SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: HBV DNA quantitative test

Device Trade Name: Aptima® HBV Quant assay

Device Procode: MKT

Applicant's Name and Address: Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P170025

Date of FDA Notice of Approval: January 23, 2018

II. INDICATIONS FOR 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. Assay performance for determining the clinical stage of HBV infection has not been established. Clinical performance characteristics have been established for individuals treated with tenofovir disoproxil fumarate or entecavir.

The Aptima HBV Quant assay is not approved 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.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Aptima HBV Quant assay labeling.

V. DEVICE DESCRIPTION

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 transcription-mediated amplification (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 the HBV genome, if present, in the test specimen. The hybridized target is then captured onto magnetic micro-particles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

Target amplification occurs via transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV (Moloney murine leukemia virus) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target sequence which contains a promoter sequence for T7 RNA polymerase. 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: polymerase gene and surface gene of the HBV genome. This dual target region approach mitigates the risk of under-quantitation from one of the target regions due to potential mutations. Amplifications of those regions are 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 most 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 hybridize specifically to the amplicon as it is generated in real-time. Each torch has a fluorophore and a quencher. The quencher suppresses the signal of the fluorophore when not hybridized to the amplicon. When the torch binds to the amplicon, the quencher is moved further away from the fluorophore and it will emit a signal at a specific wavelength when excited by a light source. More torch hybridizes when more amplicon is present creating higher fluorescent signal. The time taken for the fluorescent signal to reach a threshold is proportional to the starting HBV DNA concentration. Each reaction has an internal calibrator/internal control (IC) which controls for variations in specimen processing, amplification, and detection. The concentration of a sample is determined automatically by the Panther system software

using the HBV and IC signals for each reaction and comparing them to calibration information.

Components of the Aptima HBV Quant assay Kit
The Aptima HBV Quant assay kit (100 tests) for the Panther system consists of 4 reagent kits:

Box 1: Aptima HBV Quant assay kit which contains the following reagents:

- Amplification Reagent
- Enzyme Reagent
- Promoter Reagent
- Target Capture Reagent
- Amplification Reconstitution Reagent
- Enzyme Reconstitution Reagent
- Promoter Reconstitution Reagent

Box 2: Aptima HBV Quant Controls kit which contains the following reagents:

- Negative Control
- Low Positive Control
- High Positive Control

Box 3: Aptima HBV Quant Calibrator kit which contains the following reagent:

- Positive Calibrator

Box 4: Aptima HBV Quant Target Enhancer Reagent Box

- Target Enhancer Reagent

There is one ancillary kit required to perform the assay (available separately):
Aptima Assay Fluids kit (also known as Universal Fluids Kit) which contains the following reagents:

- Wash Solution
- Buffer for Deactivation Fluid
- Oil Reagent

The Aptima Specimen Diluent kit is one optional ancillary kit which can be procured separately. The Aptima Specimen Diluent reagent provided in this kit is used to dilute plasma and serum specimens that are tested with the Aptima HBV Quant assay on the Panther system.

Quality Control Procedures
The Aptima HBV Quant assay contains three quality controls:

1. Assay Calibration
An assay calibration must be completed to generate valid results. 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.

2. Negative and Positive Controls

A set of assay controls must be tested to generate valid results. 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 control measurements are valid for up to 24 hours. Software on the Panther system alerts the operator when controls measurements 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. 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.

3. 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 to dilute 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.

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: Results 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[c]
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.
[c]Serum and plasma specimens with value above the ULoQ may be diluted and retested to determine a quantitative result within the linear range
Note: For diluted specimens with neat concentrations greater than the ULoQ, results will be reported using scientific notation.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved in vitro diagnostic tests for the quantitation of HBV DNA from patient samples. The patient's medical history and thorough clinical examination, in addition to serology, PCR or nucleic acid testing (NAT), determination of liver enzyme levels, and biopsy of the liver will provide further information on the status of an HBV infection. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The Aptima HBV Quant assay, accessory kits, and Aptima Specimen Diluent are marketed in multiple countries. The device has not been withdrawn from marketing for any reasons related to its safety or effectiveness. The following is a list of countries where the product is distributed:

- Austria
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Hungary
- Lithuania
- Luxembourg
- Malta
- The Netherlands
- Norway
- Poland
- Romania
- Switzerland
- Belgium
- Bulgaria
- Croatia
- Cyprus
- Greece
- Iceland
- Latvia
- Liechtenstein
- Sweden
- Spain
- Italy
- Australia
- Canada

- Ireland
- United Kingdom
- Slovenia

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When used according to the instructions in the package insert, there are no known potential direct adverse effects on health. The results of the Aptima HBV Quant assay must be interpreted within the context of all relevant clinical and laboratory findings. Failure of the Aptima HBV Quant assay to perform as indicated due to human error or other cause may lead to improper patient management.

An incorrect low test result or a false negative result may lead to an inappropriate treatment decision, delay in initiation of treatment, and premature discontinuation of antiviral therapy

An incorrect high test result or a false positive may contribute to a change in therapy, unnecessary treatment, prolonged duration of therapy, or may impact the psychological well being of the patient.

Inaccurate results around clinically significant HBV DNA levels such as 2,000 IU/mL and 20,000 IU/mL may impact patient management. Because medical practitioners usually obtain multiple (serial) measurements through the course of the disease, the risk of acting on a single result is lowered. In addition, other laboratory markers (e.g. ALT) may also provide additional information to the medical practitioner to guide patient management. Thus the risks are mitigated by the availability of other medical history information and other laboratory test results.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Limit of Detection (LoD) Using the 3rd HBV WHO International Standard
The LoD was determined by testing dilutions of the 3rd HBV WHO International Standard (NIBSC 10/264, genotype A) in HBV negative human plasma and serum. A total of 9 panel members were tested over 3 days on 3 Panther systems with 3 reagent lots. Probit analysis was used to determine the LoD. The LoD for the Aptima HBV Quant assay using the 3rd HBV WHO Internation Standard standard 4.8 IU/mL for plasma and 5.9 IU/mL for serum.

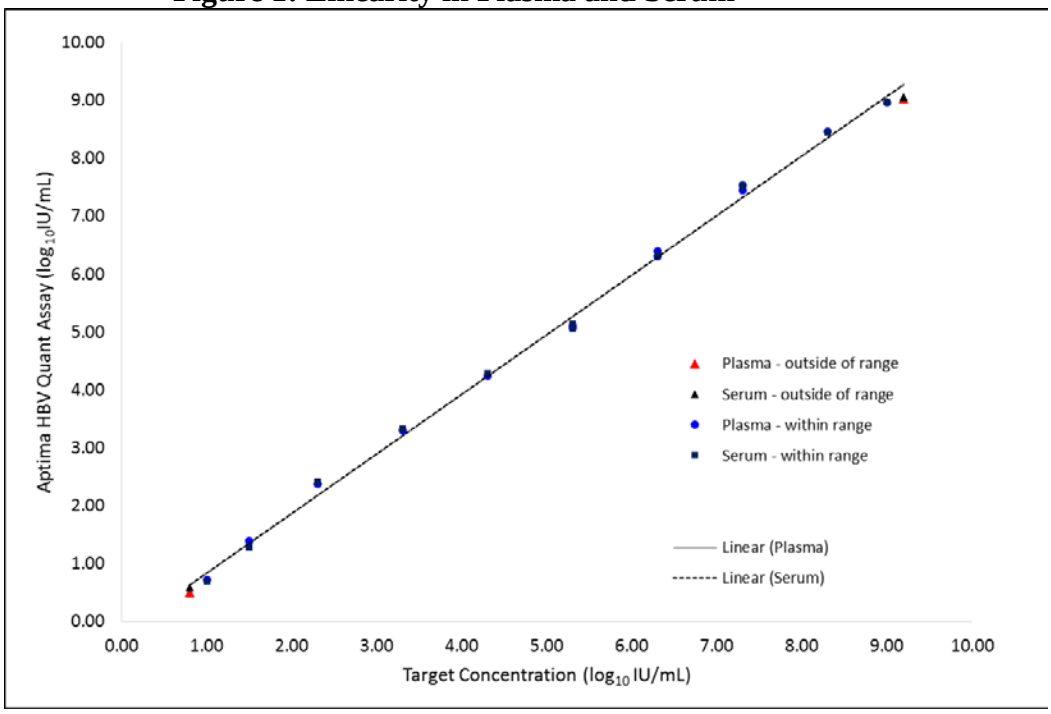
Limit of Detection by HBV Genotype
The LoD across genotypes was determined by testing dilutions of HBV positive clinical specimens for genotypes A, B, C, D, E, F, G and H in HBV negative human plasma and serum. Each panel was tested on multiple Panther systems over multiple days with multiple reagent lots. Probit analysis was used to determine the LoD. The LoD for each genotype is shown in Table 2.

Table 2: HBV DNA Genotype LoD in Plasma and Serum

Genotype	Plasma IU/mL	Serum IU/mL
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 Genotype A: The linear range was established by testing panels of HBV genotype A virus (0.78 log10 IU/mL to 7.30 log10 IU/mL) and plasmid DNA (5.30 log10 IU/mL to 9.18 log10 IU/mL) diluted in HBV negative human plasma and serum according to CLSI EP06-A. The Aptima HBV Quant assay demonstrated linearity across the range tested with an upper limit of quantitation (ULoQ) of 9.0 log10 IU/mL as shown in Figure 1.

Figure 1: 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 IU/mL and below for genotypes A-G, 3 log IU/mL and below for genotype H), and plasmid DNA was used for the upper range with a 2 log 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 2 and Table 3 for plasma and for serum. The Aptima HBV

Quant assay on the Panther system demonstrated linearity across genotypes and serum and plasma with a dynamic range of 1.0 log IU/mL to 9.0 log IU/mL.

Figure 2: Linear Range and Linearity (Plasma)

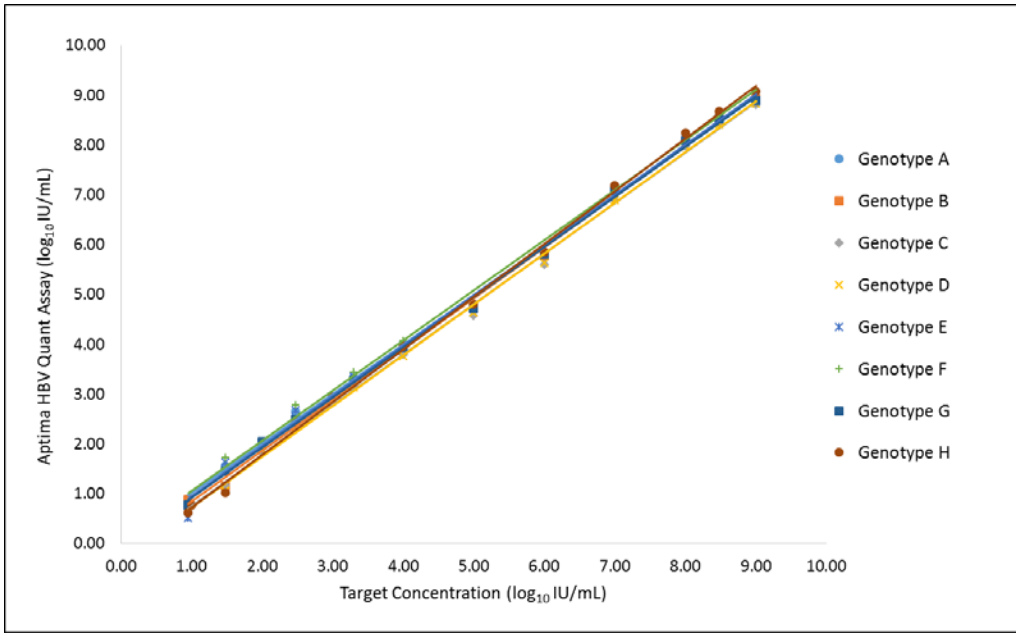


Table 3: Linear Fit Equations across Genotypes (Plasma)

Genotype	Linear Equation	Maximum Non-linearity (log10 IU/mL)
A	y = 0.9919 x + 0.0395	0.09
B	y = 1.0238 x – 0.2043	N/A*
C	y = 1.0124 x – 0.2475	0.10
D	y = 1.0196 x – 0.2987	0.07
E	y = 0.9872 x + 0.0832	0.13
F	y = 0.9958 x + 0.1273	0.14
G	y = 1.0013 x – 0.0466	0.11
H	y = 1.0561 x – 0.323	-0.05
*No 2nd/3rd order polynomial fit is statistically better than a linear fit at the significance level for genotype B.		

Lower Limit of Quantitation (LLoQ) Using the 3rd HBV 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. 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 $\log_{10}$ 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. For a given matrix, the lowest observed result within a reagent lot that met the accuracy goal (TE≤ 1 $\log_{10}$ IU/mL and TAE≤ 1 $\log_{10}$ IU/mL) with >95% detection and greater than or equal to the LOD was selected as the LLoQ for that reagent lot. The highest LLoQ observed across all reagent lots tested was selected as the final LLoQ for that matrix which is 6 IU/mL (0.79 log IU/mL) for plasma and 8 IU/mL (0.88 log IU/mL) for serum. The LLoQ was established across genotypes (see next section "Determination of the Lower Limit of Quantitation (LLoQ) Across HBV Genotypes"). This genotype data establishes the overall LLoQ for the assay as 10 IU/mL.

Determination of LLoQ Across HBV Genotype: The LLoQ across genotypes 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 of clinical specimens was determined using an FDA approved assay. Thirty-six (36) replicates of each panel member were tested with each of two reagent lots for a minimum of 72 replicates per panel member. For a given genotype and matrix, the lowest observed result within a reagent lot that met the accuracy goal (TE≤ 1 $\log_{10}$ IU/mL and TAE≤ 1 $\log_{10}$ IU/mL) with >95% detection and greater than or equal to the LOD was selected as the LLoQ for that reagent lot. The highest LLoQ observed across the two reagent lots tested for a given genotype and matrix was selected as the final LLoQ for that genotype and matrix . The calculated LLoQ for genotypes A, B, C, D, E, F, G, and H in plasma and serum are summarized in Table 4: Summary of LLoQ Across Genotypes (Plasma and Serum). Genotype D in serum had the highest LLoQ at 9 IU/mL. This supports the overall LLoQ for the assay as 10 IU/mL.

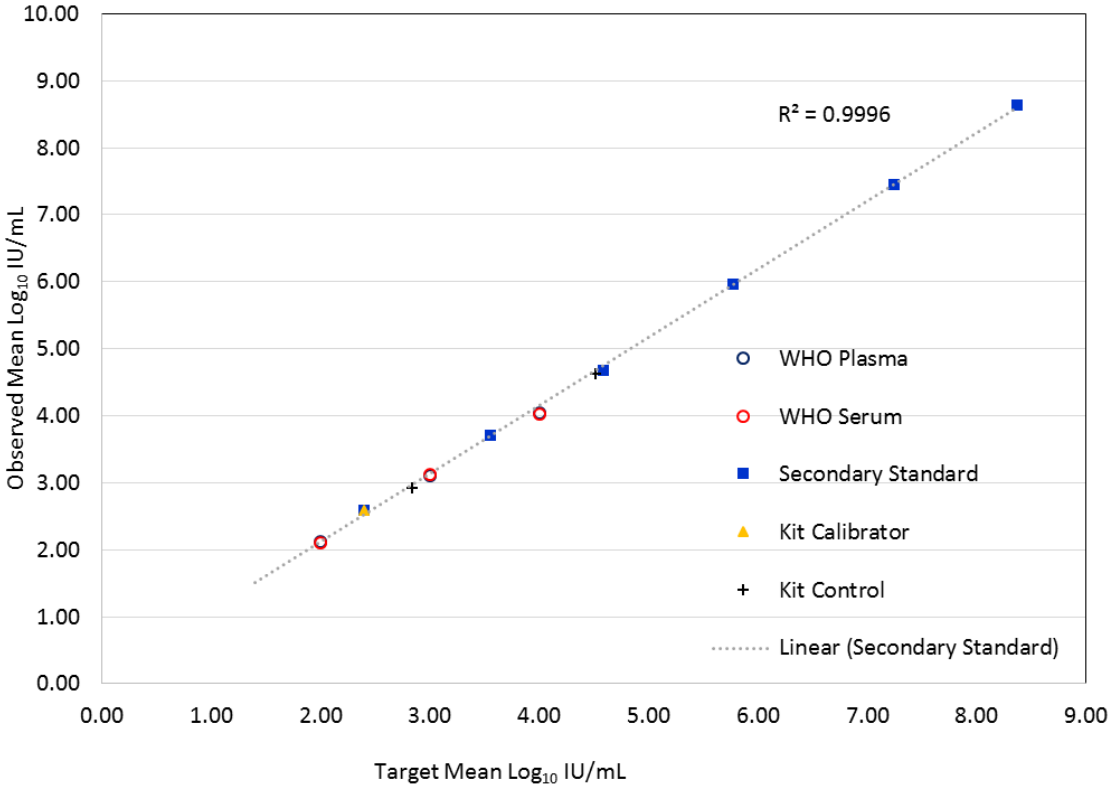
Table 4: Summary of LLoQ Across Genotypes (Plasma and Serum)

HBV Genotype	Plasma LLoQ		Serum LLoQ	
	(IU/ml)	(log10 IU/ml)	(IU/ml)	(log10 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 HBV WHO International Standard: A series of secondary standards with known concentrations were used throughout product development and product manufacturing to establish traceability to the 3rd HBV WHO International

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 3.

Figure 3: Traceability Between 3rd WHO Standard and Calibrators and Controls



Precision-Within Laboratory: 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 log10 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 5 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 5: Precision of the Aptima HBV Quant assay

Matrix	Sample	N	Mean Concentration (IU/mL)	Mean Concentration (log10 IU/mL)	Inter-Lot SD	Inter-Day SD	Inter-Run SD	Intra-Run SD	Total SD
Plasma	Virus	34[a]	32	1.21	0.07	0.07	0.05	0.28	0.30
	Virus	54	97	1.95	0.05	0.03	0.02	0.15	0.17
	Virus	54	1,474	3.16	0.00	0.02	0.02	0.07	0.07
	Virus	54	10,602	4.02	0.02	0.02	0.01	0.06	0.07
	Virus	54	429,428	5.63	0.03	0.01	0.01	0.06	0.07
	Plasmid DNA	54	652,103	5.80	0.06	0.01	0.01	0.06	0.09
	Virus	54	7,617,612	6.88	0.02	0.00	0.02	0.06	0.07
	Plasmid DNA	54	10,662,942	7.02	0.02	0.01	0.01	0.06	0.06
	Virus	54	89,149,358	7.95	0.01	0.01	0.01	0.04	0.05
	Plasmid DNA	54	103,400,000	8.01	0.04	0.02	0.01	0.05	0.07
	Plasmid DNA	54	612,200,000	8.78	0.03	0.01	0.01	0.04	0.05
Serum	Virus	29[a]	33	1.27	0.13	0.08	0.06	0.29	0.33
	Virus	54	88	1.92	0.05	0.02	0.02	0.12	0.14
	Virus	54	1,446	3.15	0.02	0.01	0.00	0.08	0.08
	Virus	54	7,873	3.89	0.02	0.01	0.01	0.06	0.06
	Virus	54	313,518	5.49	0.01	0.01	0.01	0.08	0.08
	Plasmid DNA	54	599,225	5.77	0.04	0.01	0.01	0.06	0.08
	Virus	54	7,011,440	6.84	0.02	0.01	0.01	0.07	0.08
	Plasmid DNA	54	8,845,332	6.94	0.05	0.01	0.01	0.05	0.07
	Virus	54	70,350,774	7.84	0.03	0.02	0.02	0.06	0.07
	Plasmid DNA	53[b]	122,800,000	8.08	0.04	0.01	0.01	0.04	0.06
	Plasmid DNA	54	678,700,000	8.83	0.02	0.01	0.01	0.05	0.05

SD=standard deviation
[a]Detected replicates with quantifiable result.
[b]One replicate had an invalid result.

Analytical Specificity-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).

HBV negative clinical plasma specimens from patients with elevated levels of defined substances or from patients with the indicated diseases (ten samples for each substance) listed in Table 6 were tested with the Aptima HBV Quant assay. No interference in the performance of the assay was observed.

Table 6: Clinical Specimens Tested for Interference

Antinuclear antibody (ANA)	Systemic lupus erythematosus (SLE)
Rheumatoid factor (RF)	Hyperglobulinemia
Alcoholic cirrhosis (AC)	Rheumatoid arthritis (RA)
Alcoholic hepatitis	Anti-Jo1 antibody (JO-1)
Non-alcoholic hepatitis	Multiple myeloma (MM)
Autoimmune hepatitis	Hemolyzed (elevated hemoglobin)
Elevated alanine aminotransferase (ALT)	Icteric (elevated bilirubin)
Hepatocellular carcinoma (HCC)	Lipemic (elevated lipid)
Multiple sclerosis (MS)	Elevated protein

No interference in the performance of the assay was observed in the presence of the exogenous substances listed in Table 7 at concentrations at least three times the C_{max} (human plasma).

Table 7: Exogenous Substances Tested for Interference

Exogenous Substance Pool	Exogenous Substances Tested
1	Saquinavir, ritonavir, amprenavir, indinavir, lopinavir, nelfinavir mesylate
2	Clarithromycin, valganciclovir hydrochloride, efavirenz, nevirapine
3	Paroxetine HCI, 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 – Cross Reactivity: Potential cross-reactivity to the pathogens listed in Table 8 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 IU/mL (4.3 $\log_{10}$ IU/mL) HBV DNA. No cross-reactivity or interference was observed.

Table 8: Pathogens Tested for Cross-Reactivity

Microorganism/ Pathogen	Concentration	Microorganism/ Pathogen	Concentration
Adenovirus type 5	100,000 TCID50/mL[1]	Parvo B19	100,000 IU/mL
BK human polyomavirus	1,000 TCID50/mL	Rubella virus	10,000 TCID50/mL
Cytomegalovirus	100,000 TCID50/mL	St. Louis Encephalitis virus	100,000 TCID50/mL
Dengue virus 1	10,000 TCID50/mL	vaccinia virus	1,000 TCID50/mL
Dengue virus 2	10,000 TCID50/mL	West Nile Virus	100,000 TCID50/mL
Dengue virus 3	10,000 TCID50/mL	Yellow Fever Virus	100,000 TCID50/mL

Microorganism/ Pathogen	Concentration		Microorganism/ Pathogen	Concentration	
Dengue virus 4	100,000	TCID50/mL	*Candida albicans*	1,000,000	CFU/mL[5]
Epstein-Barr virus	100,000	copies/mL	*Chlamydia trachomatis*	1,000,000	IFU/mL[6]
Flu H1N1	100,000	TCID50/mL	*Corynebacterium diphtheriae*	1,000,000	CFU/mL
Hepatitis A	100,000	TCID50/mL	*Neisseria gonorrhoeae*	1,000,000	CFU/mL
Hepatitis C	100,000	IU/mL[2]	*Propionibacterium acnes*	1,000,000	CFU/mL
Hepatitis G virus	100,000	copies/mL	*Staphylococcus aureus*	1,000,000	CFU/mL
Human Herpesvirus Type 6B	100,000	copies/mL	*Staphylococcus epidermidis*	1,000,000	CFU/mL
Human Herpesvirus Type 8	100,000	copies/mL	*Streptococcus pneumoniae*	1,000,000	CFU/mL
HIV-1	100,000	IU/mL	*Trichomonas vaginalis*	1,000,000	CFU/mL
HIV-2	10,000	TCID50/mL			
Human Palillomavirus	100,000	copies/mL			
Herpes Simplex Virus Type 1	100,000	TCID50/mL			
Herpes Simplex Virus Type 2	100,000	TCID50/mL			
Human T-lymphotropic virus 1	100,000	vp/mL[3]			
Human T-lymphotropic virus 2	100,000	vp/mL			
Japanese encephalitis virus	N/A				
Murray Valley Encephalitis virus	2,000	LD50/mL[4]			

[1] TCID50/mL = Tissue culture Infectious dose units per mL
[2] IU/mL = International units per mL
[3] vp/mL = Viral particles per mL
[4] LD50/mL = Lethal dose per mL
[5] CFU/mL = Colony forming units per mL
[6] IFU/mL = Inclusion forming units per mL

Matrix Equivalency: To demonstrate equivalent performance between serum and plasma collection tube types, one hundred eighteen sample sets of matched blood collection tubes (serum tube, ACD, K2 EDTA, K3 EDTA, PPT, SST) were assessed. Of these, 44 sets were naturally infected HBV-positive, and 74 sets were HBV-negative spiked with HBV virus. Correlation was measured by using the serum collection tube as a comparator. Deming regression analysis between serum and each of the collection tubes (SST, K2 EDTA, K3 EDTA, ACD and PPT) demonstrated that the Aptima HBV Quant assay has equivalent performance in reporting quantitative results with the 6 different collection tube types as shown in Table 9.

Table 9: 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

Sample Dilution Using Aptima Specimen Diluent (1:3): To assess the quantitation accuracy of HBV DNA in samples diluted with Aptima Specimen Diluent, samples that spanned the linear range (1.20 log10 IU/mL to 9.39 log10 IU/mL) were diluted 1:3 with Aptima Specimen Diluent (such as 240µL of sample combined with 480µL 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 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 are shown in Table 10 for plasma and Table 11 for serum. The sample concentrations were accurately quantitated in the diluted samples.

Table 10: 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 of Diluent from Plasma Matrix (log IU_{10}/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 11: 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 of Diluent from Plasma Matrix (log IU_{10}/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 the quantitation 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 12 for plasma and Table 13 for serum.

Table 12: 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 of Diluent from Plasma Matrix (log IU_{10}/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 13: Serum Specimen 1:100 Dilution Matrix Comparison Study

Serum Matrix Average Reported Concentration (log_{10} IU/mL) n = 5	**Diluent Average Reported concentration (log_{10} IU/mL) n = 10**	**Difference of Diluent from Plasma Matrix (log IU_{10}/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. Thirty-six (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 14.

Table 14: 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 15 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 15: Precision of Panels Diluted in Aptima Specimen Diluent

Matrix	Target Concentration log_{10} IU/mL	Dilution	Lot 1 Specimen Diluent (n=10)		Lot 2 Specimen Diluent (n=10)		Lot 3 Specimen Diluent (n=10)		Combined Lots (n=30)	
			Avg. log_{10} IU/mL	SD	Avg. log_{10} IU/mL	SD	Avg. log_{10} IU/mL	SD	Avg log_{10} IU/mL	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

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 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).

Specimen Stability: Specimen stability studies demonstrated that, for the Aptima HBV Quant assay, specimens should be stored as follows:

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

- In the primary collection tube or specimen aliquot tube (SAT) at 2^{O}C to 30^{O}C for up to 24 hours.
- In the primary collection tube or SAT at 2^{O}C to 8^{O}C for up to 5 days, or
- In the SAT at -20^{O}C for up to 60 days.

Real-Time Reagent (including Controls) Stability: Expiration dating for the Aptima HBV Quant assay has been established and approved as shown in Table 16:

Table 16: Real-Time Reagent Stability

Kit Description	Shelf Life
Aptima HBV Quant assay Kit	22 months at 2°C to 8°C
Aptima HBV Quant Target Enhancer Reagent Kit	22 months at 15°C to 30°C
Aptima HBV Quant Calibrator Kit	24 months at -15°C to -35°C
Aptima HBV Quant Controls Kit	24 months at -15°C to -35°C

Antimicrobial Effectiveness (AET): Testing was performed and approved for the Aptima HIV-1 Quant Assay. The Aptima HBV Quant assay uses similar base formulations with minor differences in concentrations. Because the formulations are so similar and the preservatives used are identical, the AET established for the Aptima HIV-1 Quant assay verifies the preservative effectiveness for the Aptima HBV Quant assay. Results of the study were compared to the requirements of USP51. All reagents met the USP requirements for AET. Unlike Aptima HIV-1 Quant Assay, Aptima HBV Quant assay also contains Target Enhancer Reagent which is a concentrated solution of lithium hydroxide. No microbial control is needed since this reagent is caustic and self-preserving.

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

A. Study Design

The study was designed to assess the ability of the Aptima HBV Quant assay to predict virologic, biochemical, and serologic responses to HBV treatment at clinically relevant time points during 12-, 24-, or 48-week antiviral treatment.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the study was limited to patients who met the following inclusion criteria:

- The subject is chronically infected with HBV (historical record of persistence of HBsAg for greater than 6 months).
- The subject is initiating HBV antiviral therapy with either entecavir or tenofovir as indicated in the US FDA approved label (0.5 mg once daily of entecavir or 300 mg once daily for tenofovir), and has been treated for less than 12 weeks (±7 days).
    - Treatment-naïve subjects and certain treatment-experienced subjects are eligible.
        - Treatment-experienced subjects who discontinued a previous nucleotide/nucleoside treatment regimen (eg, tenofovir, entecavir, adefovir, lamivudine, telbivudine) due to reasons other than treatment failure or resistance (ie, breakthrough, rebound, or non-response) or treatment success (eg, seroconversion) are eligible to participate (eg, discontinued due to pregnancy, cost). Previous treatment must have ended ≥6 months before the initiation of current treatment with entecavir or tenofovir.
        - For subjects who have taken their first dose of current entecavir or tenofovir treatment, a standard of care HBV quantitative assay result is available for a sample that was collected ≤180 days before the start of therapy.
- The subject is at least 18 years of age at the time of enrollment
- Adequate medical records are available for collection of protocol-defined demographics, baseline patient characteristics, medical history, virology, and specific laboratory results, and other information to verify enrollment criteria
- The subject and/or legally authorized representative is willing and able to provide consent prior to providing a specimen(s)

Patients were not permitted to enroll in the study if they met any of the following exclusion criteria:

- Treatment experienced subjects who discontinued an HBV treatment regimen due to treatment failure or resistance (ie breakthrough, rebound, nonresponse) or treatment success (eg., seroconversion)
- Subject is in one of the following patient populations:

- Acute HBV infection
- Patients who are HBV immune tolerant
- Human immunodeficiency virus (HIV) and/or hepatitis c virus (HCV) co-infection
- Solid organ or bone marrow transplant recipients
- Renal failure or dialysis
- Evidence or history of hepatic decompensation
- Evidence of history of hepatocellular carcinoma
- Underlying liver disease other than HBV
- Receiving chemotherapy, immunosuppressive agents
- Subject is unsuitable for study participation based on the Investigator's decision (eg., unlikely to comply with study visit schedule, significant medical complications)

- Participating in another investigational study that the Investigator believes might interfere with the subject's participation in this study.

2. Response to Antiviral Therapy Definitions
   Aptima HBV Quant assay clinical utility was 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 log10 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 FDA-approved HBV quantitative assay
   - Alternative Week 48 virologic response = HBV DNA <50 IU/mL as assessed by an FDA-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)

3. Demographics

The study enrolled 331 subjects from a total of 67 clinical sites. These were composed of 37 U.S. and 30 outside of U.S. clinical sites (Australia, Canada, Germany, Hungary, New Zealand, Romania, and Turkey) . Of the 331 enrolled subjects, 86 subjects were not evaluable due to withdrawal, discontinuation, early treatment halt, missing Week 48 results, or missing baseline HBV DNA viral load, or low baseline HBV DNA viral load. The remaining 245 subjects were evaluable for at least one of the clinical utility endpoints and included 126 HBeAg positive and 119 HBeAg negative subjects (Table 17).

Table 17: 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	0 (0.0)
	Native Hawaiian/Pacific	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)

Characteristics		Total
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 Tested	35 (14.3)
Cirrhotic status, n (%)	Cirrhotic	26 (10.6)
	Non-cirrhotic	201 (82.0)
	Not Tested	18 (7.3)
HBV viral load (log10 IU/mL), n	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], n (%)	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

4. Results

Predicting Week 48 Virologic Response, Defined as HBV DNA <10 IU/mL
The primary definition of virologic response being HBV DNA <10 IU/mL 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, and 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 18.

Table 18: PPV, NPV, and Odds Ratio for Virologic Response Predicated 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,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 19.

Table 19: 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, unless there were either no Week 48 responses or no Week 48 non-responses which resulted in reporting the odds ratio as NC.

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 20. 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 20: PPV, NPV, and Odds Ratio for Serologic 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]**
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 section X.A.2 Response to Antiviral Therapy Definitions). 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 21 (virologic response), Table 22 (biochemical response), and Table 23 (serologic response).

Table 21: PPV, NPV, and Odds Ratio for Virologic Response Predicted by Early Virologic Response during Treatment: Week 48 Virologic Response Defined as <50 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	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 22: PPV, NPV, and Odds Ratio for Biochemical Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <50 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	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 23: PPV, NPV, and Odds Ratio for Serologic Response Predicted by Early Virologic Response During Treatment: Week 48 Virologic Response Defined as <50 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	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 demonstrated that the Aptima HBV Quant assay can be used to assess HBV DNA viral load in subjects with chronic HBV infection at the start of and during antiviral treatment. This study demonstrated that HBV DNA levels measured at baseline, and decreases in HBV DNA levels after 12 or 24 varied by week and treatment. The Aptima HBV Quant assay can be used to quantitate HBV DNA levels to aid in the management of chronic HBV-infected patients undergoing HBV antiviral therapy.

B. 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 with 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 24 shows the reproducibility and precison 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:

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

Table 24: Reproducibility of Aptima HBV Quant assay HBV DNA Levels on the Panther System in Positive Panel Members

				Between Sites	Between Operators/ Days[a]	Between Lots	Between Runs	Within Runs	Total
GT	**N**	**IU/mL**	**Log_{10} IU/mL**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**
A	108	17.6	1.85	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	2.092	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	3.012	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	3.875	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	5.263	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	6.961	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	7.931	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	8.868	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	1.174	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	2.151	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	3.125	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	3.988	0.011	0.014	0.040	0.038	0.059	0.083

				Between Sites	Between Operators/ Days[a]	Between Lots	Between Runs	Within Runs	Total
GT	**N**	**IU/mL**	**Log_{10} IU/mL**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**	**SD (%CV)**
				(2.472)	(32.70)	(9.337)	(8.801)	(13.651)	(19.291)
	108	217400.5	5.329	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	7.069	0.046 (10.543)	0 (0)	0.02 (4.652)	0.064 (14.762)	0.073 (16.922)	0.109 (25.501)
	108	577434712.8	7.754	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	8.749	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.

C. Safety and Effectiveness Results

1. Safety Results
   There were no adverse effects of the device reported while the study was conducted.

2. Effectiveness Results
   The analysis of effectiveness of the Aptima HBV Quant assay was assessed by determining the ability of the test to measure HBV DNA levels at baseline, during treatment, and after treatment. See results in Section X. A and B, above. Overall the clinical studies demonstrate the effectiveness of the Aptima HBV Quant assay in accurately measuring HBV DNA levels in patients undergoing treatment.

3. Subgroup Analyses
   Not Applicable.

4. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

D. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 70 investigators. None of the clinical investigators had

disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the FDA Microbiology Devices Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

- The effectiveness of the Aptima HBV Quant assay has been demonstrated when used for the quantitation of HBV DNA in human plasma [EDTA, ACD, and PPTs] and serum and serum separation tubes (SST) for the management of patients undergoing treatment.
- There are no issues with endogenous interferents at physiological levels or with commonly administered medications.
- Whole blood can be stored at 2°C to 30°C and must be centrifuged within 24 hours of specimen collection. Plasma or serum may then be stored under one of the following conditions:
  - In the primary collection tube or specimen aliquot tube (SAT) at 2°C to 25°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.
- The preservatives that the Aptima HCV Quant Dx Assay reagents and controls contain have been shown to meet USP Chapter 51 criteria.
- The Aptima Quant Dx assay reagents can be stored at 2-8°C for 22 months.
- The clinical performance was evaluated in an ethnically diverse population representative of the intended use population (patients undergoing HBV therapy (see clinical performance studies, above).

B. Safety Conclusions

Based on the results of the analytical and clinical laboratory studies, the Aptima HBV Quant assay, when used according to the provided directions and in conjunction with other laboratory results and clinical information, should be safe and pose minimal risk to the patient due to incorrect test results.

C. Benefit-Risk Determination

The benefits outweigh the risks at the level of performance observed in the pivotal clinical study. Complementary analytical studies strengthen this conclusion. Accurate detection and quantitation of HBV DNA is an essential component in the management of chronic HBV infected patients undergoing antiviral therapy. Risks include inaccurant quantification. This risk is substantially mitigated by device design (i.e., use of controls), the likelihood of additional testing for incorrect results, and the overall clinical assessment.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for the Aptima HBV Quant assay as an aid in the management of chronic HBV infected patients undergoing antiviral therapy the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The device accurately measures the viral load in the patient at baseline and at intervals during antiviral therapy. Therefore, this device should benefit the physician and the patients in the management of chronic HBV infected individuals undergoing antiviral therapy when used according to the directions for use in the labeling.

XIII. CDRH DECISION

CDRH issued an approval order on January 23, 2018.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.