SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Hepatitis Viral B DNA Detection
Device Trade Name:	Alinity m HBV
Device Product Code:	MKT
Applicants Name and Address:	Abbott Molecular Inc. 1300 E. Touhy Ave Des Plaines, IL60018
Date of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P200013
Date of FDA Notice of Approval:	August 29, 2020

II. INDICATIONS FOR USE

The Alinity m HBV assay is an in vitro polymerase chain reaction (PCR) assay for use with the automated Alinity m System to quantitate Hepatitis B Virus (HBV) DNA in human plasma or serum. The Alinity m HBV assay is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy. The assay can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. The results from the Alinity m HBV assay must be interpreted within the context of all relevant clinical and laboratory findings.

This assay is not intended to be used for screening donors of blood, blood products, or cell, tissue, and cellular and tissue-based products (HCT/Ps) or as a diagnostic test to confirm the presence of HBV infection.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warning and precautions can be found in the Alinity m HBV assay package insert and Alinity m System Operations Manual

V. DEVICE DESCRIPTION

The Alinity m HBV assay utilizes real-time polymerase chain reaction (PCR) to amplify and detect HBV DNA genomic sequences that have been extracted from human plasma or serum specimens. The steps of the Alinity m HBV assay consist of sample preparation, PCR assembly, amplification/detection, and result calculation and reporting.

The Alinity m HBV assay is designed to target a highly conserved sequence within the overlap of the Surface and the Polymerase region of the HBV genome. Additionally, HBV primers and two probes ensure assay robustness against new and emerging HBV variants. All steps of the Alinity m HBV assay procedure are executed automatically by the Alinity m System. Manual dilutions may be performed for low-volume specimens to meet the minimum volume requirement, and for high-titer specimens above the upper limit of quantitation (ULoQ).

The Alinity m System is designed to be a random-access analyzer that can perform the Alinity m HBV assay in parallel with other Alinity m assays on the same instrument. HBV DNA from human plasma or serum is extracted using the Alinity m Sample Prep Kit 2, proteinase K, Alinity m Lysis Solution, and Alinity m Diluent Solution. The Alinity m System employs magnetic microparticle technology to facilitate nucleic acid capture, wash, and elution. The resulting purified nucleic acid is then combined with liquid unit-dose Alinity m HBV assay activation reagent and lyophilized unit-dose Alinity m HBV assay amplification/ detection reagents and transferred into a reaction vessel. Alinity m Vapor Barrier Solution is then added to the reaction vessel which is then transferred to an amplification/detection unit for PCR amplification and real-time fluorescence detection of HBV.

At the beginning of the Alinity m HBV assay sample preparation process, a lyophilized unit- dose Internal Control (IC) and proteinase K on the AMP TRAY 1 is rehydrated by the Alinity m System and delivered into each sample preparation reaction. IC is unrelated to HBV target sequence and is derived from the hydroxypyruvate reductase gene from the pumpkin plant, Cucurbita pepo. IC is unrelated to HBV target sequence and is derived from the hydroxypyruvate reductase gene from the pumpkin plant, Cucurbita pepo. The IC is then processed through the entire sample preparation and PCR procedure along with the specimens, calibrators, and controls to demonstrate proper sample processing and validity.

The Alinity m HBV assay amplification/detection reagents consist of enzyme, primers, probes, and activation reagents that enable polymerization and detection. The Alinity m HBV assay amplification/detection reagent also contains Uracil-DNA Glycosylase (UDG) as a contamination control for amplicons containing uracil, which may be present in molecular laboratories.

An HBV calibration curve is required for the determination of HBV DNA concentration. Two levels of calibrators are processed through sample preparation and PCR to generate the calibration curve. The concentration of HBV DNA in specimens and controls is then calculated from the stored calibration curve.

Assay controls are tested at or above an established minimum frequency to help ensure that instrument and reagent performance remains satisfactory. During each control event, a negative

control, a low-positive control, and a high-positive control are processed through sample preparation and PCR procedures that are identical to those used for specimens.

Alinity m HBV is intended for use with the Alinity m System, a fully automated, self-contained system.

A. Components of the Alinity m HBV Assay

A.1. Alinity m HBV AMP Kit:

The Alinity m HBV AMP Kit is comprised of 2 types of multi-well trays:

- Alinity m HBV AMP TRAY 1 (4 trays × 48 tests) : Alinity m HBV AMP TRAY 1 contains 48 unit-dose lyophilized amplification reagent wells and 48 unit-dose lyophilized internal control and proteinase K (PK) reagent wells.
- Alinity m HBV ACT TRAY 2 (4 trays × 48 tests) : Alinity m HBV ACT TRAY 2 contains 48 unit-dose liquid activation reagent wells.

Each Alinity m HBV Tray 1 and Tray 2 supports testing of up to 192 samples (patient specimens, assay controls, or calibrators). Both trays contain 48 unit-dose reagent wells (with reagents as listed above) of which one well of each reagent is used per test (48 tests per tray).

Additional materials required but purchased separately

B.1. Alinity m HBV CAL Kit:

The Alinity m HBV assay calibrators are for calibration of the Alinity m HBV assay on the automated Alinity m System when used for the quantitative determination of HBV DNA. The Alinity m HBV CAL Kit is composed of the following reagents:

- Alinity m HBV CAL A (4 tubes × 1.65mL)
- Alinity m HBV CAL B (4 tubes × 1.65mL)

The Alinity m HBV CAL A and Alinity m HBV CAL B tubes are intended for single-use only. The Alinity m System will process 3 replicates from each calibrator tube. The calibrators are assigned lot-specific HBV DNA concentrations based on the results of testing against the Primary Calibrators.

C.1. Alinity m HBV CTRL Kit:

The Alinity m HBV assay controls are for the validity determination of the quantitative Alinity m HBV assay on the automated Alinity m System. The Alinity m HBV CTRL Kit is composed of the following reagents:

- Alinity m HBV Negative CTRL (12 tubes × 1.15mL)
- Alinity m HBV Low Positive CTRL (12 tubes × 0.65mL)
- Alinity m HBV High Positive CTRL (12 tubes × 0.65mL)

The Alinity m HBV Negative CTRL, Alinity m HBV Low Positive CTRL, and Alinity m HBV High Positive CTRL tubes are intended for single-use only. Controls are recommended to be tested at or above the minimum frequency of once every 24 hours.

D.1. Alinity m HBV Application Specification File:

The application specification file is a data file that contains a set of parameters in a software-industry-standard JSON (JavaScript Object Notation) file format. The parameters determine how the software controls the instrument components to execute the selected assay.

To run an assay on an Alinity m System, an Application Specification File is required. The Alinity m System software interprets the assay information provided in the specific Application Specification File (including definitions for sample extraction, reagent addition, amplification/detection, dilution function, data analysis, and validity evaluation protocols), along with system information, to control the system hardware, run validity checks and identify the appropriate algorithms for data generation.

E.1. Alinity m Sample Prep Kit 2:

The Alinity m Sample Prep Kit 2 is provided in a liquid, multi-dose format and is shared with other Alinity m assays. It consists of 2 reagents:

- Alinity m Elution Buffer 2 (4 bottles × 22mL)
- Alinity m Microparticles 2 (4 bottles × 24mL)

The Alinity m Sample Prep Kit 2 is used in conjunction with Alinity m System Solutions as part of the sample preparation protocol. The Alinity m Sample Prep Kit 2 is used on the Alinity m System to extract and concentrate target molecules from biological samples for subsequent Polymerase Chain Reaction (PCR) amplification, and to remove potential inhibitors from the resulting extract.

The sample preparation procedure consists of lysis/binding, washes, and elution. The sample preparation is performed within a disposable multi-well Integrated Reaction Unit that is loaded onto an Assay Processing Unit (APU) on the Alinity m System.

F.1. Alinity m Specimen Dilution Kit I:

The Alinity m Specimen Dilution Kit I is intended to allow dilution of specimens for testing on the automated Alinity m System for the measurement of nucleic acid. The Alinity m Specimen Dilution Kit I consist of an Alinity m Specimen Diluent Tube with a pierceable cap (24 tubes × 2.45mL). Each Alinity m Specimen Dilution Kit I supports dilution of up to 24 samples (patient specimens). The Alinity m Specimen Diluent Tube is a transport tube with a pierceable cap containing Abbott Molecular Transport Buffer. The buffer contains guanidine thiocyanate (GITC) in Tris Buffer.

G.1. Alinity m Tubes and Caps:

- Alinity m LRV Tube : Low Residual Volume (LRV) Tubes closed with caps (12 capped tubes per kit)

- Alinity m Transport Tube Pierceable Capped : transport tubes closed with pierceable caps (1500 capped transport tubes per case, 10 boxes of 150 capped tubes)
- Alinity m Transport Tube : 1600 transport tubes per kit
- Alinity m Pierceable Cap : 2000 pierceable caps per kit. The pierceable cap can be used to recap a transport tube
- Alinity m Aliquot Tube : 1600 aliquot tubes per kit

H.1. Alinity m System Solution:

The Alinity m System Solutions described below are used as a part of the sample preparation protocol to extract nucleic acid and concentrate target molecules from biological samples for subsequent PCR amplification and to remove potential inhibitors from the resulting extract. Alinity m System solution consists of

- Alinity m Lysis Solution: 1 bottle × 975mL
- Alinity m Diluent Solution : 4 bottles × 975mL
- Alinity m Vapor Barrier Solution: 1 bottle × 975mL

I.1. Instrumentation and Software:

The Alinity m System is a fully integrated and automated molecular diagnostics analyzer that utilizes real-time PCR technology in clinical laboratories. It provides a sample-to-result uninterrupted processing workflow. The Alinity m System enables continuous and random-access sample processing by using multiple sample processors and PCR thermal cycler/reader modules in parallel. Each sample occupies either one sample process lane or PCR Amplification and Detection (Amp-Detect) lane. Parallel lanes are provided to enable 300 tests in approximately 8 hours.

Each Alinity m System utilizes four independent Assay Processing Units (APUs) to achieve the throughput and random-access requirements. Each APU consists of one extraction unit and one Amp-Detect unit, which automate the steps for nucleic acid purification/extraction and real-time PCR, respectively. This results in the ability to process up to twenty-four different assay types simultaneously (i.e., up to 12 different assay types for purification/extraction and up to 12 different assay types for amplification and detection).

The Alinity m System software is the set of computer instructions that interprets system and assay information, calculates results, and provides the interface for controlling the system hardware. The Alinity m System software interprets the assay information provided in the specific Application Specification File, along with system information, to control the system hardware and identify the appropriate algorithms for data reduction.

Using application specifications, customers create orders for calibrators, controls, and specimens. Customers load racks of calibrators, controls, and specimens in the sample input to begin processing. Once the samples are processed, the results are reviewed and released through the software user interface.

Additional details can be found in the Alinity m Operator’s Manual.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved Class III in vitro diagnostic tests for the quantitation of HBV DNA. 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 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 CE certified Alinity m HBV AMP Kit (List No. 08N47-090), Alinity m HBV CAL Kit (List No. 08N47-070), and Alinity m HBV CTRL Kit (List No. 08N47-080) are identical in formulation to the US kits except for kit labeling and were introduced to markets outside of the United States as listed in Table 1. Additionally, Alinity m Sample Prep Kit 2 (List No. 09N12-001), Alinity m Specimen Dilution Kit I (List No. 09N50-001), Alinity m Tubes and Caps (List No. 09N49) and Alinity m System Solutions (List No. 09N20) received CE certification and are available in markets outside of the United States as listed in Table 1. The devices have not been withdrawn from marketing for any reason related to safety and effectiveness.

B. **Table 1. Alinity m HBV assay in Foreign Markets Outside of the United States**

Australia	Greece	Poland
Austria	Hungary	Portugal
Belgium	Iceland	Romania
Brazil	Ireland	Saudi Arabia
Bulgaria	Italy	Slovakia
Croatia	Latvia	Slovenia
Cyprus	Liechtenstein	South Korea
Czech Republic	Lithuania	Spain
Denmark	Luxembourg	Sweden
Estonia	Malta	Switzerland
Finland	Netherlands	Turkey
France	Norway	UK
Germany	Peru	

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When used according to the instructions in the package insert, there are no known direct adverse effects of this device on the health of the user. No specific adverse effects occurred during the conduct of clinical studies.

An erroneous test result—too low or too high— may occur with the Alinity m HBV assay. An erroneously low result may lead a clinician to believe that the current therapy is effective when it is not. Consequently, the clinician could fail to implement more appropriate therapy. An

erroneously high result may lead a clinician to believe that the current therapy is not effective. Consequently, the clinician could implement an inappropriate change in therapy.

There were no specific adverse events that occurred in clinical studies.

IX. SUMMARY OF NON-CLINICAL STUDIES

C. Laboratory Studies

1. Limit of Detection (LoD)

The LoD was determined by testing dilutions of the 3rd World Health Organization (WHO) International Standard for Hepatitis B Virus for Nucleic Acid Amplification Techniques (NIBSC code: 10/264; genotype A) prepared in HBV negative human plasma and serum. A total of 7 panel members were tested for each specimen matrix (plasma and serum) in 3 testing runs with each of the 4 Alinity m HBV AMP Kit lots performed across 3 days including a minimum of 8 replicates per day (i.e., 4 AMP Kit lots × 8 replicates /day × 3 days = minimum 96 total replicates per panel member tested). The analytical sensitivity of Alinity m HBV assay in plasma and serum are included in Table 3.

Probit analysis of the data determined that the concentration of HBV DNA in plasma detected with 95% probability (LoD by Probit) was 4.29 IU/mL (95% CI: 3.61 to 5.34 IU/mL).

Probit analysis of the data determined that the concentration of HBV DNA in serum detected with 95% probability (LoD by Probit) was 6.85 IU/mL (95% CI 5.44 IU/mL to 9.19 IU/mL).

The claimed LoD for Alinity m HBV assay is 10 IU/mL (1.00 Log IU/mL) in plasma and serum for HBV genotype A.

Table 3. Alinity m HBV assay Limit of Detection (LoD) in Plasma and Serum (Genotype A)

Matrix	HBV DNA (IU/mL)	Number of Valid Replicates	Number of detected Replicates	Detection Rate (%)	LoD by Probit [95% CI]
Plasma	15.00	111	111	100.0	4.29 IU/mL [3.61 – 5.34 IU/mL]
	10.00	112	112	100.0	
	7.00	110	110	100.0	
	4.00	109	101	92.7	
	2.00	109	83	76.1	
	1.00	112	47	42.0	
	0.50	111	25	22.5	
Serum	15.00	113	112	99.1	6.85 IU/mL [5.44 – 9.19 IU/mL]
	10.00	114	112	98.2	
	7.00	112	108	96.4	
	4.00	113	101	89.4	
	2.00	108	74	68.5	
	1.00	113	64	56.6	
	0.50	112	46	41.1	

2. Limit of Detection for Genotypes

The LoD of the Alinity m HBV assay was verified for HBV Genotypes B, C, D, E, F, G, H, and I in plasma and serum. For each HBV Genotype, 3 panel members were prepared by diluting a clinical specimen into HBV negative human serum and plasma. Panel quantitation values were established using internal reference standards that are traceable to 3rd WHO International Standard for Hepatitis B Virus (NIBSC code: 10/264). Testing was performed across 3 days including 8 replicates per day for a total of 24 replicates per panel member. This study design ensures a minimum of 20 valid replicates per panel member according to CLSI EP17-A2. The analytical sensitivity of Alinity m HBV assay for genotypes B, C, D, E, F, G, H, and I are summarized in Table 4 and Table 5. Alinity m HBV assay detected >95% HBV replicates at and above 10 IU/mL (1.00 Log IU/mL) in plasma and serum. The study demonstrates that the Alinity m HBV assay detects genotypes B, C, D, E, F, G, H, and I at the claimed LoD.

Table 4. Alinity m HBV assay Genotype Limit of Detection (LoD) in Plasma

Genotype	HBV DNA (IU/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
B	20	20	20	100.0
	10	24	24	100.0
	7	24	24	100.0
C	20	24	24	100.0
	10	24	24	100.0
	7	24	24	100.0
D	20	24	24	100.0
	10	24	24	100.0
	7	23	21	91.3
E	20	24	24	100.0
	10	23	23	100.0
	7	24	24	100.0
F	20	24	24	100.0
	10	24	24	100.0
	7	24	24	100.0
G	20	23	23	100.0
	10	24	24	100.0

	7	24	24	100.0
H	20	23	23	100.0
	10	24	24	100.0
	5	24	20	83.3
I	20	24	24	100.0
	10	24	24	100.0
	7	23	22	95.7

Table 5. Alinity m HBV assay Genotype Limit of Detection (LoD) in Serum

Genotype	HBV DNA (IU/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
B	20	22	22	100.0
	10	23	23	100.0
	7	23	23	100.0
C	20	24	24	100.0
	10	24	24	100.0
	7	24	24	100.0
D	20	24	24	100.0
	10	22	22	100.0
	7	24	24	100.0
E	20	24	24	100.0
	10	24	24	100.0
	7	24	23	95.8
F	20	24	24	100.0
	10	24	24	100.0
	7	23	23	100.0
G	20	24	24	100.0
	10	24	24	100.0
	7	24	23	95.8
H	20	23	22	95.7
	10	24	24	100.0

	5	24	18	75.0
I	20	22	22	100.0
	10	23	23	100.0
	7	22	20	90.9

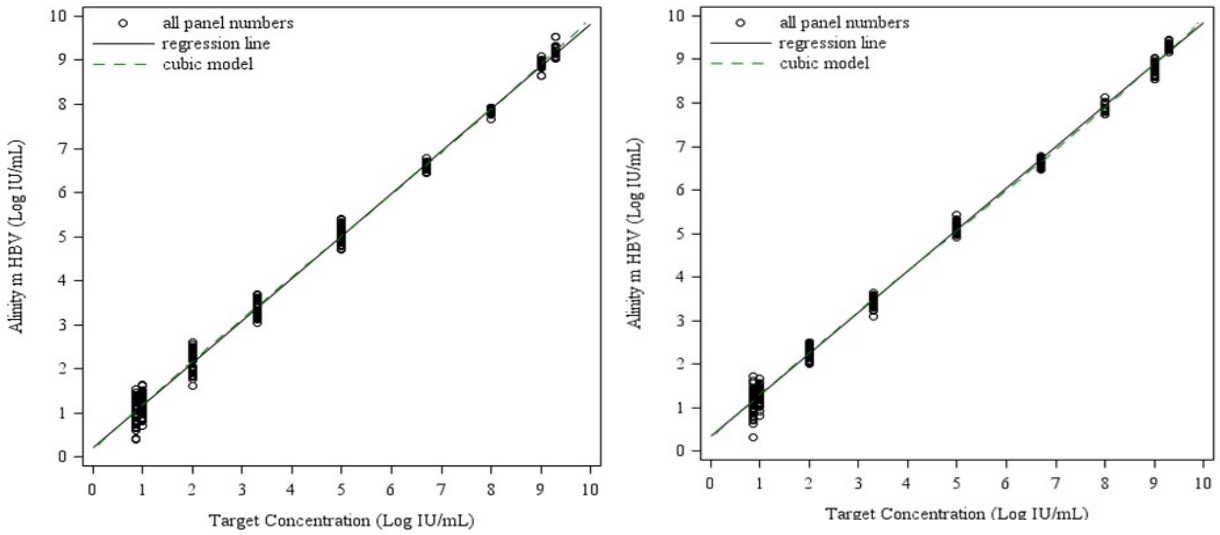
3. Linear Range

The linearity of Alinity m HBV assay was assessed by testing a dilution series of HBV genotype A in negative human plasma and serum, each consisting of 9 panel levels spanning from 7 to 2,000,000,000 IU/mL (0.85 to 9.30 Log IU/mL). Two kinds of panel members were created: panel members with concentrations from 7 to 100,000 IU/mL (0.85 to 5.00 Log IU/mL) were prepared using an HBV positive clinical sample, while panel members with concentrations 100 to 2,000,000,000 IU/mL (2.00 to 9.30 Log IU/mL) were prepared using synthetic DNA. Panel quantitation values were traceable to the 3rd WHO International Standard for Hepatitis B Virus for Nucleic Acid Amplification Techniques, (NIBSC code: 10/264).

There was no significant difference in the linearity between the clinical sample and the synthetic DNA based panel members. Similarly, there was no significant difference in the linearity between the plasma and serum (Figure 1). The difference in the predicted concentration between the fitted nonlinear model and the linear model was less than or equal to 0.50 Log IU/mL for each panel member. The Alinity m HBV assay demonstrated linearity in plasma and serum across the range tested 7 to 2,000,000,000 IU/mL (0.85 to 9.3 Log IU/mL) for HBV Genotype A . The results support plasma and serum linearity claim of 10 IU/mL (LLoQ) to 1,000,000,000 IU/mL (ULoQ) for Alinity m HBV assay.

Figure 1: Linearity of Plasma and Serum. The markers in the plot represent the mean (in Log IU/mL) Alinity m HBV assay concentration for each panel member.

Plasma **Serum**



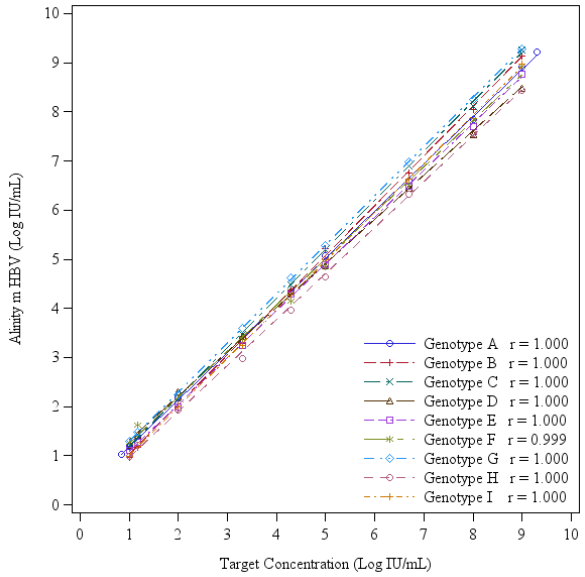
4. Linearity Across HBV Genotypes

The Linearity of Alinity m HBV assay was assessed for HBV genotypes A, B, C, D, E, F, G, H, and I in plasma and serum. For each HBV genotype and each matrix (plasma and serum), linearity was evaluated by testing 9 panel members that spanned the intended dynamic range of the assay: levels spanning from 10 to 1,000,000,000 IU/mL (1.00 to 9.00 Log IU/mL). Two kinds of panels were created: a panel with levels with lower concentrations (10 IU/mL to 20,000 IU/mL) were prepared using either an HBV positive clinical sample or synthetic DNA, and a panel with levels with higher concentrations (10 IU/mL to 1,000,000,000 IU/mL) were prepared using synthetic DNA. Least squares linear regression analysis was performed for Genotypes separately.

The Alinity m HBV assay was linear in plasma and serum across the range of HBV DNA concentrations from 10 IU/mL (LLoQ) to 1,000,000,000 IU/mL (ULoQ) for genotypes A, B, C, D, E, F, G, H, and I. The results are shown in Figure 2 and Table 6.

Figure 2. Linearity Across HBV Genotypes in Plasma and Serum. The markers in the plot represent the mean (in Log IU/mL) Alinity m HBV assay concentration for each panel member

Plasma



Serum

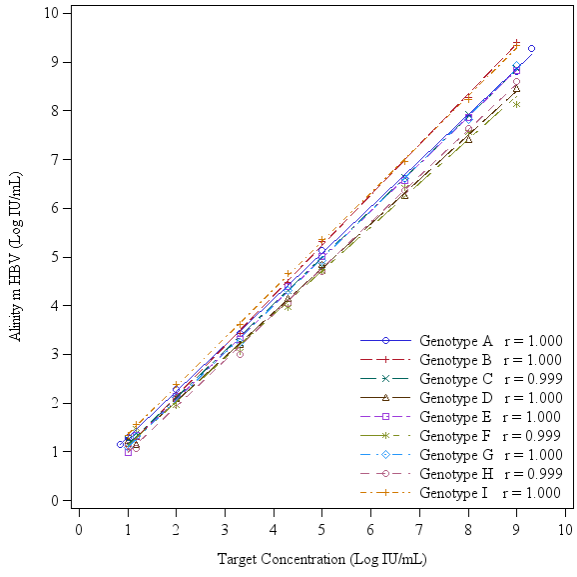


Table 6. Linear Fit Equations across Genotypes

Genotype	Plasma		Serum	
	Linear Equation	Maximum Non-linearity (log IU/mL)	Linear Equation	Maximum Non-linearity (log IU/mL)
A	Y = 0.96X + 0.22	0.04	Y = 0.95X + 0.35	0.05
B	Y = 1.01X + 0.00	0.03	Y = 1.03X + 0.07	NA*
C	Y = 1.00X + 0.19	NA*	Y = 0.96X + 0.20	0.08
D	Y = 0.90X + 0.41	0.08	Y = 0.91X + 0.23	NA*
E	Y = 0.95X + 0.14	NA*	Y = 0.97X + 0.14	0.05
F	Y = 0.93X + 0.36	0.20	Y = 0.89X + 0.23	0.18
G	Y = 1.00X + 0.29	NA*	Y = 0.97X + 0.14	0.07
H	Y = 0.93X + 0.05	0.08	Y = 0.94X + 0.08	0.08
I	Y = 0.99X + 0.02	NA*	Y = 0.99X + 0.37	0.04

**No 2nd/3rd order polynomial fit is statistically better than a linear fit at the significance level.*

5. Lower Limit of Quantitation

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which HBV DNA is reliably quantitated within a total error. Total error was estimated by 2 methods: Total Analytical Error (TAE) = |bias| + 2 × SD, and Total Error (TE) = SQRT (2) × 2 × SD.

TAE and TE of Alinity m HBV assay for genotypes A, B, C, D, E, F, G, H, and I in plasma and serum were calculated for panel members with observed concentrations at or near 10 IU/mL (1.00 Log IU/mL) tested in multiple non-clinical studies, as shown in Table 7 and Table 8. The concentrations of the panel members were traceable to the 3rd WHO International Standard for Hepatitis B Virus for Nucleic Acid Amplification Techniques (NIBSC code: 10/264).

The results of these analyses demonstrated that Alinity m HBV assay can determine the concentration of HBV DNA for genotypes A, B, C, D, E, F, G, H, and I in plasma and serum at 10 IU/mL (1.00 Log IU/mL) with an acceptable level of accuracy and precision, i.e., TAE and TE less than or equal to 1.00 Log IU/mL. The LLoQ of the Alinity m HBV is 10 IU/ml.

Table 7. Total Error for Plasma

Study	Genotype	Target Conc. (Log IU/mL)	Mean Conc. (Log IU/mL)	Bias[a] (Log IU/mL)	SD (Log IU/mL)	TAE (Log IU/mL)	TE (Log IU/mL)
Limit of Detection	A	1.00	1.07	0.07	0.25	0.57	0.71
Genotype Limit of Detection	B	1.00	1.18	0.18	0.20	0.58	0.57
	C	1.00	1.46	0.46	0.17	0.81	0.49
	D	1.00	1.24	0.24	0.24	0.72	0.67
	E	1.00	1.25	0.25	0.17	0.59	0.48
	F	1.00	1.63	0.63	0.14	0.91	0.40
	G	1.00	1.35	0.35	0.13	0.61	0.37
	H	1.00	0.98	-0.02	0.29	0.59	0.82
	I	1.00	0.94	-0.06	0.21	0.49	0.61
Linearity	A	1.00	1.19	0.19	0.23	0.65	0.65
Genotype Linearity	B	1.00	0.97	-0.03	0.22	0.46	0.61
	C	1.00	1.18	0.18	0.31	0.79	0.87
	D	1.00	1.21	0.21	0.32	0.85	0.91
	E	1.00	1.11	0.11	0.18	0.47	0.50
	F	1.00	1.28	0.28	0.32	0.93	0.91
	G	1.00	1.29	0.29	0.12	0.53	0.35
	H	1.00	0.99	-0.01	0.32	0.66	0.92
	I	1.00	1.00	-0.00	0.28	0.57	0.80
Precision	C	1.00	1.23	0.23	0.27	0.76	0.75

[a] *Bias = Mean Concentration – Target Concentration.*

Table 8. Total Error for Serum

Study	Genotype	Target Conc. (Log IU/mL)	Mean Conc. (Log IU/mL)	Bias[a] (Log IU/mL)	SD (Log IU/mL)	TAE (Log IU/mL)	TE (Log IU/mL)
Limit of Detection	A	1.00	0.96	-0.04	0.26	0.56	0.72
Genotype Limit of Detection	B	1.00	1.19	0.19	0.20	0.59	0.57
	C	1.00	1.26	0.26	0.22	0.70	0.63
	D	1.00	1.19	0.19	0.19	0.57	0.54
	E	1.00	1.21	0.21	0.23	0.67	0.65
	F	1.00	1.48	0.48	0.20	0.87	0.56
	G	1.00	1.67	0.67	0.13	0.93	0.36
	H	1.00	1.14	0.14	0.20	0.55	0.58
	I	1.00	1.16	0.16	0.20	0.56	0.56
Linearity	A	1.00	1.30	0.30	0.17	0.65	0.49
Genotype Linearity	B	1.00	1.12	0.12	0.20	0.52	0.57
	C	1.00	1.24	0.24	0.22	0.69	0.63
	D	1.00	1.23	0.23	0.27	0.76	0.76
	E	1.00	0.99	-0.01	0.34	0.68	0.95
	F	1.00	1.10	0.10	0.26	0.63	0.75
	G	1.00	1.09	0.09	0.21	0.52	0.60
	H	1.00	1.13	0.13	0.25	0.63	0.70
	I	1.00	1.34	0.34	0.18	0.69	0.50
Precision	C	1.00	1.19	0.19	0.28	0.76	0.80

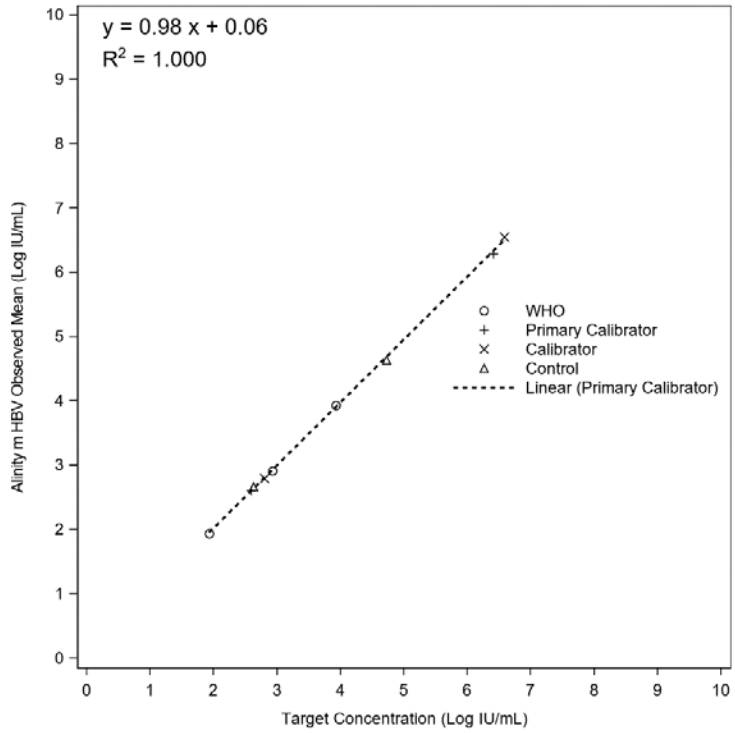
[a] *Bias = Mean Concentration – Target Concentration.*

6. Traceability to the WHO Standard

Primary calibrators and assay calibrators with known concentrations were used throughout product development and product manufacturing to establish traceability to the 3rd World Health Organization (WHO) International Standard for Hepatitis B Virus for Nucleic Acid Amplification Techniques (NIBSC code: 10/264; genotype A). The concentrations tested for the WHO standard were 1.93, 2.93, and 3.93 Log IU/mL. The concentrations tested for the primary calibrators ranged from 2.53 to 6.42 Log IU/mL. The Alinity m HBV assay calibrators and controls were also tested along with the primary calibrators and the WHO standard. All of the panels had

observed HBV concentrations similar to the target concentrations and were linear across the assay's quantitation range. The results are shown in Figure 3.

Figure 3. Traceability to the WHO Standard



7. Precision

The Alinity m HBV assay variability was assessed in an internal precision study (serum and plasma) and the external reproducibility study using plasma on the Alinity m system at 3 external sites with three reagent lots.

a. Internal (within laboratory) Precision Study

The precision of the Alinity m HBV assay was determined by analyzing an 8-member plasma panel and an 8-member serum panel. Panel members spanning a range from 1 to 5 Log IU/mL (10 to 100,000 IU/mL) were prepared using an HBV positive genotype C sample, while panel members with concentrations greater than 5 Log IU/mL (100,000 IU/mL) were prepared using synthetic DNA for HBV genotype C. Each panel member was tested in 4 replicates, twice each day for 12 days, on 3 Alinity m Systems with 1 Alinity m AMP Kit operated by 3 operators (one operator per instrument), for a total of 288 replicates per panel member.

The study results for Alinity m HBV assay precision in plasma and serum are shown in Table 9 and Table 10. The analyses demonstrated that the Alinity m HBV assay has a within-laboratory standard deviation (SD) was less than or equal to 0.25 Log IU/mL for HBV DNA from 2 to 9 Log

IU/mL (100 to 1,000,000,000 IU/mL), and less than or equal to 0.35 Log IU/mL near the LLoQ (1.00 to 1.48 Log IU/mL or 10 to 30 IU/mL).

Table 9. Precision in Plasma

Panel Member	N[a]	Mean Conc (Log IU/mL)	Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[b]		Between-Instrument/ Operator Component		Total[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
08	284	8.87	0.09	1.0	0.04	0.5	0.04	0.4	0.10	1.2	0.09	1.0	0.14	1.5
07	282	7.66	0.06	0.8	0.02	0.2	0.03	0.4	0.07	0.9	0.06	0.8	0.10	1.3
06	283	6.53	0.07	1.1	0.02	0.3	0.04	0.5	0.08	1.3	0.05	0.8	0.10	1.5
05	283	5.03	0.07	1.4	0.02	0.5	0.03	0.7	0.08	1.7	0.02	0.4	0.09	1.7
04	285	3.38	0.08	2.4	0.04	1.1	0.05	1.6	0.10	3.1	0.04	1.3	0.11	3.3
03	277	2.26	0.12	5.3	0.03	1.2	0.08	3.5	0.15	6.5	0.10	4.3	0.18	7.8
02	271	1.76	0.16	8.8	0.00	0.0	0.09	5.0	0.18	10.2	0.11	6.3	0.21	11.9
01	278	1.23	0.23	18.6	0.09	6.9	0.11	8.6	0.27	21.6	0.13	10.3	0.29	24.0

[a] *Number of valid replicates.*
[b] *Within-Laboratory includes Within-Run, Between-Run, and Between-Day components.*
[c] *Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument/Operator components.*

Table 10. Precision in Serum

Panel Member	N[a]	Mean Conc (Log IU/mL)	Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[b]		Between-Instrument/ Operator Component		Total[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
08	284	8.82	0.09	1.0	0.02	0.2	0.05	0.6	0.10	1.2	0.13	1.5	0.17	1.9
07	284	7.73	0.08	1.0	0.03	0.4	0.00	0.0	0.08	1.1	0.08	1.0	0.12	1.5
06	285	6.49	0.08	1.2	0.00	0.0	0.00	0.0	0.08	1.2	0.06	0.9	0.10	1.5
05	287	4.97	0.08	1.6	0.02	0.3	0.01	0.3	0.08	1.7	0.05	1.1	0.10	2.0
04	283	3.32	0.09	2.6	0.06	1.8	0.07	2.2	0.13	3.8	0.08	2.4	0.15	4.5
03	279	2.19	0.12	5.5	0.00	0.0	0.09	3.9	0.15	6.8	0.07	3.3	0.17	7.5
02	282	1.71	0.16	9.3	0.06	3.8	0.07	4.2	0.19	10.9	0.11	6.5	0.22	12.7
01	286	1.19	0.25	20.9	0.04	3.4	0.13	10.6	0.28	23.7	0.10	8.3	0.30	25.1

[a] *Number of valid replicates.*
[b] *Within-Laboratory includes Within-Run, Between-Run, and Between-Day components.*
[c] *Total includes Within-Run, Between-Run, Between-Day, and Between-Instrument/Operator components.*

b. Multi-site (external) Reproducibility Study

The reproducibility study was performed at three external U.S sites. Nine unique panel members for Genotype A and nine unique panel members for Genotype C covering the quantitation range of the assay (1 to 8.5 Log IU/mL) were formulated in plasma to make an 18-member panel. Each panel member was tested 5 times per test run. The Alinity m System is a random-access analyzer, therefore a run is defined as testing a batch of 5 replicates of each of the 18 panel members consecutively on the system within a day using the same Alinity m HBV assay reagent lots. A total of 3 Alinity m HBV AMP Kit lots were used. Each of the 3 clinical sites tested 2 Alinity m HBV AMP Kit lots, on 5 non-consecutive days for each lot. Each of the 3 clinical sites used a unique lot of Alinity m HBV CAL Kit, Alinity m HBV CTRL Kit, and Alinity m Sample Prep Kit 2. The study design (3 sites x 5 replicates in one-panel x 1 run/day x 5 days x 2 lots/site) accounts for a total of 150 replicates per panel member. The analyses demonstrated that the Alinity m HBV assay standard deviation (SD) was less than or equal to 0.25 Log IU/mL for HBV DNA from 2.29 to 7.98 Log IU/mL, and for near LLoQ to 3x LLoQ SD was less than or equal to 0.35 Log IU/mL. The precision/reproducibility results are acceptable, and the results are summarized in Table 11.

Table 11. Reproducibility of Alinity m HBV assay

Geno-type	N[a]	Mean Conc.(Log IU/mL)	Within-Run/Day Component[a]		Between-Run/Day Component[a]		Within-Laboratory[b]		Between-Lot Component		Between-Site Component		Total[c]	
			SD[d]	%CV	SD[d]	%CV	SD[d]	%CV	SD[d]	%CV	SD[d]	%CV	SD[d]	%CV
A	150	7.98	0.11	1.4	0.02	0.2	0.11	1.4	0.05	0.7	0.04	0.5	0.13	1.6
	150	6.44	0.10	1.6	0.05	0.7	0.11	1.8	0.00	0.0	0.04	0.7	0.12	1.9
	150	5.11	0.13	2.6	0.08	1.6	0.16	3.1	0.06	1.2	0.21	4.0	0.27	5.2
	150	4.15	0.12	2.8	0.08	2.0	0.14	3.5	0.04	0.9	0.12	2.9	0.19	4.6
	150	3.17	0.14	4.4	0.08	2.4	0.16	5.0	0.04	1.3	0.15	4.6	0.22	6.9
	150	2.29	0.15	6.5	0.08	3.3	0.17	7.3	0.06	2.6	0.16	7.1	0.24	10.5
	150	1.74	0.22	12.8	0.09	5.0	0.24	13.7	0.00	0.0	0.14	7.8	0.28	15.8
	146	1.05	0.35	33.7	0.00	0.0	0.35	33.7	0.06	5.9	0.20	19.1	0.41	39.1
	146	1.09	0.27	24.8	0.00	0.0	0.27	24.8	0.09	8.5	0.12	10.6	0.31	28.3
B	150	7.96	0.11	1.4	0.02	0.2	0.11	1.4	0.04	0.5	0.11	1.4	0.16	2.0
	150	6.36	0.12	1.9	0.00	0.0	0.12	1.9	0.04	0.7	0.05	0.9	0.14	2.2
	150	5.03	0.13	2.7	0.06	1.3	0.15	3.0	0.00	0.0	0.06	1.1	0.16	3.2
	150	4.12	0.12	2.9	0.04	1.0	0.12	3.0	0.00	0.0	0.04	0.9	0.13	3.2
	150	3.13	0.11	3.5	0.03	1.0	0.11	3.6	0.00	0.0	0.07	2.1	0.13	4.2
	150	2.27	0.12	5.3	0.01	0.6	0.12	5.3	0.04	1.6	0.10	4.2	0.16	6.9
	150	1.82	0.16	8.5	0.06	3.5	0.17	9.2	0.00	0.0	0.12	6.6	0.21	11.3
	150	1.17	0.19	16.1	0.04	3.8	0.19	16.5	0.00	0.0	0.17	14.5	0.26	22.0
	141	0.85	0.30	35.7	0.08	9.3	0.31	36.9	0.00	0.0	0.20	23.0	0.37	43.5

[a] *Number of valid replicates.*
[b] *Within-Laboratory includes Within-Run, and Between-Run Components.*
[c] *Total includes Within-Run, Between-Run, Between-Lot, and Between-Site Variance Components.*
[d] *SD = Standard deviations in Log IU/mL*

8. Performance with HBV-Negative Specimens

The analytical specificity of Alinity m HBV assay was determined by testing 250 HBV-negative plasma and 250 HBV-negative serum specimens (as determined by an FDA approved HBV Ag/Ab screening, and HBsAg Confirmatory assay) from individual donors. HBV DNA was not detected in any of the 500 specimens tested (specificity 100.0%; 95% CI: 99.2 to 100.0%).

9. Cross-Reactivity of the Alinity m HBV assay with Other Microorganisms

The impact of potential cross-reactivity and/or interference of pathogens in the Alinity m HBV assay was evaluated. The study was designed according to CLSI EP7-A2. HBV-negative or positive plasma specimens were spiked with microorganisms or purified nucleic acid from microorganisms to achieve a final titer of 10^5 units/mL for viruses, protozoans, and yeast or 10^6 CFU/mL for bacteria (Table 12). Cross-reactivity was analyzed using an HBV negative sample and microbial interference was analyzed using an HBV-positive sample at 30 IU/mL (3x LLoQ) and at 2000 IU/mL. Three replicates for each cross reactant were tested.

No cross-reactivity or microbial interference was observed in the presence of the tested microorganisms.

Table 12. Microorganisms

Viruses	**Viruses**
Adenovirus Type 5 (AV5) BK Polyomavirus Dengue Virus 1 (DENV 1) Dengue Virus 2 (DENV 2) Dengue Virus 3 (DENV 3) Dengue Virus 4 (DENV 4) FSME Virus GB Virus C (GBV-C: Hepatitis G Virus, HGV) Hepatitis A Virus (HAV) Hepatitis C Virus (HCV) Hepatitis D Virus (HDV) Human Herpesvirus 1 (HHV-1)/ Herpes Simplex Virus 1 (HSV-1) Human Herpesvirus 2 (HHV-2)/ Herpes Simplex Virus 2 (HSV-2) Human Herpesvirus 5 (HHV-5)/ Human Cytomegalovirus (CMV) Human Herpesvirus 4 (HHV-4)/ Epstein Barr Virus (EBV) Human Herpesvirus 6B (HHV-6B) Human Herpesvirus 8 (HHV8)/ Kaposi Sarcoma Virus Human Immunodeficiency Virus 2 (HIV-2) Human Immunodeficiency Virus 1 (HIV-1) Human Papilloma Virus 16 (HPV-16) Human Papilloma Virus 18 (HPV-18) Human T-Lymphotropic Virus 1 (HTLV-1) Human T-Lymphotropic Virus 2 (HTLV-2) Influenza A	Japanese Encephalitis Murray Valley Encephalitis Virus Parvo Virus B19 Rubella Virus St. Louis Encephalitis Vaccinia Virus (VACV) Varicella-Zoster Virus (VZV) West Nile Virus (WNV) Yellow Fever Virus Zika Virus
	Bacteria
	Chlamydia trachomatis *Corynebacterium diphtheriae* *Mycobacterium gordonae* *Mycobacterium smegmatis* *Neisseria gonorrhoeae* *Propionibacterium acnes* *Staphylococcus aureus* *Staphylococcus epidermidis* *Streptococcus pneumoniae*
	Protozoan
	Trichomonas vaginalis
	Yeast
	Candida albicans

a. Potentially Interfering Substances (Endogenous)

The impact of potentially interfering endogenous substances, the presence of autoimmune disorders, and markers of other diseases on the analytical specificity and detection/quantitation of the Alinity m HBV assay was evaluated by testing spiked samples as well as patient samples with

naturally elevated levels of endogenous substances. Ten donor samples were tested for each interfering substance with one replicate each. As a control, one replicate of each donor sample was also tested without the addition of any potentially interfering endogenous substance. Potential interference was assessed by testing 10 HBV negative samples, and 10 positive samples containing 30 or 2,000 IU/mL HBV DNA, except hepatocellular carcinoma (HCC), for which 4 samples were tested at each HBV concentration.

No interference was observed in the presence of albumin (60 mg/mL), hemoglobin (2 mg/mL), triglycerides (37 mM), conjugated bilirubin (0.342 mM), unconjugated bilirubin (0.342 mM) or human genomic DNA (2 mg/L) that were introduced in the sample. In addition, no interference was observed in specimens collected from individual donors containing the naturally elevated interfering substances, albumin (>5.1 g/dL), bilirubin (>2 mg/dL), hemoglobin (>2 g/L) or triglycerides (> 325 mg/dL).

No interference was observed for specimens collected from patients with the following disease states: systemic lupus erythematosus (SLE), anti-nuclear antibodies (ANA), rheumatoid factor (RF), alcoholic hepatitis, non-alcoholic steatohepatitis (NASH), cirrhosis, auto-immune hepatitis, and hepatocellular carcinoma (HCC). In addition, no interference was observed for specimens collected from patients that have received Influenza and Hepatitis B vaccines.

b. Potentially Interfering Substances (Exogenous)

The impact of potentially interfering drugs commonly prescribed for the treatment of HBV and other related disease states on the performance of Alinity m HBV assay was evaluated. Ten donor samples were tested for each interfering drug pool or single drug with one replicate each. As a control, one replicate of each donor sample was also tested without the addition of any potentially interfering drug compounds. Potential interference was assessed by testing 10 HBV-negative samples, and 10 positive samples containing 30 (3x LLoQ) or 2,000 IU/mL HBV DNA. The drug compounds listed in Table 13 were tested at three times the reported maximum concentration (Cmax) evaluated with and without HBV viral targets.

No interference was observed in the presence of drug compounds tested in pools at a concentration of 3 times the reported Cmax or higher.

Table 13. Drug Compounds

Pools Tested	Drug Compounds
1	Abacavir sulfate, Acetaminophen, Acyclovir, Adefovir, Amitriptyline, Amlodipine, Aspirin, Atazanavir, Atenolol, Atorvastatin, Azithromycin, Celecoxib, Cidofovir, Clarithromycin, Clopidogrel
2	Didanosine, Efavirenz, Entecavir, Fluconazole, Fluoxetine, Ibuprofen, Indinavir, Kaletra (Lopinavir and Ritonavir), Lamivudine, Levofloxacin, Maraviroc, Nelfinavir, Nevirapine, Paroxetine
3	Prednisone, Raltegravir, Ribavirin, Rifamate (Rifampin and Isoniazid), Saquinavir, Sertraline, Stavudine, Stribild (Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir), Bactrim (Sulfamethoxazole and Trimethoprim)
4	Darunavir, Ethambutol, Etravirine, Flucytosine, Fluticasone propionate / Salmeterol xinafoate, Furosemide, Hydrochlorothiazide, Levothyroxine, Rifabutin, Rilpivirine, Simeprevir, Sofosbuvir, Telaprevir, Tenofovir alafenamide, Trazodone, Warfarin, Zalcitabine
5	Fosamprenavir, Keflex (Cephalexin), Metformin, Naproxen, Pyrazinamide
6	Tipranavir
7	Ceftriaxone, Ciprofloxacin, Foscarnet, Lisinopril, Peginterferon alfa-2a, Enfuvirtide, Imipramine
8	Cyclosporine, Telbivudine, Valacyclovir, Valganciclovir, Zidovudine, Amphotericin B, Ganciclovir
9	Acetaminophen, Hydrocodone
10	Biotin

10. Carryover

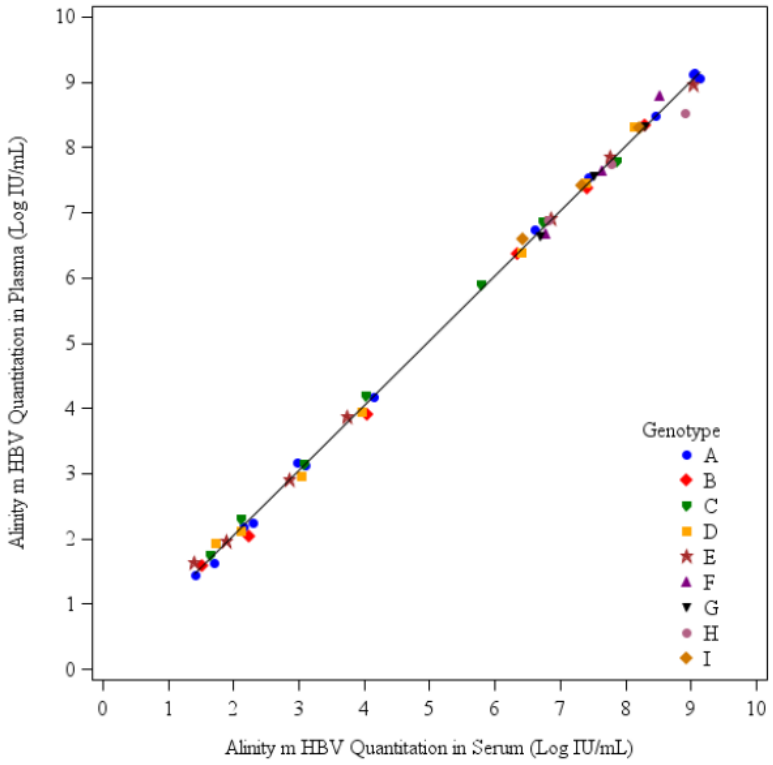
The carryover rate for Alinity m HBV assay was determined by analyzing 360 valid replicates of HBV negative samples processed from alternating positions with 360 valid replicates of high concentrated HBV-positive samples at 100,000,000 IU/mL, across a total of 17 runs. HBV DNA was not detected in any of the HBV- negative samples, resulting in an overall carryover rate of 0.0% (95% CI: 0.0 to 1.1%).

11. Matrix Equivalency

a. Serum/Plasma Equivalency Across the Linear Range

Serum/Plasma matrix equivalency in the Alinity m HBV assay was evaluated by analyzing 30 negative plasma and serum pairs and 53 positive plasma and serum pairs. Negative plasma and serum samples in each pair were collected from the same normal donor (individuals that report no history of HIV or liver disease, such as hepatitis). The positive pairs were prepared by spiking high titer virus (for <5 Log IU/mL samples) or synthetic DNA (for >5 Log IU/mL samples) in serum and plasma matched pairs collected from normal donors. The HBV DNA concentrations for the HBV positive serum/plasma pairs were distributed across the quantitation range of the assay, with the lowest concentration at 1.40 Log IU/mL and the highest concentration at 9.14 Log IU/mL. HBV genotypes A, B, C, D, E, F, G, H, and I were included in the positive samples.

Figure 4: Least Squares Regression Plot with Genotype (Outliers included)



All HBV negative plasma and serum samples were not detected, and all HBV positive plasma and serum samples were detected, resulting in an overall percent agreement between plasma and serum samples of 100.0 % (95% CI: 95.6 to 100.0%).

The least-squares regression analysis of samples including genotypes between serum and plasma demonstrated that the Alinity m HBV Assay has an equivalent performance in reporting quantitative results with serum and plasma since the regression demonstrated a slope of 1.0, intercept of 0.1, r = 1.0, and mean bias of 0.03 Log IU/mL when compared to the results in serum (Figure 4).

b. Specimen and Collection Tube Type Equivalency

To demonstrate equivalent performance between plasma and serum collection tube types, 25 matched sets of HBV negative and positive samples were obtained in each of the following sample collection tubes and evaluated using the Alinity m HBV Assay. For testing, plasma samples were collected in either Di-Potassium Ethylenediaminetetraacetic Acid (K2 EDTA) tubes, Tri-Potassium EDTA (K3 EDTA) tubes, Acid Citrate Dextrose (ACD) tubes, Plasma Preparation Tubes (PPT), and serum samples were collected in serum tubes, Serum Separator Tubes (SST), and serum rapid-clot tubes (both Z-clot and thrombin-clot). For each tube type, an HBV-positive clinical sample was prepared at a concentration of 30 IU/mL (3x LLoQ) or 2000 IU/mL. The paired difference in concentration between the test condition (sample collection tubes except for K2 EDTA tube) and control condition (K2 EDTA tube) was calculated for each donor. The mean and SD of the paired difference were calculated for each test condition.

Plasma (K2 EDTA, K3 EDTA, ACD, and PPT) and serum (serum and SST) collection tubes demonstrated a 100% Not Detected interpretation for HBV-negative samples for each tube type. 100% of the Positive samples were detected at 30 IU/mL (3x LLoQ) and at 2000 IU/mL. For the 30 IU/mL samples' quantification, the mean difference between the test condition and the K2 EDTA control condition across tube types ranged from -0.08 to 0.16 Log IU/mL. For the 2000 IU/mL samples, the 95% confidence interval of the mean difference between each test condition and the K2 EDTA control condition was within [-0.50, 0.50] Log IU/mL with a maximum standard deviation of 0.18. The largest mean difference between the test condition and the control serum tube across all tube types was 0.22 Log IU/mL. The results are summarized in Table 14 below. The matrix and sample collection tube equivalence studies demonstrated acceptable performance.

Table 14: HBV Positive samples tube equivalency summary

Panel	Collection Tube Type[a]	N	Mean Difference[b] (Log IU/mL)	SD	95% CI of the Mean Difference
Low 30 IU/mL	K3 EDTA	25	-0.08	0.236	N/P
	ACD Plasma Tube	25	0.12	0.267	N/P
	Plasma Preparation Tube (PPT)	25	-0.03	0.183	N/P
	Serum Tube	25	0.16	0.216	N/P
	Serum Separator Tube (SST)	25	0.09	0.229	N/P
	Serum Rapid-clot Tube (Z-clot)	25	0.10	0.209	N/P
	Serum Rapid-clot tube (thrombin-clot)	25	0.10	0.184	N/P
High 2000 IU/mL	K3 EDTA	25	-0.00	0.135	(-0.06, 0.05)
	ACD Plasma Tube	25	0.06	0.144	(-0.00, 0.12)
	Plasma Preparation Tube (PPT)	25	-0.00	0.108	(-0.05, 0.04)
	Serum Tube	25	0.04	0.160	(-0.03, 0.10)
	Serum Separator Tube (SST)	25	0.03	0.136	(-0.03, 0.08)
	Serum Rapid-clot Tube (Z-clot)	25	0.04	0.181	(-0.04, 0.11)
	Serum Rapid-clot tube (thrombin-clot)	25	0.02	0.131	(-0.04, 0.07)

[a] *Twenty-five samples from K2 EDTA tubes were used as Control*
[b] *Test condition - control condition, N/P: Not provided*

12. Alinity m HBV assay Testing Using Dilution Procedure

a. Quantitation of Manually Diluted Specimens

The Alinity m HBV assay design provides optional manual dilution procedures for low volume or high viral load specimens (upper limit of quantification). To verify that the Alinity m HBV assay provides accurate quantitation, dilution procedures were evaluated by comparing quantitation of neat specimens and specimens tested using the Alinity m HBV assay dilution procedure of 1:2.5

and 1:50. Specimens were diluted using Alinity m Specimen Dilution Kit I. Ten plasma panel members and 10 serum panel members, consisting of HBV concentrations ranging from 75 to 2,000,000,000 IU/mL. Panel members were prepared by spiking unique HBV negative plasma and serum specimens with a high titer HBV clinical specimen or synthetic HBV DNA stock, to a final target concentration ranging from 1.88 Log IU/mL (75 IU/mL) to 9.30 Log IU/mL (2,000,000,000 IU/mL). Synthetic HBV DNA stock was only used for panel members with high concentrations (≥ 5 Log IU/mL). Panel members were then manually diluted in the Specimen Diluent provided in the Alinity m Specimen Dilution Kit. Dilutions of 1:2.5 and 1: 50 were performed according to the instructions for use. For each HBV panel member, 5 undiluted replicates and 5 replicates diluted in Specimen Diluent were tested and compared. Results are summarized in Table 15A and Table 15B.

For the 10 plasma panel members, the differences between the mean of the diluted samples and the mean of the neat samples ranged from -0.15 to 0.09 Log IU/mL for the 1:2.5 dilution, and from -0.12 to 0.14 Log IU/mL for the 1:50 dilution. For the 10 serum panel members, the differences between the mean of the diluted samples and the mean of the neat samples ranged from -0.04 to 0.27 Log IU/mL for the 1:2.5 dilution, and from -0.18 to 0.13 Log IU/mL for the 1:50 dilution. The observed differences in viral load quantitation between the replicates of the diluted and undiluted samples are negligible (<0.3 Log IU/mL) and clinically insignificant. This study demonstrates that the Alinity m HBV assay can accurately quantify specimens diluted 1:2.5 and 1:50 as recommended in the package insert.

Table 15A: Quantitation of Manual Diluted Plasma Samples

Specimen Type	Dilution Factor	Panel	Test Condition (Diluted)			Control Condition (Undiluted)			Mean Difference (Test - Control)	95% CI of the Mean Difference
			N	Mean (Log IU/mL)	SD	N	Mean (Log IU/mL)	SD		
Plasma	2.5	01	5	2.07	0.133	5	2.22	0.256	-0.15	(-0.45, 0.15)
		02	5	2.25	0.070	5	2.39	0.090	-0.14	(-0.26, -0.02)
		03	5	3.51	0.145	4	3.50	0.066	0.02	(-0.17, 0.20)
		04	5	4.30	0.037	5	4.22	0.049	0.09	(0.02, 0.15)
		05	5	4.56	0.072	5	4.52	0.077	0.04	(-0.07, 0.15)
		06	5	5.33	0.049	5	5.40	0.069	-0.07	(-0.16, 0.02)
		07	5	6.37	0.084	4	6.33	0.037	0.04	(-0.07, 0.15)
		08	5	7.36	0.059	5	7.30	0.049	0.06	(-0.02, 0.14)
		09	5	8.38	0.038	5	8.29	0.030	0.09	(0.04, 0.14)
		10	4	8.62	0.070	5	8.63	0.038	-0.01	(-0.10, 0.08)

Specimen Type	Dilution Factor	Panel	Test Condition (Diluted)			Control Condition (Undiluted)			Mean Difference (Test - Control)	95% CI of the Mean Difference
			N	Mean (Log IU/mL)	SD	N	Mean (Log IU/mL)	SD		
Plasma	50	03	5	3.42	0.124	4	3.50	0.066	-0.07	(-0.23, 0.09)
		04	5	4.15	0.058	5	4.22	0.049	-0.07	(-0.14, 0.01)
		05	5	4.40	0.044	5	4.52	0.077	-0.12	(-0.21, -0.03)
		06	5	5.53	0.080	5	5.40	0.069	0.13	(0.02, 0.23)
		07	5	6.33	0.079	4	6.33	0.037	0.01	(-0.09, 0.11)
		08	5	7.45	0.074	5	7.30	0.049	0.14	(0.05, 0.23)
		09	5	8.37	0.054	5	8.29	0.030	0.08	(0.02, 0.15)
		10	5	8.76	0.073	5	8.63	0.038	0.13	(0.04, 0.22)
		11	5	9.42	0.090	5	9.46	0.064	-0.04	(-0.15, 0.08)
		12	5	9.67	0.011	5	9.77	0.077	-0.10	(-0.20, -0.01)

Table 15B: Quantitation of Manual Diluted Serum Samples

Specimen Type	Dilution Factor	Panel	Test Condition (Diluted)			Control Condition (Undiluted)			Mean Difference(Test - Control)	95% CI of the Mean Difference
			N	Mean (Log IU/mL)	SD	N	Mean (Log IU/mL)	SD		
Serum	2.5	01	5	2.28	0.160	4	2.07	0.090	0.21	(-0.00, 0.43)
		02	5	2.45	0.090	5	2.38	0.134	0.07	(-0.10, 0.24)
		03	5	3.36	0.076	5	3.34	0.078	0.02	(-0.10, 0.13)
		04	5	4.37	0.051	5	4.30	0.078	0.07	(-0.03, 0.17)
		05	5	4.92	0.456	5	4.64	0.108	0.27	(-0.29, 0.83)
		06	5	5.32	0.047	5	5.27	0.109	0.05	(-0.07, 0.17)
		07	5	6.23	0.144	5	6.25	0.075	-0.02	(-0.18, 0.15)
		08	5	7.31	0.075	4	7.35	0.059	-0.04	(-0.15, 0.07)
		09	4	8.37	0.024	5	8.22	0.091	0.14	(0.03, 0.26)
		10	5	8.53	0.023	4	8.51	0.063	0.02	(-0.05, 0.10)

Specimen Type	Dilution Factor	Panel	Test Condition (Diluted)			Control Condition (Undiluted)			Mean Difference(Test - Control)	95% CI of the Mean Difference
			N	Mean (Log IU/mL)	SD	N	Mean (Log IU/mL)	SD		
Serum	50	03	5	3.34	0.182	5	3.34	0.078	-0.01	(-0.21, 0.20)
		04	5	4.12	0.075	5	4.30	0.078	-0.18	(-0.29, -0.07)
		05	5	4.53	0.089	5	4.64	0.108	-0.11	(-0.26, 0.03)
		06	5	5.30	0.063	5	5.27	0.109	0.02	(-0.11, 0.15)
		07	5	6.22	0.056	5	6.25	0.075	-0.02	(-0.12, 0.07)
		08	5	7.34	0.026	4	7.35	0.059	-0.00	(-0.07, 0.06)
		09	5	8.35	0.054	5	8.22	0.091	0.13	(0.02, 0.23)
		10	5	8.58	0.076	4	8.51	0.063	0.07	(-0.04, 0.18)
		11	5	9.32	0.064	5	9.45	0.039	-0.13	(-0.21, -0.05)
		12	5	9.51	0.101	5	9.66	0.104	-0.15	(-0.30, 0.00)

b. On-Board Stability of Diluted Specimens

The onboard/off-board stability of the diluted sample was evaluated in both serum and plasma. For each dilution factor, 12 aliquots of plasma and serum panel members (~20,000 IU/mL) were prepared and stored frozen. The design of this study is as follows. Two test conditions were performed. For Test condition (X1) 4 aliquots were thawed and placed onboard the Alinity m System for a minimum of 4 hours, then diluted in Specimen Diluent, placed off-board (i.e., at 15°C to 30°C) for a minimum of 2 hours, and placed onboard for a minimum of another 4 hours before testing. For Test condition (X2), 4 aliquots per panel member were thawed and placed onboard for a minimum of 4 hours, then diluted in Specimen Diluent and placed onboard for a minimum of another 4 hours before testing. For the undiluted control condition (X3), 4 aliquots were thawed once the X1 aliquots had already been incubating for a minimum of 6 hours and X3 was placed onboard for a minimum of 4 hours before testing. All conditions for each dilution factor were tested in the same run. The results from both test conditions X1 and X2 were compared to the results from the control condition X3.

The difference between the mean quantitation results from testing diluted specimens with onboard/off-board storage (test conditions X1 and X2) and the mean quantitation results from testing undiluted specimens (X3 control condition) across all panel members, dilution factors and matrices ranged from -0.10 Log IU/mL to +0.03 Log IU/mL. The data demonstrated that serum and plasma specimens diluted in specimen diluent may be stored onboard the Alinity m System for 4 hours before testing with the Alinity m HBV assay. The data also demonstrated that diluted

serum and plasma specimens may be stored in specimen diluent for 2 hours at 15°C to 30°C and an additional 4 hours onboard the instrument before testing with the Alinity m HBV assay.

c. Precision of Diluted Specimens

The precision of Alinity m HBV assay, using the dilution procedures, was determined by analyzing 3 panel members prepared by spiking HBV clinical specimen (panel member 1) or synthetic DNA (panel members 2 and 3) in HBV negative human plasma. The panel members were prepared with HBV concentrations, such that when diluted in Specimen Diluent, the concentrations (target concentration in Specimen Diluent) were within the linear range of the Alinity m HBV assay. Each panel member was tested in 5 replicates, twice each day for 12 days, on 3 Alinity m Systems with 3 Specimen Diluent lots by 3 operators (1 Specimen Diluent lot and 1 operator per instrument), for a total of 360 replicates.

The analyses demonstrated that the Alinity m HBV assay has a within-laboratory SD of 0.25 Log IU/mL or less for plasma samples tested using dilution procedures. These results further support the use of dilution procedure as recommended for low sample volume or high viral load HBV samples. The results are summarized in Table 16

Table 16. Precision of Alinity m HBV assay using Dilution Procedures

Panel Member	Dilution Factor	N[a]	Mean Conc. (Log Copies/mL)	Within-Run Component		Between-Run Component		Between-Day Component		Within-Laboratory[b]		Between-Instrument/ Operator/ Diluent Lot Component		Total[c]	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	1:2.5	355	2.96	0.10	3.2	0.07	2.2	0.07	2.5	0.14	4.7	0.08	2.8	0.16	5.4
2	1:50	359	8.43	0.09	1.0	0.01	0.2	0.01	0.2	0.09	1.0	0.07	0.8	0.11	1.3
3	1:50	358	6.04	0.09	1.4	0.02	0.3	0.04	0.7	0.10	1.6	0.07	1.1	0.12	2.0

[a] Number of valid replicates with detectable viral load.
[b] Within-Laboratory includes Within-Run, Between-Run, and Between-Day components
[c] Total includes Within-Run, Between-Run, Between-Day, and Between Instrument/Operator/ Diluent Lot components.

d. Confirmation of the LLoQ in Diluted Specimens

The LLoQ for the Alinity m HBV assay using dilution procedures was confirmed in serum and plasma by testing 2 panel members for each dilution factor, 1:2.5 and 1:50. The HBV concentrations in the panel members were targeted at 10 and 15 IU/mL (1.00 and 1.18 Log IU/mL) after dilution in Specimen Diluent and were traceable to the 3rd WHO International Standard for Hepatitis B Virus for Nucleic Acid Amplification Techniques (NIBSC code: 10/264). A minimum of 14 replicates of each panel member were tested using the dilution procedure in 3 runs across 3 days (one run per day). The study was performed using 1 Alinity m HBV AMP Kit lot, 1 Specimen Diluent lot, and 1 Alinity m System. For each specimen type and each panel member, the detection rate, the mean, standard deviation (SD), bias, Error in Difference of two measurements (TE), and Total Analytical Error (TAE) were calculated. The

accuracy and precision at 10 and 15 IU/mL were confirmed for Alinity m HBV assay testing of serum and plasma using 1:2.5 and 1:50 dilution procedures.

The detection rate for the plasma and serum panel members with the target concentration in Specimen Diluent at expected LLoQ (10 IU/mL) and above the assay's LLoQ (15 IU/mL) was 100% for both dilution factors 2.5 and 50. The TE and TAE for the plasma and serum panel members was <1 Log IU/mL which is acceptable for an HBV viral load at or near the LLoQ. The results are summarized in Table 17.

Table 17. LLoQ Verification for Specimens in Specimen Diluent

Specimen Type	Panel Member	Dilution Factor	Target Conc.in Specimen Diluent (Log IU/mL)	Target Conc. Neat (Log IU/mL)	Tested (N)	Detected (N)	Detection Rate (%)	Mean Conc.[a] (Log IU/mL)	Bias[b] (Log IU/mL)	SD (Log IU/mL)	TAE (Log IU/mL)	TE (Log IU/mL)
Plasma	1	2.5	1.00	1.40	48	48	100	1.44	0.04	0.29	0.62	0.83
	2	2.5	1.18	1.57	47	47	100	1.55	-0.02	0.20	0.42	0.57
	3	50	1.00	2.70	48	48	100	2.67	-0.03	0.24	0.50	0.67
	4	50	1.18	2.88	48	48	100	2.69	-0.19	0.18	0.55	0.51
Serum	1	2.5	1.00	1.40	46	46	100	1.48	0.08	0.31	0.71	0.89
	2	2.5	1.18	1.57	48	48	100	1.55	-0.02	0.27	0.56	0.77
	3	50	1.00	2.70	47	47	100	2.74	0.04	0.21	0.47	0.61
	4	50	1.18	2.88	48	48	100	2.91	0.03	0.14	0.31	0.40

[a] *Reported concentration for undiluted samples.*
[b] *Bias = Mean concentration - Target concentration neat.*

13. Stability Studies

a. Specimen Stability

Specimen stability studies demonstrated that, for the Alinity m HBV Assay, specimens should be stored as indicated in Table 18 and can be stored in a primary or secondary tube.

Table 18: Final Sample Storage Claims

Specimen	Temperature	Max. Storage Time	Special Instructions
Whole Blood	2 to 8°C	0-72 hours	Whole blood may be stored between draw and plasma/serum separation. Whole blood storage plus separated plasma/serum storage at 2 to 8°C must not exceed a combined total of 72 hours.
	15 to 30°C	24 hours	
Plasma/ Serum	2 to 8°C	0-72 hours	Plasma/Serum may be stored in primary tubes (with or without gel) or secondary tubes after separation from blood cells (plasma) or clot (serum). Plasma/Serum may further stay onboard Alinity m System for up to 4 hours prior to processing.
	15 to 30°C	20 hours	
	-20°C	60 days	Plasma/Serum may be stored frozen in primary gel tubes or secondary tubes after separation from blood cells (plasma) or clot (serum). Plasma can be subjected to at most 2 freeze-thaw cycles. The serum can be subjected to at most 3 freeze-thaw cycles. Defrosted samples may be stored at 2 to 8°C for up to 6 hours prior to loading on Alinity m System. Plasma/Serum may further stay onboard Alinity m System for up to 4 hours prior to processing.
	-70°C	Long term	Plasma/Serum may be stored frozen in primary gel tubes or secondary tubes after separation from blood cells (plasma) or clot (serum). Plasma can be subjected to at most 2 freeze-thaw cycles. The serum can be subjected to at most 3 freeze-thaw cycles. Defrosted samples may be stored at 2 to 8°C for up to 6 hours prior to loading on Alinity m System. Plasma/Serum may further stay onboard Alinity m System for up to 4 hours prior to processing.

b. Real-time Reagent Stability (Shelf-life)

Real-time stability studies were performed to establish the shelf-life for the Alinity m HBV assay. Three lots of the reagent kits were stored at the intended storage temperature indicated in Table 19 and then tested at various time points throughout the study. The performance was assessed against clinically relevant acceptance criteria (change in viral load within 0.5 or 0.7 Log IU/mL) using controls and calibrators and an internal stability panel consisting of four panel members between ~3x LLoQ of the Alinity m HBV assay and ~4.5 Log IU/mL.

The Shelf-life study included the assessment of an inverted condition as well as a condition that simulated fluctuating (hot/cold) temperature extremes during shipping.

Study results demonstrate that reagents are stable at their intended storage condition and continue to meet acceptance criteria fifteen months after the date of manufacture including when shipped upon exposure to fluctuating temperature extremes. Shelf-life conditions are summarized in Table 19.

On-Board Storage

The effect of the On-Board Storage (OBS) on reagent performance was assessed by testing one lot of reagents at the maximum on-board (34 days) temperature/humidity conditions allowed by the Alinity m Instrument System (i.e., 30ºC [± 2 ºC], 65% [± 10%] relative humidity [RH]) for the intended OBS of the reagents. Results of the OBS conditions were compared to the results when the reagents were stored at their intended storage condition.

Study results demonstrate that reagents are stable on-board the Alinity m Instrument and continue to meet acceptance criteria for the intended on-board storage time summarized in Table 19.

Table 19: Reagent Shelf Life and On-Board Stability for the Alinity m HBV and Alinity m Accessory Kits

Kit/Accessory	Intended Storage Condition (Shelf Life)	On-Board Storage
HBV AMP Kit	15 months 2°C to 8°C	30 days
HBV CTRL Kit	15 months -15°C to -25°C	4 hours
HBV CAL Kit	15 months -15°C to -25°C	4 hours
Sample Prep Kit 2 (Elution Buffer 2, Microparticles 2)	15 months 15°C to 30°C	10 days
System Solutions (Lysis Solution, Diluent Solution, Vapor Barrier Solution)	15 months 15°C to 30°C	Lysis Solution: 30 days Diluent Solution: 30 days Vapor Barrier Solution: until expiration
Specimen Dilution Kit I	15 months	Not Applicable

Expiration dating for the Alinity m HBV assay has been established and approved at 15 months when reagents are stored at the intended storage conditions.

14. Antimicrobial effectiveness testing

The purpose of the Antimicrobial Effectiveness Testing (AET) is to determine the antimicrobial effectiveness of a preservative system and establish the level of antimicrobial protection provided by the preservative system at 3 months, 12 months, and 25 months. For the Alinity m HBV assay AET is only required for reagents containing preservatives (i.e., Proclin 950). The following reagents containing preservatives were evaluated in this study;

- Alinity m HBV-1 ACT TRAY 2
- Controls and Calibrators
- Alinity m Diluent Solution
- Alinity m Elution Buffer 2

Aliquots of the reagents were inoculated at bioburden concentration levels of 10^5 to 10^6 CFU/mL of 7 bacterial, 2 fungal microbial organisms, and one uninoculated control. Effectiveness of the preservative for each tested organism was classified as cidal, static or neither cidal nor static depending on the microbial count at the testing time point.

This study demonstrated the effectiveness of the preservatives in the preservative containing reagents as cidal (i.e., no growth) at the intended testing duration.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A. Study Design

The applicant performed a clinical study to establish a reasonable assurance of safety and effectiveness of the Alinity m HBV assay for use with the automated Alinity m System in the US. A summary of the clinical study is presented below.

The clinical performance was evaluated by assessing the antiviral therapy response in chronic HBV-infected subjects. The de-identified, remnant specimens tested in this study were previously collected from subjects treated with adefovir dipivoxil or placebo (collected during GS-98-437 and GS-98-438 studies). The relationship between treatment and HBV DNA viral loads at various treatment time points as measured by Alinity m HBV results and clinical responses (histological, bio-chemical and/or serological) was determined in both HBeAg-positive subjects with compensated liver function (GS-98-437) and HBeAg-negative subjects with compensated liver function (GS-98-438). The results were used to determine whether a viral response is informative for determining the response to treatment in HBeAg-positive and HBeAg-negative subjects with chronic hepatitis B infection.

Testing with the Alinity m HBV assay was performed across 3 Alinity m Systems and 3 sites in US, using a total of 4 Alinity m HBV AMP Kit lots. Bar-coded aliquots were provided to each site for testing. Sites were provided with a minimum of one aliquot of each specimen for testing. A total of 412 subjects were included in the study.

B. Study population and baseline demographics

The demographics and baseline clinical characteristics of the test subjects were summarized in Table 20. A majority of the HBeAg-positive subjects in this study were Asian and HBV Genotypes A and C, while a majority of the HBeAg-negative subjects in this study were White and HBV Genotype D.

Table 20. Summary of Subject Demographics and Baseline Characteristics

Characteristic	Category	Summary Statistics	HBeAg Positive	HBeAg Negative	Total
Number of Subjects		n	228	184	412
Treatment Arm	Antiviral	n (%)	168 (73.7%)	123 (66.8%)	291 (70.6%)
	Placebo	n (%)	60 (26.3%)	61 (33.2%)	121 (29.4%)
Total Number of Subjects with Demographic Information		n	219	183	402
Age (Yr)		Median (Min, Max)	34 (16, 65)	46 (18, 65)	40 (16, 65)
Weight (kg)		Median (Min, Max)	71 (43, 118)	75 (46, 135)	73 (43, 135)
Sex	Male	n (%)	164 (74.9%)	151 (82.5%)	315 (78.4%)
	Female	n (%)	55 (25.1%)	32 (17.5%)	87 (21.6%)
Race	Asian	n (%)	128 (58.4%)	56 (30.6%)	184 (45.8%)
	White	n (%)	80 (36.5%)	122 (66.7%)	202 (50.2%)
	Other	n (%)	11 (5.0%)	5 (2.7%)	16 (4.0%)
Genotype	A	n (%)	64 (29.2%)	11 (6.0%)	75 (18.7%)
	B	n (%)	40 (18.3%)	31 (16.9%)	71 (17.7%)
	C	n (%)	82 (37.4%)	24 (13.1%)	106 (26.4%)
	D	n (%)	27 (12.3%)	114 (62.3%)	141 (35.1%)
	Other	n (%)	6 (2.7%)	3 (1.6%)	9 (2.2%)
Number of Subjects with Knodell Score		n	198	163	361
Total Knodell Score		Mean (SD)	9.38 (3.29)	9.26 (3.30)	9.33 (3.29)
Necro-inflammatory Score		Mean (SD)	7.70 (2.73)	7.42 (2.71)	7.57 (2.72)
Fibrosis Score		Mean (SD)	1.68 (1.09)	1.85 (1.15)	1.75 (1.12)

From these subjects, a total of 1830 specimens (from each subject at multiple time points) were tested, of which 1821 specimens were included in analysis. A Summary of the treatment arm subjects and associated specimens is presented in Table 21.

Table 21. Summary of Subjects and Specimens.

Population	Total Subjects	Subjects-Placebo	Subjects-Antiviral	Total Specimens[a]
HBeAg-Positive	228	60	168	949
HBeAg-Negative	184	61	123	872
Overall	412	121	291	1821

[a] *Number of specimens included in the analysis of clinical performance.*

Within-Subject Variability in Absence of Treatment

The objective of this study was to assess the change in viral load (in Log IU/mL) between two successive time points (baseline and Week 12) in placebo subjects as assessed by the Alinity m HBV assay. In the placebo arm, there were fifty-one HBeAg-negative and fifty-one HBeAg-positive subjects that had available results for both weeks 0 and 12. These results were used to estimate within-subject variability. As shown in Table 22 within-subject variability in the absence of treatment is < 2 Log IU/mL.

Table 22. Within Subject Variability

Study Population	N	Within-Subject Variability (Log IU/mL)	Median difference (Week 12 - Week 0)	Percent with <2 Log Change [a]
HBeAg Positive	51	0.72	-0.01	90.2%
HBeAg Negative	51	0.74	-0.17	88.2%

[a] *Percentage of subjects with the viral load change (absolute difference) of less than 2 Log IU/mL between Week 12 and Week 0.*

C. Safety and Effectiveness Results

Safety results

The analysis of safety was based on the tested cohort of 412 patients in the Patient Management Study available for evaluation. There were no adverse effects during the study

Effectiveness results

The analysis of effectiveness was based on the tested cohort of 412 patients in the Patient Management Study. Key effectiveness outcomes are presented below.

Determination of Response to Antiviral Treatment (Clinical Utility)

Alinity m HBV assay testing was performed using specimens collected at baseline and at weeks 12, 24, and 48 during treatment. The HBV viral load results were evaluated against histologic, biochemical, and serological responses at Week 48. Data from HBeAg-positive and HBeAg-negative subjects were analyzed separately.

Clinical Response Definitions:

Viral Response:

- HBV DNA <2000 IU/mL at weeks 12, 24 and 48

Alternate Response:

- HBV DNA ≥2 Log IU/mL decrease from baseline at weeks 12, 24 and 48

Clinical Response at week 48:

- Histologic response: Improvement of histologic status by at least 2 units of the Knodell necro-inflammatory score without deterioration of the fibrosis score compared to the histologic status at baseline
- Biochemical response: Normalization of ALT test result compared to the biochemical status at baseline
- HBeAg Loss: HBeAg undetectable
- Anti-HBe Gain: Antibody against HBeAg detected
- Seroconversion: HBeAg undetectable and antibody against HBeAg detected

Measures of association, Predictive Value and Odds Ratios:

- Positive Predictive Value (PPV) = True Positive/(True Positive + False Positive) or the probability of clinical response at various time points given the presence of viral response.
- Negative Predictive Value (NPV) = True Negative/(False Negative + True Negative) or the probability of absence of clinical response at various time points given the absence of viral response.
- Odds Ratio (OR) = (True Positive × True Negative)/(False Positive × False Negative)

Statistical Analysis:

Viral load response was defined as either an HBV DNA viral load less than 2000 IU/mL or greater than or equal to 2 Log IU/mL decrease from baseline. Statistical analysis (PPV) was performed to evaluate the association between clinical responses at Weeks 48 and a viral load response at Weeks 12, 24, or 48. Statistical analysis (NPV) was performed to evaluate whether there is an association between the clinical non-responses at Weeks 48 and a viral load non-response at Weeks 12, 24, or 48.

Acceptance criteria:

HBeAg-positive subjects: Odds Ratio from at least one of the following analyses shall be statistically significant (lower bound of two-sided 95% CI >1) : Association between the viral response (<2000 IU/mL and/or ≥2 Log decrease) at week 12, 24 or 48 and at least one of clinical responses at week 48 should be demonstrated.

HBeAg-Positive Subjects:

Table 23 summarizes the associations of the baseline covariates (Race, Sex, Age and Genotype) with clinical responses to treatment (histological, biochemical, HBeAg Loss, Anti-HBe Gain, and Seroconversion) at Week 48.

Table 23. Association Between Baseline Covariates and Clinical Responses to treatment at Week 48 for HBeAg-Positive Subjects.

Clinical Response to Treatment	Covariate	Category	N	No. of Subjects with Response	Proportion (%) of Subjects with Response	Unadjusted Odds Ratio (95%CI)
Histologic	Race	Asian	66	43	65.2%	1.59 (0.70 ,3.61)
		Other	50	27	54.0%	
	Sex	Male	92	56	60.9%	1.11 (0.40 ,3.03)
		Female	24	14	58.3%	
	Age	<= 30	45	29	64.4%	1.33 (0.58 ,3.10)
		> 30	71	41	57.7%	
	Genotype	B or C	64	42	65.6%	1.64 (0.72 ,3.71)
		Other	52	28	53.8%	
Biochemical	Race	Asian	72	42	58.3%	1.72 (0.78 ,3.82)
		Other	49	22	44.9%	
	Sex	Male	95	50	52.6%	0.95 (0.36 ,2.48)
		Female	26	14	53.8%	
	Age	<= 30	50	30	60.0%	1.63 (0.74 ,3.63)
		> 30	71	34	47.9%	
	Genotype	B or C	70	41	58.6%	1.72 (0.78 ,3.80)
		Other	51	23	45.1%	
HBeAg Loss	Race	Asian	74	19	25.7%	0.83 (0.35 ,2.00)
		Other	51	15	29.4%	
	Sex	Male	97	25	25.8%	0.73 (0.27 ,2.09)
		Female	28	9	32.1%	
	Age	<= 30	52	12	23.1%	0.70 (0.28 ,1.68)
		> 30	73	22	30.1%	
	Genotype	B or C	71	17	23.9%	0.69 (0.29 ,1.64)
		Other	54	17	31.5%	
Anti-HBe Gain	Race	Asian	74	7	9.5%	0.43 (0.13 ,1.37)
		Other	51	10	19.6%	
	Sex	Male	97	14	14.4%	1.41 (0.35 ,8.21)
		Female	28	3	10.7%	
	Age	<= 30	52	6	11.5%	0.74

Clinical Response to Treatment	Covariate	Category	N	No. of Subjects with Response	Proportion (%) of Subjects with Response	Unadjusted Odds Ratio (95%CI)
		> 30	73	11	15.1%	(0.21 ,2.37)
	Genotype	B or C	71	5	7.0%	0.27 (0.07 ,0.89)
Seroconversion	Race	Asian	74	7	9.5%	0.43 (0.13 ,1.37)
		Other	51	10	19.6%	
	Sex	Male	97	14	14.4%	1.41 (0.35 ,8.21)
		Female	28	3	10.7%	
	Age	<= 30	52	6	11.5%	0.74 (0.21 ,2.37)
		> 30	73	11	15.1%	
	Genotype	B or C	71	5	7.0%	0.27 (0.07 ,0.89)
		Other	54	12	22.2%	

The statistical significance of the associations of Race, Sex, Age, and Genotype covariates with viral response was studied and the results are summarized in Table 23 and Table 24. All lower limits of the 95% confidence intervals in these two tables are less than 1, except for Race and Genotype at Weeks 12, 24, and 48 (when a response is defined as <2000 IU/mL). This is in agreement with logistic regression analyses resulting in no statistically significant (P>0.05) associations between the four covariates and viral load.

The associations of the baseline covariates (Race, Sex, Age, and Genotype) with a viral response to treatment (unadjusted OR) are summarized in Table 24 for a viral response of <2000 IU/mL.

Table 24. Association Between Baseline Covariates and Viral Response to Treatment (<2000 IU/mL) at Weeks 12, 24, and 48 for HBeAg Positive Subjects.

Week	Covariate	Category	N	No. with < 2,000 IU/mL	Proportion (%) with < 2,000 IU/mL	Unadjusted Odds Ratio (95% CI)
12	Race	Asian	81	17	21.0%	2.87 (0.93 ,10.54)
		Other	59	5	8.5%	
	Sex	Male	107	16	15.0%	0.79 (0.26 ,2.72)
		Female	33	6	18.2%	
	Age	<= 30	58	7	12.1%	0.61 (0.20 ,1.75)
		> 30	82	15	18.3%	
	Genotype	B or C	77	16	20.8%	2.49 (0.85 ,8.28)
		Other	63	6	9.5%	
24	Race	Asian	76	26	34.2%	3.64 (1.36 ,10.78)
		Other	56	7	12.5%	
	Sex	Male	100	23	23.0%	0.66 (0.25 ,1.79)
		Female	32	10	31.3%	
	Age	<= 30	57	14	24.6%	0.96 (0.40 ,2.28)
		> 30	75	19	25.3%	
	Genotype	B or C	73	25	34.2%	3.32

		Other	59	8	13.6%	(1.28 ,9.29)
48	Race	Asian	74	33	44.6%	2.35 (1.02 ,5.60)
		Other	51	13	25.5%	
	Sex	Male	97	34	35.1%	0.72 (0.28 ,1.88)
		Female	28	12	42.9%	
	Age	<= 30	52	18	34.6%	0.85 (0.38 ,1.90)
		> 30	73	28	38.4%	
	Genotype	B or C	71	31	43.7%	2.01 (0.89 ,4.66)

All lower limits of the 95% confidence intervals in Table 25 are smaller than 1 (when a viral response is defined as ≥ 2 log decrease). When the response is defined as ≥ 2 log decrease, logistic regression analyses resulted in only Sex at Week 12 showing a borderline statistically significant association (p = 0.0383) with viral load. Generally, the virological responses at Weeks 12, 24, and 48 do not appear to be correlated with Race, Sex, Age, and HBV Genotype.

The associations of the baseline covariates (Race, Sex, Age and Genotype) with a viral response to treatment (unadjusted OR) are summarized in Table 25 for a viral response of ≥ 2 Log IU/mL decrease from baseline.

Table 25. Association Between Baseline Covariates and Viral Response to Treatment (≥ 2 Log Decrease) at Weeks 12, 24, and 48 for HBeAg Positive Subjects.

Week	Covariate	Category	N	No. With ≥ 2 Log Decrease	Proportion (%) With ≥ 2 Log Decrease	Unadjusted Odds Ratio (95% CI)
12	Race	Asian	81	43	53.1%	1.02 (0.49 ,2.11)
		Other	59	31	52.5%	
	Sex	Male	107	51	47.7%	0.40 (0.15 ,0.97)
		Female	33	23	69.7%	
	Age	<= 30	58	33	56.9%	1.32 (0.64 ,2.75)
		> 30	82	41	50.0%	
	Genotype	B or C	77	40	51.9%	0.92 (0.45 ,1.89)
		Other	63	34	54.0%	
24	Race	Asian	76	47	61.8%	0.71 (0.32 ,1.56)
		Other	56	39	69.6%	
	Sex	Male	100	61	61.0%	0.44 (0.15 ,1.18)
		Female	32	25	78.1%	
	Age	<= 30	57	37	64.9%	0.98 (0.45 ,2.16)
		> 30	75	49	65.3%	
	Genotype	B or C	73	45	61.6%	0.71 (0.32 ,1.55)
		Other	59	41	69.5%	
48	Race	Asian	74	50	67.6%	1.14 (0.50 ,2.57)
		Other	51	33	64.7%	
	Sex	Male	97	60	61.9%	0.35 (0.10 ,1.07)

		Female	28	23	82.1%	
	Age	<= 30	52	36	69.2%	1.24 (0.55 ,2.87)
		> 30	73	47	64.4%	
	Genotype	B or C	71	47	66.2%	0.98 (0.43 ,2.21)

Viral Response <2000 IU/mL:

The associations between individual clinical responses at Week 48 and viral response (<2000 IU/mL) at Weeks 12, 24, and 48 are summarized in Table 26. The PPV is the highest for the association of viral response and the histologic and biochemical responses while NPV is the highest for the association of viral response and the serological responses (HBeAg loss, anti-HBe gain, and seroconversion).

The viral response at Weeks 12, 24, and 48 was informative for determining Week 48 clinical responses depending on clinical response type and week of viral response as indicated by the lower bound of 95% CI for OR greater than 1.00. Viral response at Week 24 is informative in predicting histologic improvement at Week 48. Viral response at Week 48 is also informative in predicting anti-HBe gain and seroconversion at Week 48 of treatment.

Table 26. PPV, NPV, and Odds Ratio (OR) for Individual Clinical Responses During Treatment at Week 48 Associated with Viral Response (<2000 IU/mL) in HBeAg-Positive Subjects.

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV %		NPV %		OR
			Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	48	Histologic	80.0(55.7, 93.4)	16/20	43.2(32.8, 54.2)	38/88	3.04(0.87, 13.39)
		Biochemical	81.0(57.4, 93.7)	17/21	54.3(43.7, 64.7)	50/92	5.06(1.47, 21.98)
		HBeAg Loss	57.1(34.4, 77.4)	12/21	78.1(68.3, 85.7)	75/96	4.76(1.57, 14.52)
		Anti-HBe Gain	38.1(19.0, 61.3)	8/21	90.6(82.5, 95.4)	87/96	5.95(1.64, 20.74)
		Seroconversion	38.1 (19.0, 61.3)	8/21	90.6(82.5, 95.4)	87/96	5.95(1.64, 20.74)
24	48	Histologic	83.3(64.5, 93.7)	25/30	49.3(37.7, 61.0)	37/75	4.87(1.58, 17.79)
		Biochemical	87.1(69.2, 95.8)	27/31	59.0(47.3, 69.8)	46/78	9.70(2.92, 41.02)
		HBeAg Loss	64.5(45.4, 80.2)	20/31	86.6(76.8, 92.8)	71/82	11.74(4.02, 34.82)
		Anti-HBe Gain	29.0(14.9, 48.2)	9/31	92.7(84.2, 97.0)	76/82	5.18(1.44, 19.45)
		Seroconversion	29.0(14.9, 48.2)	9/31	92.7(84.2, 97.0)	76/82	5.18(1.44, 19.45)
48	48	Histologic	73.3(57.8, 84.9)	33/45	47.9(36.0, 60.0)	34/71	2.53(1.06, 6.24)
		Biochemical	78.3(63.2, 88.5)	36/46	62.7(50.7, 73.3)	47/75	6.04(2.44, 15.63)
		HBeAg Loss	65.2(49.7, 78.2)	30/46	94.9(86.9, 98.4)	75/79	35.16(10.03, 150.23)
		Anti-HBe Gain	32.6(20.0, 48.1)	15/46	97.5(90.3, 99.6)	77/79	18.63(3.88, 173.08)
		Seroconversion	32.6(20.0, 48.1)	15/46	97.5(90.3, 99.6)	77/79	18.63(3.88, 173.08)

[a] *Bold indicates statistical significance (lower bound of 95% CI >1.00).*

The following two tables (Table 27 and Table 28) demonstrate that the NPV is high (>86% for Week 48 of clinical response) for the associations between viral response with the combination of

all three responses (histologic, biochemical, and HBeAg loss or seroconversion). These data indicate that the associations between clinical responses (histologic, biochemical, HBeAg Loss, Anti-HBe Gain or Seroconversion) at Week 48 and viral response (<2000 IU/mL) as measured by Alinity m HBV assay at Weeks 24 or 48 were informative for determining Week 48 clinical response HBeAg Loss or seroconversion, where the lower bound of 95% CI for OR was greater than 1.00.

Table 27. PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and HBeAg Loss Responses During Treatment at Week 48, Associated with Viral Response (<2000 IU/mL) in HBeAg-Positive Subjects.

Week of Viral Response	Week of Clinical Response	PPV %		NPV %		OR
		Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	48	35.0 (16.3,59.1)	7/20	86.2 (76.8,92.4)	75/87	3.37 (0.93,11.37)
24	48	43.3 (26.0,62.3)	13/30	94.6 (86.0,98.3)	70/74	**13.38 (3.46,61.47)**
48	48	37.8 (24.2,53.5)	17/45	97.1 (89.1,99.5)	68/70	**20.64 (4.33,190.61)**

[a] *Bold indicates statistical significance (lower bound of 95% CI > 1.00).*

Table 28. PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and Seroconversion Responses During Treatment at Week 48, Associated with Viral Response (<2000 IU/mL) in HBeAg-Positive Subjects.

Week of Viral Response	Week of Clinical Response	PPV %		NPV %		OR
		Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI) [a]
12	48	25.0 (9.6,49.4)	5/20	96.6 (89.5,99.1)	84/87	**9.33 (1.57,64.43)**
24	48	20.0 (8.4,39.1)	6/30	98.6 (91.7,99.9)	73/74	**18.25**
48	48	15.6 (7.0,30.1)	7/45	98.6 (91.2,99.9)	69/70	**12.71**

[a] *Bold indicates statistical significance (lower bound of 95% CI > 1.00).*

Viral Response ≥ 2 Log IU/mL Decrease:

The associations between individual clinical responses at Week 48 and viral response (≥ 2 Log IU/mL decrease) at Weeks 12, 24, and 48 are summarized in Table 29. High NPV (>83.6%) was observed for the association of viral response and the serological responses (HBeAg loss, anti-HBe gain, and seroconversion).

The viral response at Weeks 12, 24, and 48 was informative for determining Week 48 clinical responses depending on clinical response type and week of viral response, as indicated by the lower bound of 95% CI for OR greater than 1.00 (indicated in bold).

Table 29. PPV, NPV, and Odds Ratio (OR) for Individual Clinical Responses During Treatment at Week 48, Associated with Viral Response (≥ 2 Log IU/mL Decrease) in HBeAg-Positive Subjects.

Week of Viral Response	Clinical Response	PPV %		NPV %		OR
		Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	Histologic	69.5 (56.0, 80.5)	41/59	49.0 (34.6, 63.5)	24/49	2.19 (0.93, 5.19)
	Biochemical	63.9 (50.6, 75.5)	39/61	61.5 (47.0, 74.4)	32/52	**2.84 (1.24, 6.55)**
	HBeAg Loss	38.7 (26.9, 52.0)	24/62	83.6 (70.7, 91.8)	46/55	**3.23 (1.25, 8.80)**
	Anti-HBe Gain	19.4 (10.8, 31.7)	12/62	90.9 (79.3, 96.6)	50/55	2.40 (0.72, 9.29)
	Seroconversion	19.4 (10.8, 31.7)	12/62	90.9 (79.3, 96.6)	50/55	2.40 (0.72, 9.29)
24	Histologic	66.2 (53.9, 76.7)	47/71	52.9 (35.4, 69.8)	18/34	2.20 (0.88, 5.51)
	Biochemical	66.7 (54.5, 77.1)	48/72	70.3 (52.8, 83.6)	26/37	**4.73 (1.86, 12.35)**
	HBeAg Loss	40.5 (29.5, 52.6)	30/74	97.4 (84.9, 99.9)	38/39	25.91 (3.84, 1084.22)
	Anti-HBe Gain	20.3 (12.2, 31.5)	15/74	100.0 (88.8, 100.0)	39/39	>9.66[b]
	Seroconversion	20.3 (12.2, 31.5)	15/74	100.0 (88.8, 100.0)	39/39	>9.66[b]
48	Histologic	66.7 (55.0, 76.7)	52/78	52.6 (36.0, 68.7)	20/38	2.22 (0.93, 5.29)
	Biochemical	66.7 (55.2, 76.5)	54/81	75.0 (58.5, 86.8)	30/40	**6.00 (2.39, 15.67)**
	HBeAg Loss	41.0 (30.5, 52.3)	34/83	100.0 (89.6, 100.0)	42/42	>28.45[b]
	Anti-HBe Gain	20.5 (12.7, 31.0)	17/83	100.0 (89.6, 100.0)	42/42	>10.56[b]
	Seroconversion	20.5 (12.7, 31.0)	17/83	100.0 (89.6, 100.0)	42/42	>10.56[b]

[a] *Bold indicates statistical significance (lower bound of 95% CI >1.00).*

[b] *The odds ratio calculations are undefined when NPV is 100% or PPV is 100%. Where the denominators for both the NPV and PPV are greater than 5, a minimum odds ratio was determined by subtracting one specimen from the numerator of the 100% parameter estimate (NPV or PPV).*

The following two tables (Table 30 and Table 31) demonstrate that the NPV is very high (greater than or equal to 91.7% for clinical response at Week 48) for the association of viral response with the combination of all three responses (histologic, biochemical, and serological - HBeAg loss or seroconversion). These data indicate that the associations between clinical responses (histologic, biochemical, HBeAg Loss, Anti-HBe Gain or Seroconversion) at Week 48 and viral response (defined as ≥ 2 log IU/mL decrease) as measured by Alinity m HBV assay at Weeks 12, 24 or 48 were informative for determining Week 48 clinical response HBeAg Loss or seroconversion, where the lower bound of 95% CI for OR was greater than 1.00.

Table 30. PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and HBeAg Loss Responses During Treatment at Week 48, Associated with Viral Response (≥ 2 Log IU/mL Decrease) in HBeAg-Positive Subjects.

Week of Viral Response	PPV %		NPV %		OR
	Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	25.4 (15.4, 38.7)	15/59	91.7 (79.1, 97.3)	44/48	**3.75 (1.07, 16.57)**
24	24.3 (15.2, 36.3)	17/70	100.0 (87.4, 100.0)	34/34	>10.58[b]
48	24.7 (15.9, 36.0)	19/77	100.0 (88.6, 100.0)	38/38	>12.12[b]

[a] *Bold indicates statistical significance (lower bound of 95% CI >1.00).*
[b] *The odds ratio calculations are undefined when NPV is 100% or PPV is 100%. Where the denominators for both the NPV and PPV are greater than 5, a minimum odds ratio was determined by subtracting one specimen from the numerator of the 100% parameter estimate (NPV or PPV).*

The associations between the combined clinical responses based on positive histologic, positive biochemical and positive seroconversion responses at Week 48 and viral response (≥ 2 Log decrease) at Weeks 12, 24, and 48 are summarized in Table 31.

Table 31. PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and Seroconversion Responses During Treatment at Week 48, Associated with Viral Response (≥ 2 Log IU/mL Decrease) in HBeAg-Positive Subjects

Week of Viral Response	PPV %		NPV %		OR
	Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)
12	11.9 (5.3, 23.5)	7/59	97.9 (87.5, 99.9)	47/48	6.33 (0.76, 291.09)
24	10.0 (4.5, 20.1)	7/70	100.0 (87.4, 100.0)	34/34	>3.67[a]
48	10.4 (4.9, 20.0)	8/77	100.0 (88.6, 100.0)	38/38	>4.29[a]

[a] *The odds ratio calculations are undefined when NPV is 100% or PPV is 100%. Where the denominators for both the NPV and PPV are greater than 5, a minimum odds ratio was determined by subtracting one specimen from the numerator of the 100% parameter estimate (NPV or PPV).*

HBeAg-Negative Subjects

The following Table 32 summarizes the associations of the baseline covariates (Race, Sex, Age, and Genotype) with clinical responses to treatment (histological and biochemical,) at Week 48.

Table 32. Association Between Baseline Covariates and Clinical Responses to Treatment at Week 48 for HBeAg-Negative Subjects.

Clinical Response to Treatment	Covariate	Category	N	No. of Subjects with Response	Proportion (%) of Subjects with Response	Unadjusted Odds Ratio (95%CI)
Histologic	Race	Asian	28	16	57.1%	0.43 (0.15 ,1.23)
		Other	62	47	75.8%	
	Sex	Male	75	54	72.0%	1.71 (0.44 ,6.16)
		Female	15	9	60.0%	
	Age	<= 30	3	3	100.0%	*
		> 30	87	60	69.0%	
	Genotype	B or C	28	16	57.1%	0.43 (0.15 ,1.23)
		Other	62	47	75.8%	
Biochemical	Race	Asian	26	19	73.1%	0.75 (0.24 ,2.54)
		Other	65	51	78.5%	
	Sex	Male	77	57	74.0%	0.22 (<0.01 ,1.66)
		Female	14	13	92.9%	
	Age	<= 30	3	3	100.0%	*
		> 30	88	67	76.1%	
	Genotype	B or C	26	19	73.1%	0.75 (0.24 ,2.54)
		Other	65	51	78.5%	

[*] *Undefined (division by zero)*

Statistical significance of the associations of Race, Sex, Age, and Genotype covariates with viral response was studied and the results are summarized in the Table 32 and Table 33. All lower limits of the 95% confidence intervals in these two tables are smaller than 1. This is in concordance with logistic regression analyses of viral response as a function of covariates indicating no statistically significant associations between the four covariates and viral load. Therefore, the virological responses at Weeks 12, 24, and 48 do not appear to be correlated with Race, Sex, Age and HBV Genotype.

The associations of baseline covariates (Race, Sex, Age, and Genotype) with viral response to treatment (unadjusted OR) are summarized in Table 33 for a viral response of <2000 IU/mL.

Table 33. Association Between Baseline Covariates and Viral Response to Treatment (<2000 IU/mL) at Weeks 12, 24, and 48 for HBeAg-Negative Subjects.

Week	Covariate	Category	N	No. with <2,000 IU/mL	Proportion (%) with < 2,000 IU/mL	Unadjusted Odds Ratio (95% CI)
12	Race	Asian	30	20	66.7%	2.19 (0.82, 6.07)
		Other	65	31	47.7%	
	Sex	Male	82	45	54.9%	1.42 (0.37, 5.58)
		Female	13	6	46.2%	
	Age	<= 30	6	3	50.0%	0.85 (0.11, 6.74)
		> 30	89	48	53.9%	
	Genotype	B or C	30	20	66.7%	2.19 (0.82, 6.07)
		Other	65	31	47.7%	
24	Race	Asian	30	22	73.3%	1.14 (0.40, 3.48)
		Other	65	46	70.8%	
	Sex	Male	79	58	73.4%	1.66 (0.44, 5.77)
		Female	16	10	62.5%	
	Age	<= 30	6	5	83.3%	2.06 (0.21, 101.30)
		> 30	89	63	70.8%	
	Genotype	B or C	30	22	73.3%	1.14 (0.40, 3.48)
		Other	65	46	70.8%	
48	Race	Asian	30	25	83.3%	1.94 (0.60, 7.39)
		Other	68	49	72.1%	
	Sex	Male	81	63	77.8%	1.91 (0.50, 6.58)
		Female	17	11	64.7%	
	Age	<= 30	3	3	100.0%	*
		> 30	95	71	74.7%	
	Genotype	B or C	30	25	83.3%	1.94 (0.60, 7.39)
		Other	68	49	72.1%	

[*] *Undefined (division by zero).*

Associations of baseline covariates (Race, Sex, Age and Genotype) with viral response to treatment (unadjusted OR) are summarized in Table below for a viral response of ≥ 2 Log IU/mL decrease from baseline. All lower limits of the 95% confidence intervals in Table 34 are smaller than 1 (when a viral response is defined as ≥ 2 log decrease). Generally, the virological responses at Weeks 12, and 24 do not appear to be correlated with Race, Sex, Age, and HBV Genotype.

Table 34. Association Between Baseline Covariates and Viral Response to Treatment (≥ 2 Log Decrease) at Weeks 12, 24, and 48 for HBeAg-Negative Subjects.

Week	Covariate	Category	N	No. With ≥ 2 Log Decrease	Proportion (%) With ≥ 2 Log Decrease	Unadjusted Odds Ratio (95% CI)
12	Race	Asian	30	20	66.7%	0.55 (0.19 ,1.63)
		Other	65	51	78.5%	
	Sex	Male	82	61	74.4%	0.87 (0.14 ,3.84)
		Female	13	10	76.9%	
	Age	<= 30	6	5	83.3%	1.74 (0.18 ,85.94)
		> 30	89	66	74.2%	
	Genotype	B or C	30	20	66.7%	0.55 (0.19 ,1.63)
		Other	65	51	78.5%	
24	Race	Asian	30	24	80.0%	0.91 (0.27 ,3.31)
		Other	65	53	81.5%	
	Sex	Male	79	64	81.0%	0.98 (0.16 ,4.27)
		Female	16	13	81.3%	
	Age	<= 30	6	5	83.3%	1.18 (0.12 ,59.08)
		> 30	89	72	80.9%	
	Genotype	B or C	30	24	80.0%	0.91 (0.27 ,3.31)
		Other	65	53	81.5%	
48	Race	Asian	30	25	83.3%	0.86 (0.24 ,3.56)
		Other	68	58	85.3%	
	Sex	Male	81	68	84.0%	0.70 (0.07 ,3.63)
		Female	17	15	88.2%	
	Age	<= 30	3	3	100.0%	*
		> 30	95	80	84.2%	
	Genotype	B or C	30	25	83.3%	0.86 (0.24 ,3.56)
		Other	68	58	85.3%	

Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Odds Ratio (OR) Analysis for HBeAg-Negative Patients.

For each patient, two responses - histologic and biochemical were measured at various times during treatment. Also, Viral load response was defined as either HBV DNA less than 2000 IU/mL or greater than or equal to 2 Log IU/mL decrease from baseline. Statistical analysis (PPV) was performed to evaluate the association between the clinical responses at Weeks 48 and a viral load response at Weeks 12, 24, or 48. Statistical analysis (NPV) was performed to evaluate whether there is an association between the clinical non-responses at Weeks 48 and a viral load non-response at Weeks 12, 24, or 48.

Viral Response <2000 IU/mL:

The associations between individual clinical responses at Week 48 and viral response (<2000 IU/mL from the baseline viral load result) at Weeks 12, 24, and 48 are summarized in Table 35. The PPV is the highest for the association of viral response and individual clinical responses (histologic and biochemical). Thus, the viral response was informative for determining Week 48 clinical responses depending on clinical response type and week of viral response, as indicated by the lower bound of 95% CI for OR greater than 1.00.

Table 35. PPV, NPV, and Odds Ratio (OR) for Individual Clinical Responses During Treatment at Week 48, Associated with Viral Response (<2000 IU/mL) in HBeAg-Negative Subjects.

Week of Viral Response	Clinical Response	PPV %		NPV %		OR
		Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	Histologic	73.7 (56.6, 86.0)	28/38	33.3 (19.6, 50.3)	13/39	1.40 (0.47, 4.23)
	Biochemical	79.5 (63.1, 90.1)	31/39	25.0 (13.2, 41.5)	10/40	1.29 (0.40, 4.32)
24	Histologic	75.0 (61.4, 85.2)	42/56	39.1 (20.5, 61.2)	9/23	1.93 (0.59, 6.05)
	Biochemical	79.6 (66.1, 88.9)	43/54	33.3 (16.4, 55.3)	8/24	1.95 (0.57, 6.47)
48	Histologic	72.9 (60.7, 82.5)	51/70	42.1 (21.1, 66.0)	8/19	1.95 (0.58, 6.27)
	Biochemical	86.4 (75.2, 93.2)	57/66	50.0 (29.6, 70.4)	12/24	**6.33 (1.92, 21.01)**

[a] *Bold indicates statistical significance (lower bound of 95% CI >1.00).*

The associations between the combined clinical responses based on positive histologic and positive biochemical responses at Week 48 and viral response (<2000 IU/mL) at Weeks 12, 24, and 48 are summarized in Table 36.

The viral response (<2000 IU/mL) as measured by Alinity m HBV assay at Week 48 was informative for determining Week 48 clinical responses (histologic and biochemical), where the lower bound of 95% CI for OR was greater than 1.00.

Table 36. PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic and Biochemical Responses During Treatment at Week 48, Associated with Viral Response (<2000 IU/mL) in HBeAg-Negative Subjects

Week of Viral Response	Week of Clinical Response	PPV %		NPV %		OR
		Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	48	67.6 (49.4, 82.0)	23/34	44.7 (29.0, 61.5)	17/38	1.69 (0.58, 4.98)
24	48	68.0 (53.2, 80.1)	34/50	56.5 (34.9, 76.1)	13/23	2.76 (0.89, 8.65)
48	48	68.8 (55.8, 79.4)	44/64	73.7 (48.6, 89.9)	14/19	**6.16 (1.75, 24.39)**

[a] *Bold indicates statistical significance (lower bound of 95% CI >1.00).*

Viral Response ≥ 2 Log Decrease:

The association between individual clinical responses (histologic and biochemical) at Week 48 and viral response (≥ 2 Log decrease) at Weeks 12, 24, and 48 are summarized in Table 37. The PPV for the association of viral response (weeks 12, 24, and 48) and histologic response at week 48 was >70%. The PPV for the association of viral response and bio-chemical response was >81% and remained consistent throughout the study. The viral response as measured by Alinity m HBV assay was informative for determining Week 48 clinical responses depending on clinical response type and week of viral response, as indicated by the lower bound of 95% CI for OR greater than 1.00 (indicated in bold).

Table 37. PPV, NPV, and Odds Ratio (OR) for Individual Clinical Responses During Treatment at Week 48, Associated with Viral Response (≥ 2 Log Decrease) in HBeAg-Negative Subjects.

Week of Viral Response	Clinical Response	PPV %		NPV %		OR
		Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	Histologic	80.4 (67.2, 89.3)	45/56	57.1 (34.4, 77.4)	12/21	**5.45 (1.61, 18.59)**
	Biochemical	85.0 (72.9, 92.5)	51/60	47.4 (25.2, 70.5)	9/19	**5.10 (1.38, 18.55)**
24	Histologic	73.8 (61.2, 83.6)	48/65	42.9 (18.8, 70.4)	6/14	2.12 (0.52, 8.10)
	Biochemical	81.5 (69.6, 89.7)	53/65	57.1 (29.6, 81.2)	8/14	**5.89 (1.44, 24.29)**
48	Histologic	70.5 (59.0, 80.0)	55/78	33.3 (11.3, 64.6)	4/12	1.20 (0.24, 5.01)
	Biochemical	84.4 (74.0, 91.3)	65/77	64.3 (35.6, 86.0)	9/14	**9.75 (2.36, 42.56)**

[a] *Bold indicates statistical significance (lower bound of 95% CI >1.00).*

The associations between the combined clinical responses based on positive histologic and biochemical responses at Week 48 and viral response (≥ 2 Log decrease) at Weeks 12, 24, and 48 are summarized in Table 38. PPV for the association of viral response at week 12 (defined as ≥ 2 log IU/mL decrease) as measured by Alinity m HBV assay at Weeks 12 was informative for determining Week 48 clinical response with the combination of both responses (histologic and biochemical), where the lower bound of 95% CI for OR was greater than 1.00. However, the association of viral response (at weeks 24 and 48) and combined clinical response at week 48, 95% CI is wide, and OR is not significant for the tested population.

Table 38. PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic and Biochemical Responses During Treatment at Week 48, Associated with Viral Response (≥ 2 Log Decrease) in HBeAg-Negative Subjects.

Week of Viral Response	Week of Clinical Response	PPV %		NPV %		OR
		Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	48	72.2 (58.1, 83.1)	39/54	72.2 (46.4, 89.3)	13/18	**6.76 (1.81, 27.79)**
24	48	65.0 (51.5, 76.6)	39/60	61.5 (32.3, 84.9)	8/13	2.97 (0.74, 12.91)
48	48	63.0 (50.9, 73.8)	46/73	63.6 (31.6, 87.6)	7/11	2.98 (0.67, 15.01)

[a] *Bold indicates statistical significance (lower bound of 95% CI >1.00).*

D. Safety Results

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

E. Effectiveness Results

The Alinity m HBV assay can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. The effectiveness of the Alinity m HBV assay was assessed for whether on-treatment viral response as determined by Alinity m HBV assay was informative for determining clinical responses in patients with chronic HBV infection undergoing anti-viral therapy as discussed in the results in Section X.C above. Overall, a clinical study demonstrates the effectiveness of the Alinity m HBV assay for determining on-treatment responses to antiviral therapy in the management of patients with chronic HBV infection.

F. Pediatric Extrapolation

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

G. 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 clinical study included 3 investigators. None of the clinical investigators were full-time or part-time employees of the sponsor and all 3 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 provision 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 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. Safety Conclusions

The risk of the device is based on data collected in the non-clinical and clinical studies conducted to support PMA approval as described above. Based on the results from both studies, the Alinity m HBV assay, when used according to the provided directions and in conjunction with all relevant clinical and laboratory findings, should be safe to use and poses minimal risk to the patient due to false test results.

B. Effectiveness Conclusions

The effectiveness of the Alinity m HBV assay has been demonstrated for use in quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma.

HBeAg-Positive Subjects:

For both viral responses (<2000 IU/mL and ≥2 Log decrease), the associations between clinical responses (histologic, biochemical, HBeAg Loss, Anti-HBe Gain or Seroconversion) at Week 48 and viral response at Weeks 12, 24 or 48 demonstrated that the viral responses as measured by Alinity m HBV assay were informative for determining Week 48 clinical response (histological, bio-chemical and serological), where the lower bound of 95% CI for OR was greater than 1.00.

HBeAg-Negative Subjects:

For both viral responses (<2000 IU/mL and ≥2 Log decrease), the association between clinical responses (histologic or biochemical) at Week 48 and viral response at Weeks 12, 24 or 48 demonstrated that the viral responses as measured by Alinity m HBV assay were informative for determining Week 48 clinical response (histological and bio-chemical), where the lower bound of 95% CI for OR was greater than 1.00.

This study demonstrated that HBV DNA levels measured at baseline, and decreases in HBV DNA levels after 12, 24 and 48 weeks of therapy and to predict clinical responses at specific threshold week 48.The study identified subjects who achieved Virologic Response, Histologic and Biochemical Response, or loss of HBeAg at Week 48 of therapy. The results of this study demonstrate the clinical utility of the Alinity m HBV assay for determining treatment responses to therapy in the management of patients with chronic HBV infection.

Clinical study results, in combination with non-clinical performance evaluations, support the effectiveness of the Alinity m HBV assay for the intended use as an aid in the management of patients with chronic HBV-infection undergoing anti-viral therapy.

C. Benefit-Risk Determination

The benefit of the assay is quantitation of Hepatitis B Virus (HBV) DNA in samples from patients with chronic HBV infection. The assay will be used as an aid in the management of patients with

chronic HBV infection undergoing antiviral therapy. The assay can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. Treatment of HBV infection is associated with normalization of liver enzymes, decrease in necroinflammation, and a decrease in fibrosis. Treatment with antiviral drugs will mitigate the sequelae of hepatitis B infection and may result in improved morbidity and mortality in infected patients. Known sequelae of hepatitis B infection include continued symptoms, increases in all-cause mortality, liver disease-related complications and death, hepato-cellular carcinoma, and need for liver transplantation. Additionally, treatment can potentially decrease transmission and disease burden in the general population and particularly in populations at high risk for hepatitis B infection. Performance of the device in the clinical study suggests that patients will benefit from the assay.

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device. Risks of erroneously high results include improper patient management, such as treatment for hepatitis B with antiviral medication. Administration of antiviral medication has risks including toxicity and more rarely allergic reactions. Risks of erroneously low results include improper patient management, such as potentially missing and under-treating a patient who has hepatitis B infection. Under-treating a patient with hepatitis B infection whose clinical picture warrants antiviral treatment could result in the known sequelae of HBV infection and may result in higher morbidity and mortality in these patients. Under-treating hepatitis B in patients whose clinical picture otherwise warrants treatment will lead to continued symptoms, increases in all-cause mortality, liver disease-related complications and death, hepato-cellular carcinoma rates, and need for liver transplantation.

The clinical benefits outweigh the potential risks for the proposed assay, considering the mitigations of the risks provided in the premarket approval as well as general controls. The required premarket approval helps to ensure that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results. The device's performance observed in the clinical study suggests that errors will be uncommon and that the assay will provide substantial benefits to patients as an aid in the management of patients with chronic HBV infection undergoing antiviral therapy.

D. Patient Perspectives

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

E. 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 data from the nonclinical studies demonstrated acceptable analytical sensitivity, linearity, precision, and analytical specificity of the Alinity m HBV assay when used according to the instructions for use as stated in the labeling, the warnings, and precautions, and limitations sections of the labeling. The clinical studies and the statistical analysis of clinical data in this application have shown that viral response measured with Alinity m HBV assay is informative for determining clinical response in

patients with chronic hepatitis B under anti-viral therapy and that the assay is safe and effective when used according to the directions for use in the labeling.

In conclusion, given the available information above, the data support that for the diagnosis of HBV infection and for the management of HBV patients who are undergoing antiviral therapy, the probable benefits outweigh the probable risks.

XIII. CDRH DECISION

- CDRH issued an approval order on August 29, 2020.

- 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.