		1:3	3.45	0.08	3.40	0.06	3.39	0.08	3.41	0.07
	4.30	Neat	4.35	0.05	4.37	0.06	4.43	0.06	4.38	0.06
		1:3	4.35	0.05	4.37	0.05	4.41	0.04	4.37	0.05
	9.18	Neat	9.08	0.03	9.14	0.05	9.31[b]	0.12	9.17[b]	0.12
		1:100	9.18	0.02	9.14	0.03	9.33	0.09	9.22	0.10

SD = standard deviation.

[a] 1 replicate excluded (above calculable range).

[b] 2 replicates excluded (above calculable range).

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 $\log_{10}$ IU/mL) interspersed between HBV negative samples in a checkerboard pattern. Testing was carried out over fifteen runs. The overall carryover rate was 0.14% (1/705).

Reproducibility

Reproducibility was evaluated on the Panther system at three external U.S. sites. Two operators performed testing at each site. Each operator performed two runs per day over three days, using three reagent lots over the course of testing. Each run had three replicates of each panel member. Overall, 108 replicates of each panel member were tested.

Reproducibility was tested using panel members prepared using HBV-negative plasma. The positive panel members were positive for HBV genotypes A or C. HBV DNA concentrations spanned the linear range of the assay.

Table 21 shows the reproducibility and precision of assay results for each positive panel member between sites, between operators/days, between lots, between runs, within runs, and overall.

The coefficient of variation was calculated using the following equation where σ^2 is the sample variance of the data after $\log_{10}$ transformation.

$$\%CV = 100 \times \sqrt{(10^{\sigma^2 \ln(10)} - 1)}$$

For all HBV-positive panel members, agreement values were 100%.

Table 21: Reproducibility of Aptima HBV Quant Assay HBV DNA Levels on the Panther System in Positive Panel Members

GT	N	Observed Mean		Percent Contribution to Total Variance SD (%CV)					
		IU/mL	Log_{10} IU/mL	Between Sites	Between Operators/ Days[a]	Between Lots	Between Runs	Within Runs	Total Variance SD (%CV)
A	108	17.6	1.2	0.059 (13.578)	<0.001 (<0.001)	0.138 (32.693)	0.090 (20.869)	0.178 (42.883)	0.250 (62.666)
	108	129.4	2.1	0.009 (2.162)	0 (0)	0.074 (17.109)	0.051 (11.869)	0.106 (24.736)	0.139 (32.886)
	107	1056.0	3.0	0.035 (7.994)	0.032 (7.432)	0.014 (3.246)	0.032 (7.356)	0.085 (19.666)	0.103 (24.060)
	108	7663.0	3.9	0 (0)	0.027 (6.262)	0.040 (9.235)	0.044 (10.088)	0.066 (15.194)	0.092 (21.540)
	108	188172.1	5.3	0.027 (6.281)	0.042 (9.707)	0.042 (9.787)	0.030 (6.829)	0.072 (16.689)	0.102 (23.772)
	108	9389094.1	7.0	0.038 (8.846)	0 (0)	0.031 (7.237)	0.064 (14.791)	0.068 (15.756)	0.106 (24.692)
	107	86664677.2	7.9	0.038 (8.692)	0.029 (6.753)	0.020 (4.584)	0.037 (8.635)	0.049 (11.375)	0.081 (18.725)
	107	753726183.2	8.9	0.024 (5.476)	0.052 (11.997)	0.015 (3.499)	0.045 (10.304)	0.053 (12.187)	0.091 (21.163)
C	107	17.0	1.2	0.041 (9.521)	0.041 (9.392)	0.074 (17.147)	0.092 (21.438)	0.189 (45.704)	0.230 (57.010)
	108	152.9	2.2	0.035 (8.127)	0 (0)	0.055 (12.706)	0.064 (14.925)	0.131 (30.748)	0.160 (38.013)
	108	1363.8	3.1	0.042 (9.583)	0.023 (5.316)	0 (0)	0.061 (14.033)	0.055 (12.623)	0.094 (22.002)
	108	9871.9	4.0	0.011 (2.472)	0.014 (3.270)	0.040 (9.337)	0.038 (8.801)	0.059 (13.651)	0.083 (19.291)
	108	217400.5	5.3	0.031 (7.255)	0.047 (10.843)	0.016 (3.791)	0.026 (6.023)	0.063 (14.685)	0.090 (21.044)
	108	12087179.5	7.1	0.046 (10.543)	0 (0)	0.020 (4.652)	0.064 (14.762)	0.073 (16.922)	0.109 (25.501)
	108	57743712.8	7.8	0.044 (10.232)	0.028 (6.472)	0.013 (2.944)	0.043 (10.026)	0.052 (12.010)	0.087 (20.146)
	108	572184754.9	8.7	0.042 (9.711)	0.048 (11.160)	0.028 (6.374)	0.034 (7.740)	0.048 (11.081)	0.091 (21.208)

%CV=log-normal coefficient of variation, GT=genotype, SD=standard deviation.

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.

[a] Between Operators may be confounded with Between Days; therefore, Between Operators and Between Days estimates are combined in Between Operators/Days.

Clinical Performance

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 9.

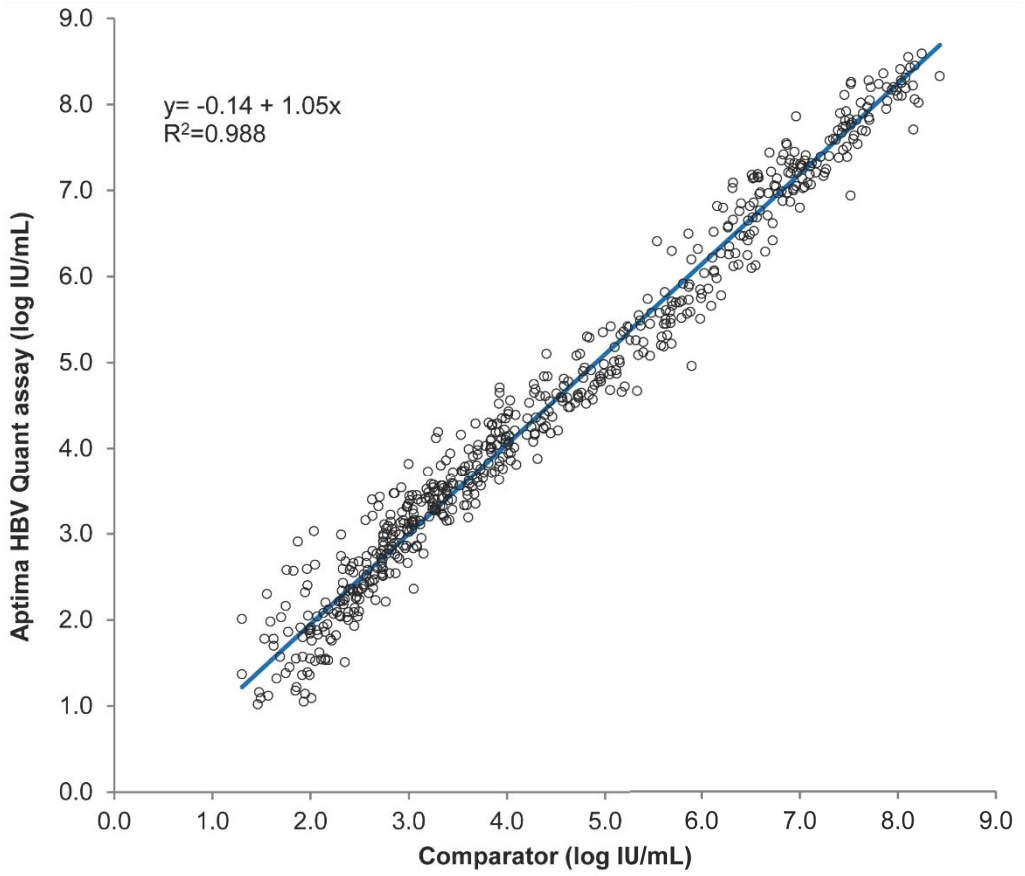

Figure 9. Correlation between the Aptima HBV Quant Assay and Comparator Assay

Clinical Utility

The study was designed to assess the Aptima HBV Quant assay's ability to predict virologic, biochemical, and serologic clinical utility endpoints 48 weeks after initiation of therapy. Specimens were prospectively collected from subjects with chronic HBV infection who were initiating monotherapy with entecavir or tenofovir as part of their standard of care treatment.

Of the 331 subjects enrolled, 86 subjects were not evaluable due to withdrawal, discontinuation, early treatment halt, missing Week 48 results, or missing or low baseline HBV DNA viral loads. The remaining 245 subjects were evaluable for at least one of the clinical utility endpoints and included 126 HBeAg(+) and 119 HBeAg(-) subjects. Table 22 shows the demographic and baseline clinical characteristics of evaluable subjects. The demographic distribution of subjects in this study was consistent with that of patients with chronic HBV in the US.[13] Data from HBeAg(+) and HBeAg(-) subjects were analyzed separately.

Table 22: Demographics and Baseline Clinical Characteristics of Evaluable Subjects

Characteristics		Total
Total, N	N	245
Entecavir	n (%)	94 (38.4)
Tenofovir	n (%)	151 (61.6)
Sex, n (%)	Male	154 (62.9)
	Female	91 (37.1)
Age (years)	Mean ± SD	43.5 ± 13.63
	Median	44.0
	Range	18 – 83
Age category (years), n (%)	18 – 29	40 (16.3)
	30 – 49, n (%)	120 (49.0)
	50 – 70, n (%)	80 (32.7)
	>70, n (%)	5 (2.0)
Ethnicity, n (%)	Hispanic or Latino	7 (2.9)
	Not Hispanic or Latino	236 (96.3)
	Unknown/Refused	2 (0.8)
Race[a], n (%)	White	89 (36.3)
	Black or African American	16 (6.5)
	Asian	132 (53.9)
	American Indian/Alaska native	0 (0.0)
	Native Hawaiian/Pacific Islander	6 (2.4)
	Other	1 (0.4)
	Unknown/Refused	1 (0.4)
Genotype, n (%)	A	28 (11.4)
	B	64 (26.1)
	C	36 (14.7)
	D	47 (19.2)
	E	2 (0.8)
	F	0 (0.0)
	G	0 (0.0)
	H	3 (1.2)
	Unknown	65 (26.5)
HBV treatment status, n (%)	Experienced	27 (11.0)
	Naïve	218 (89.0)
Previous drug treatment, n (%)	Tenofovir	5 (18.5)
	Entecavir	4 (14.8)
	Adefovir	2 (7.4)
	Lamivudine	1 (3.7)
	Telbivudine	0 (0.0)
	Interferon	10 (37.0)
	Other [b]	5 (18.5)
Previous treatment outcome, n (%)	Failure	1 (3.7)
	Success	0 (0.0)
	Discontinued for other reasons	26 (96.3)
HBsAg serostatus, n (%)	Positive/reactive	210 (85.7)
	Not done	35 (14.3)

Table 22: Demographics and Baseline Clinical Characteristics of Evaluable Subjects (continued)

Characteristics		Total
Cirrhotic status, n (%)	Cirrhotic	26 (10.6)
	Non-cirrhotic	201 (82.0)
	Not done	18 (7.3)
HBV viral load (log_{10} IU/mL)	Mean ± SD	6.3 ± 1.93
	Median	6.4
	Range	3 – 9
ALT (U/L)	Mean ± SD	102.4 ± 175.97
	Number above ULN[c]	177 (85.9)

[a] Subjects may report multiple races.
[b] Various combinations of the specific drugs listed.
[c] The upper limit of normal range (ULN) for alanine aminotransferase (ALT) was 30 U/L for males and 19 U/L for females.

Prediction of Response to Antiviral Therapy

Aptima HBV Quant assay clinical utility has been assessed for individuals treated with tenofovir and entecavir. No information is available on the assay's clinical utility when other HBV antiviral therapies are used.

Definitions:

Early virologic response outcomes

Week 12 and Week 24 virologic response = HBV DNA <10 IU/mL (<LLoQ) as assessed by the Aptima HBV Quant assay on the Panther system

Week 12 alternative virologic response = HBV DNA ≥2 log_{10} decrease from baseline

Week 24 alternative virologic response = HBV DNA <2000 IU/mL (for HBeAg+) or <50 IU/mL (for HBeAg-)

Clinical utility endpoints

Week 48 virologic response = HBV DNA <10 IU/mL (<LLoQ) as assessed by an approved HBV quantitative assay

Alternative Week 48 virologic response = HBV DNA <50 IU/mL as assessed by an approved HBV quantitative assay

Biochemical response = Normalization of ALT test results at Week 48 (ALT <30 U/L for males and <19 U/L for females)

Serologic response = Loss of HBeAg (HBeAg-negative results) at Week 48

Measures of association and predictive value

Positive Predictive Value (PPV) = True Positive / (True Positive + False Positive) or the probability of response at Week 48 (for the clinical utility endpoint being assessed) in subjects with virologic response at the early time point

Negative Predictive Value (NPV) = True Negative / (False Negative + True Negative) or the probability of non-response at Week 48 (for the clinical utility endpoint being assessed) in subjects with virologic non-response at the early time point

Odds Ratio (OR) = (True Positive × True Negative) / (False Positive × False Negative)

Predicting Week 48 Virologic Response, Defined as HBV DNA <10 IU/mL

In this study, the primary definition of virologic response was HBV DNA <10 IU/mL, and this definition was used for both early virologic response at Weeks 12 and 24, as well as the virologic response at Week 48. The association between early virologic responses at Weeks 12 and 24 and Week 48 clinical utility endpoints (virologic response, biochemical response, serologic response) were assessed.

Predicting Virologic Response at Week 48

Associations between virologic response at Week 48 and virologic response at Week 12 and Week 24 are summarized in Table 23.

Early virologic responses at Weeks 12 and 24, as predictors of Week 48 virologic response, varied by week and treatment.

Table 23: PPV, NPV, and Odds Ratio for Virologic Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <10 IU/mL

			PPV (%)		NPV (%)		OR
HBeAg Status	Week of Early Virologic Response	Treatment	Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a,b]
HBeAg(+)	12	Entecavir	0.0 (0.0, 93.2)	0/1	82.5 (80.0, 88.4)	33/40	1.49 (<0.01, 30.95)
		Tenofovir	100 (27.3, 100)	2/2	74.4 (72.6, 78.7)	61/82	14.30 (1.11, >999.99)
		All	66.7 (15.4, 98.2)	2/3	77.0 (75.7, 79.9)	94/122	6.71 (0.62, 147.55)
	24	Entecavir	50.0 (6.4, 93.2)	2/4	88.2 (83.5, 95.4)	30/34	7.50 (0.74, 79.76)
		Tenofovir	75.0 (52.7, 92.3)	12/16	84.1 (78.5, 90.0)	58/69	15.81 (4.62, 65.54)
		All	70.0 (50.3, 88.1)	14/20	85.4 (81.3, 90.1)	88/103	13.69 (4.74, 44.16
HBeAg(-)	12	Entecavir	94.1 (87.1, 99.0)	32/34	22.2 (7.6, 36.0)	4/18	4.57 (0.80, 35.85)
		Tenofovir	83.3 (70.5, 93.2)	25/30	46.9 (35.0, 58.9)	15/32	4.41 (1.42, 15.71)
		All	89.1 (82.1, 94.7)	57/64	38.0 (29.0, 47.1)	19/50	4.99 (1.96, 14.00)
	24	Entecavir	93.0 (88.0, 98.1)	40/43	37.5 (6.4, 67.2)	3/8	8.00 (1.21, 55.54)
		Tenofovir	82.6 (74.3, 90.4)	38/46	75.0 (54.1, 92.0)	12/16	14.25 (3.92, 62.71)
		All	87.6 (82.7, 92.6)	78/89	62.5 (44.8, 78.0)	15/24	11.82 (4.30, 34.94)

CI=95% profile-likelihood confidence interval.

[a] Shading indicates statistical significance of odds ratios.

[b] For calculation of odds ratios and their confidence intervals, 0.5 was added to all cells whenever at least one cell was zero.

Predicting Biochemical Response at Week 48

Associations between biochemical response at Week 48 and virologic response at Week 12 and Week 24 are summarized in Table 24.

The value of early virologic responses at Weeks 12 and 24 as a predictor of Week 48 biochemical response varied by week and treatment.

Table 24: PPV, NPV, and Odds Ratio for Biochemical Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <10 IU/mL

HBeAg Status	Week of Early Virologic Response	Treatment	PPV (%)		NPV (%)		OR
			Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
HBeAg(+)	12	Entecavir	NC (NC)	0/0	67.9 (63.4-76.1)	19/28	2.05 (0.01-393.52)
		Tenofovir	100 (27.6, 100)	2/2	58.5 (55.1, 63.9)	31/53	7.00 (0.54, 983.90)
		All	100 (27.3, 100)	2/2	61.7 (59.7, 65.5)	50/81	8.02 (0.63, >999.99)
	24	Entecavir	66.7 (16.4-98.2)	2/3	68.2 (60.1-80.0)	15/22	4.29 (0.35-101.82)
		Tenofovir	58.3 (33.2, 81.0)	7/12	61.4 (54.3, 69.7)	27/44	2.22 (0.61, 8.61)
		All	60.0 (36.6, 80.9)	9/15	63.6 (58.2, 70.0)	42/66	2.62 (0.84, 8.70)
HBeAg(-)	12	Entecavir	43.8 (25.1, 61.2)	7/16	50.0 (25.1, 74.9)	6/12	0.78 (0.17, 3.53)
		Tenofovir	52.9 (35.1, 72.2)	9/17	76.2 (61.2, 89.8)	16/21	3.60 (0.93, 15.39)
		All	48.5 (36.0, 60.9)	16/33	66.7 (54.5, 78.6)	22/33	1.88 (0.70, 5.20)
	24	Entecavir	45.8 (36.0, 56.0)	11/24	75.0 (25.2, 98.7)	3/4	2.54 (0.28, 55.45)
		Tenofovir	42.9 (32.8, 53.1)	12/28	80.0 (53.0, 97.1)	8/10	3.00 (0.61, 22.34)
		All	44.2 (37.8, 50.8)	23/52	78.6 (55.1, 95.0)	11/14	2.91 (0.80, 13.98)

CI=95% profile-likelihood confidence interval, NC=not calculable.

[a] For calculation of odds ratios and their confidence intervals, 0.5 was added to all cells whenever at least one cell was zero.

Predicting Serologic Response at Week 48

Associations between serologic response at Week 48 and virologic response at Week 12 and Week 24 are summarized in Table 25.

The value of early virologic responses at Weeks 12 and 24 as a predictor of Week 48 serologic response varied by week and treatment.

Table 25: PPV, NPV, and Odds Ratio for Serologic Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <10 IU/mL

HBeAg Status	Week of Early Virologic Response	Treatment	PPV (%) Estimate (95% CI)	PPV (%) n/N	NPV (%) Estimate (95% CI)	NPV (%) n/N	OR Estimate (95% CI)[a]
HBeAg(+)	12	Entecavir	100 (6.5, 100)	1/1	86.8 (84.2, 93.9)	33/38	18.27 (0.86, >999.99)
		Tenofovir	0.0 (0.0, 72.0)	0/2	82.9 (81.7, 86.0)	68/82	0.95 (<0.01, 12.45)
		All	33.3 (1.8, 84.3)	1/3	84.2 (83.1, 86.8)	101/120	2.66 (0.12, 29.11)
	24	Entecavir	50.0 (6.4, 93.2)	2/4	88.2 (83.5, 95.4)	30/34	7.50 (0.74, 79.76)
		Tenofovir	18.8 (3.1, 39.1)	3/16	84.1 (80.6, 89.1)	58/69	1.22 (0.25, 4.59)
		All	25.0 (8.5, 43.6)	5/20	85.4 (82.5, 89.4)	88/103	1.96 (0.57, 5.94)

CI=95% profile-likelihood confidence interval.

[a] For calculation of odds ratios and their confidence intervals, 0.5 was added to all cells whenever at least one cell was zero.

Predicting Week 48 Virologic Response, Defined as HBV DNA <50 IU/mL (Alternative Definition)

Alternative definitions of early (Weeks 12 and 24) and Week 48 virologic responses also were assessed (see alternative response definitions above).

Associations between clinical utility endpoints and virologic response at Week 12 and Week 24, using these alternate definitions of virologic response, are summarized in Table 26 (virologic response), Table 27 (biochemical response), and Table 28 (serologic response).

Table 26: PPV, NPV, and Odds Ratio for Virologic Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <50 IU/mL

HBeAg Status	Week of Early Virologic Response	Treatment	PPV (%)		NPV (%)		OR
			Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a,b]
HBeAg(+)	12	Entecavir	34.2 (26.9, 39.2)	13/38	66.7 (16.1, 98.2)	2/3	1.04 (0.09, 23.60)
		Tenofovir	55.1 (51.9, 59.4)	43/78	83.3 (43.5, 99.2)	5/6	6.14 (0.93, 120.54)
		All	48.3 (45.6, 51.3)	56/116	77.8 (45.3, 97.1)	7/9	3.27 (0.75, 22.54)
	24	Entecavir	65.0 (51.3, 81.2)	13/20	100 (86.3, 100)	18/18	66.59 (7.20, >999.99)
		Tenofovir	72.4 (64.7, 80.6)	42/58	88.9 (74.6, 97.2)	24/27	21.00 (6.28, 97.36)
		All	70.5 (63.5, 77.9)	55/78	93.3 (84.0, 98.3)	42/45	33.47 (10.81, 148.13)
HBeAg(-)	12	Entecavir	100 (NC)	52/52	NC (NC)	0/0	NC
		Tenofovir	93.0 (89.1, 97.5)	53/57	60.0 (21.3, 93.3)	3/5	19.87 (2.62, 191.49)
		All	96.3 (94.4, 98.7)	105/109	60.0 (21.1, 93.3)	3/5	39.37 (5.24, 376.77)
	24	Entecavir	100 (NC)	47/47	0.0 (NC)	0/4	NC
		Tenofovir	93.9 (88.6, 98.3)	46/49	30.8 (6.7, 52.4)	4/13	6.81 (1.30, 39.97)
		All	96.9 (93.8, 99.2)	93/96	23.5 (7.0, 39.8)	4/17	9.54 (1.91, 53.22)

CI=95% profile-likelihood confidence interval, NC=not calculable.

[a] Shading indicates statistical significance of odds ratios.

[b] For calculation of odds ratios and their confidence intervals, 0.5 was added to all cells whenever at least one cell was zero, unless there were either no Week 48 responses or no Week 48 non-responses, which resulted in reporting the odds ratio as NC.

Table 27: PPV, NPV, and Odds Ratio for Biochemical Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <50 IU/mL

HBeAg Status	Week of Early Virologic Response	Treatment	PPV Estimate (95% CI)	PPV n/N	NPV Estimate (95% CI)	NPV n/N	OR Estimate (95% CI)[a,b]
HBeAg(+)	12	Entecavir	33.3 (25.1-39.0)	9/27	100 (6.7-100)	1/1	1.54 (0.07-233.77)
		Tenofovir	44.2 (39.6, 48.6)	23/52	66.7 (15.7, 98.2)	2/3	1.59 (0.14, 35.36)
		All	40.5 (37.1, 43.7)	32/79	75.0 (23.9, 98.7)	3/4	2.04 (0.25, 42.30)
	24	Entecavir	50.0 (31.2-69.5)	7/14	81.8 (58.9-97.1)	9/11	4.50 (0.79-37.15)
		Tenofovir	52.5 (44.0, 61.8)	21/40	81.3 (60.1-96.7)	13/16	4.79 (1.30, 23.28)
		All	51.9 (44.1, 60.2)	28/54	81.5 (66.4, 93.1)	22/27	4.74 (1.66, 15.82)
HBeAg(-)	12	Entecavir	46.4 (39.5, 52.6)	13/28	NC (NC)	0/0	0.87 (<0.01, 166.17)
		Tenofovir	40.0 (33.6, 46.3)	14/35	100 (41.4, 100)	3/3	4.72 (0.41, 653.11)
		All	42.9 (39.6, 46.7)	27/63	100 (41.2, 100)	3/3	5.27 (0.48, 720.38)
	24	Entecavir	44.0 (34.4, 52.7)	11/25	66.7 (16.3-98.2)	2/3	1.57 (0.13, 36.42)
		Tenofovir	41.4 (31.3, 51.1)	12/29	77.8 (48.6, 97.1)	7/9	2.47 (0.49, 18.57)
		All	42.6 (36.4, 48.9)	23/54	75.0 (48.8, 93.7)	9/12	2.23 (0.59, 10.87)

CI=95% profile-likelihood confidence interval.

[a] Shading indicates statistical significance of odds ratios.

[b] For calculation of odds ratios and their confidence intervals, 0.5 was added to all cells whenever at least one cell was zero.

Table 28: PPV, NPV, and Odds Ratio for Serologic Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <50 IU/mL

HBeAg Status	Week of Early Virologic Response	Treatment	PPV Estimate (95% CI)	PPV n/N	NPV Estimate (95% CI)	NPV n/N	OR Estimate (95% CI)[a,b]
HBeAg(+)	12	Entecavir	16.7 (10.4, 19.6)	6/36	100 (44.1, 100)	3/3	1.49 (0.12, 209.89)
		Tenofovir	16.7 (12.9, 18.6)	13/78	83.3 (44.2, 99.2)	5/6	1.00 (0.14, 19.98)
		All	16.7 (13.9, 18.1)	19/114	88.9 (58.8, 99.5)	8/9	1.60 (0.27, 30.56)
	24	Entecavir	30.0 (16.5, 41.6)	6/20	100 (86.3, 100)	18/18	16.59 (1.72, >999.99)
		Tenofovir	22.4 (16.9, 26.8)	13/58	96.3 (84.7, 99.9)	26/27	7.51 (1.37, 140.31)
		All	24.4 (20.0, 28.4)	19/78	97.8 (90.4, 99.9)	44/45	14.17 (2.77, 259.25)

CI=95% profile-likelihood confidence interval.

[a] Shading indicates statistical significance of odds ratios.

[b] For calculation of odds ratios and their confidence intervals, 0.5 was added to all cells whenever at least one cell was zero.

Conclusion

Overall, the results demonstrate that the Aptima HBV Quant assay can quantitate HBV DNA levels at baseline and during treatment to aid in assessing viral response to treatment. This study demonstrated that early virologic responses at Weeks 12 and 24, as predictors of Week 48 virologic response, varied by week and treatment.

The Aptima HBV Quant assay can be used as an aid in the management of chronic HBV-infected patients undergoing HBV antiviral drug therapy.

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Serious incidents occurring in relation to the device in the European Union should be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

AW-28215-001 Rev. 003
2025-10

Revision History	Date	Description
AW-28215-001 Rev. 001	July 2025	• Initial release of new Aptima HBV assay IFU AW-28215 Rev. 001 for regulatory compliance with IVDR and will replace AW-13182.
AW-28215-001 Rev. 002	August 2025	• Trademark updates to meet BSI requirements.
AW-28215-001 Rev. 003	October 2025	• Added tube rocker option. • Updated SDS.