Alinity m

HBV AMP Kit

en
HBV
REF 08N47-095
53-608162/R1

Created February 2020

REF 08N47-095
53-608162/R1

Rx ONLY

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Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

NAME

Alinity m HBV

INTENDED 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 in screening 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.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B virus (HBV) is a DNA virus containing a partially double-stranded genome of approximately 3.2 kb.[1-3] HBV is one of the world's most widespread infectious agents and the most common of several viruses worldwide that can cause lifelong, chronic infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death.[1] As of 2015, approximately 257 million people globally have chronic hepatitis B and approximately 887,000 people die each year due to complication of hepatitis B including cirrhosis and liver cancer.[4] A common route of infection is mother-to-child transmission at birth or during early childhood.[5-7] Other populations with high risk of HBV infection are associated with intravenous drug use, hemophilia, high-risk sexual activity, hemodialysis, needle stick injury in health care staff, and body piercing and tattooing.[8] Hepatitis is largely diagnosed via serology (e.g., hepatitis B surface antigen, hepatitis core antigen and hepatitis B surface antibody). Nucleic acid tests for HBV DNA are used as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy and to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment.[9] Once a diagnosis of HBV infection is made, patients may receive treatment. Two classes of antivirals are available for treatment, interferon-based therapy (IFN) or nucleic acid-based therapy (NA), based on current clinical practice guidelines. Treatment duration and monitoring of viral response varies for each

drug treatment class. The HBV viral load, in addition to other laboratory findings, is used in monitoring the response to both IFN and NA pharmacotherapy.[9] The Alinity m HBV assay is designed to target highly conserved sequences of the HBV genome. HBV has significant genetic diversity within nine known HBV genotypes (A-I).[10-18] The diversity arises in part due to low fidelity of the HBV DNA polymerase.[11,13,19] To ensure assay robustness against this genetic diversity, the assay is designed to target a highly conserved region of the HBV genome. This design ensures detection and quantitation of all 9 HBV genotypes. In addition to the HBV primers and probes, the assay utilizes an internal control (IC) primer/probe set for amplification and detection of the IC target sequence, which is not related to HBV. The IC controls for substantial reagent failure and sample inherent inhibitions of the PCR reaction. The IC probe is labeled with a different fluorophore than the HBV probes. This allows for simultaneous real-time detection and discrimination of both the HBV and IC amplified product targets within the same reaction vessel.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Alinity m HBV assay requires 3 separate assay specific kits:

- Alinity m HBV AMP Kit (08N47-095) consisting of 2 types of multi-well assay trays. The amplification trays (AMP TRAYS) contain lyophilized, unit-dose real-time PCR amplification/detection reagents and lyophilized, unit-dose IC with proteinase K in separate wells, and the activation trays (ACT TRAYS) contain liquid activation reagent. The intended storage condition for the Alinity m HBV AMP Kit is 2 to 8°C.
- Alinity m HBV CAL Kit (08N47-075) consisting of 2 calibrator levels, each supplied as liquid in single-use tubes. The intended storage condition for the Alinity m HBV CAL Kit is – 25 to – 15°C.
- Alinity m HBV CTRL Kit (08N47-085) consisting of negative controls, low-positive controls and high-positive controls, each supplied as liquid in single-use tubes. The intended storage condition for the Alinity m HBV CTRL Kit is – 25 to – 15°C.

The Alinity m HBV assay utilizes real-time polymerase chain reaction (PCR) with fluorescence labeled probes 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. 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 activation reagent and lyophilized unit-dose Alinity m HBV 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 sample preparation process, a lyophilized unit-dose IC and proteinase K on the AMP TRAY is rehydrated by the Alinity m System and delivered into each sample preparation reaction. 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 amplification/detection reagents consist of enzymes, primers, probes and activation reagents that enable polymerization and detection. The Alinity m HBV 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 the 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 remain 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.

The possibility of nucleic acid contamination on the Alinity m System is minimized because:

- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- PCR amplification and detection is carried out automatically in a sealed reaction vessel.
- Disposal of the reaction vessel is performed automatically by the Alinity m System.

For additional information on system and assay technology, refer to the Alinity m System Operations Manual, Section 3.

REAGENTS

Kit Contents

Alinity m HBV AMP Kit List No. 08N47-095

The Alinity m HBV AMP Kit is comprised of 2 types of multi-well trays: Alinity m HBV AMP TRAY 1 and Alinity m HBV ACT TRAY 2.

Each Alinity m HBV AMP TRAY 1 (individually packed in a foil pouch with a desiccant bag) contains 48 unit-dose lyophilized amplification reagent wells and 48 unit-dose lyophilized IC and proteinase K reagent wells. One well of each is used per test.

- Amplification reagent wells consist of synthetic oligonucleotides, DNA Polymerase, Uracil-DNA Glycosylase, and dNTPs in a buffered solution with a reference dye.
- Internal control (IC) and proteinase K wells consist of noninfectious non-HBV related linearized plasmid DNA and proteinase K, in a buffered solution with carrier DNA.

Each Alinity m HBV ACT TRAY 2 (individually packed in a foil pouch without a desiccant bag) contains 48 unit-dose liquid activation reagent wells. One reagent well is used per test.

- Activation reagent wells consist of magnesium chloride and tetramethylammonium chloride. Preservative: 0.15% ProClin® 950.

	Quantity
Σ	192 tests
Alinity m HBV AMP TRAY 1	4 trays / 48 tests each
Alinity m HBV ACT TRAY 2	4 trays / 48 tests each

WARNINGS AND PRECAUTIONS

IVD

- **For In Vitro Diagnostic Use**

Safety Precautions

Human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[20] OSHA Standard on Bloodborne Pathogens,[21] CLSI Document M29-A4,[22] and other appropriate biosafety practices.[23] Therefore, all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.

- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[20]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[23]

The following warnings and precautions apply to:
Alinity m HBV AMP TRAY 1.

DANGER	Contains: Tris hydoxymethyl aminomethane and proteinase K.
H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves / protective clothing / eye protection.
P284	In case of inadequate ventilation wear respiratory protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P312	Call a POISON CENTER/doctor if you feel unwell.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P337+P313	If eye irritation persists: Get medical advice / attention.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
P362+P364	Take off contaminated clothing and wash it before reuse.
Storage	
P405	Store locked up.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: Alinity m HBV ACT TRAY 2.	
	
DANGER	Contains: Tetramethylammonium chloride and 2-Methyl-4-isothiazolin-3-one
H302	Harmful if swallowed.
H316	Causes mild skin irritation.[a]
H317	May cause an allergic skin reaction.
H370	Causes damage to organs.
H412	Harmful to aquatic life with long lasting effects.
Prevention	
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P272	Contaminated work clothing should not be allowed out of the workplace.
P273	Avoid release to the environment.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P301+P312	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
P302+P352	IF ON SKIN: Wash with plenty of water.
P308+P311	IF exposed or concerned: Call a POISON CENTER / doctor.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

[a] Not applicable where regulation EU 1272/2008 (CLP) or OSHA Hazard Communication 29CFR1910.1200 (HCS) 2012 have been implemented.

Important information regarding the safe handling, transport and disposal of this product is contained in the Safety Data Sheet.

Safety Data Sheets are available from your Abbott Representative.

For a detailed discussion of safety precautions during system operation, refer to the Alinity m System Operations Manual, Section 7 and Section 8.

Reagent Shipment

	Shipment Condition
Alinity m HBV AMP Kit	On dry ice

Reagent Storage

In order to minimize damage to foil pouches, it is recommended that the Alinity m HBV AMP TRAY 1 (AMP TRAY 1) and Alinity m HBV ACT TRAY 2 (ACT TRAY 2) are stored in the original kit packaging. Open the foil pouch for the reagent trays just prior to loading on the instrument. Onboard storage time begins when reagents are loaded on the Alinity m System.

	Storage Temperature	Maximum Storage Time
Unopened	2 to 8°C	Until expiration date
Onboard	System Temperature	30 days (not to exceed expiration date)

Reagent Handling

- Do not use reagents that have been damaged.
- Minimize contact with the surface of reagent trays during handling.
- Only load AMP TRAY 1 and ACT TRAY 2 from the same AMP Kit lot on the same Alinity m Assay Tray Carrier. Do not load AMP TRAY 1 and ACT TRAY 2 from different AMP Kit lots on the same Alinity m Assay Tray Carrier.
- The Alinity m System will track the onboard storage time of AMP TRAY 1 and ACT TRAY 2 while on the instrument. The Alinity m System will not allow the use of AMP TRAY 1 and ACT TRAY 2 if the maximum onboard storage time has been exceeded.
- For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity m System Operations Manual, Section 8.

Indications of Reagent Deterioration

- Deterioration of the reagents may be indicated when a calibration or control error occurs, or controls are repeatedly out of the specified ranges.
- Reagents are shipped on dry ice and are stored at 2 to 8°C upon arrival. If reagents arrive in a condition contrary to this recommendation or are damaged, immediately contact your Abbott Representative.
- For troubleshooting information, refer to the Alinity m System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The Alinity m HBV assay application specification file must be installed on the Alinity m System prior to performing the assay.

For a detailed description of system operating instructions, refer to the Alinity m System Operations Manual, Section 5.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The plasma and serum specimen types listed below can be used with this assay on the Alinity m System. For the Alinity m HBV assay, only use the collection tubes as described in the following table for the corresponding specimen type. Alinity m HBV assay performance with other specimen types or collection tubes has not been evaluated.

Specimen Types[a]	Blood Collection Tubes
Plasma	Acid Citrate Dextrose (ACD) K_2 EDTA K_3 EDTA Plasma Preparation Tube (PPT)[b]
Serum	Serum Rapid-clot Tube (z-clot, thrombin) Serum Separator Tube (SST)[b]

[a] The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to use the correct specimen types in the assay.
[b] The Plasma Preparation Tube and Serum Separator Tube are gel tubes.

Specimen Storage: Plasma or Serum Testing

Specimen	Temperature	Maximum Storage Time	Special Instructions
Whole Blood	2 to 8°C	0 to 72 hours	Whole blood may be stored between draw and plasma/serum separation.
	15 to 30°C	24 hours	Whole blood storage plus separated plasma/serum storage at 2 to 8°C must not exceed a combined total of 72 hours.
Plasma/Serum	2 to 8°C	0 to 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). Whole blood storage plus separated plasma/serum storage at 2 to 8°C must not exceed a combined total of 72 hours. 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/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/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.

Specimen Shipping

Ship specimens according to the recommended storage temperature and time listed in the **Specimen Storage** section. Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

Preparation for Analysis

Freshly Drawn Whole Blood Specimens:

- Follow the specimen collection tube manufacturer instructions for blood collection and centrifugation. Separate plasma and serum from cells or clot by centrifugation.
- After centrifugation, plasma/serum may be stored on the blood cells/clot (in tube with or without gel) prior to being loaded onto the Alinity m System or used for dilution.
- Whole blood storage plus separated plasma/serum storage at 2 to 8°C must not exceed a combined total of 72 hours.

  NOTE: Specimens stored on the blood cells or on the clot cannot be frozen without a gel.
- Plasma and serum specimens may also be transferred to a secondary tube for storage prior to being loaded onto the Alinity m System or used for dilution. Please refer to the table above for storage times and temperatures.

- If longer storage is required, plasma and serum specimens in primary gel tubes or secondary tubes may be stored frozen.

Frozen Specimens: Primary Gel Tubes

- Thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.
- Vortex specimens 3 times for 2 to 3 seconds.
- Centrifuge specimens stored in primary gel tubes at 2000*g* for 5 minutes before loading onto the Alinity m System or before preparing a specimen dilution. If any clot or debris is observed, transfer the supernatant of the specimen into a new tube. Avoid transferring any debris or clot into the new tube.

Frozen Specimens: Secondary Aliquot Tubes

- Thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.
- Vortex each specimen 3 times for 2 to 3 seconds. If any debris is observed, transfer the specimen into a new tube. Avoid transferring any debris into the new tube.

All specimen tubes (primary and secondary tubes) must be labeled with specimen ID barcodes or must be identified with a specimen ID and rack and position. Refer to the **Assay Procedure** section of this package insert or the Alinity m System Operations Manual, Section 4, for tube sizes and requirements for minimum sample volume and use of caps. Avoid touching the inside of the cap when opening tubes.

PROCEDURE

Materials Provided

08N47-095 Alinity m HBV AMP Kit

Materials Required but not Provided

- 08N47-075 Alinity m HBV CAL Kit
- 08N47-085 Alinity m HBV CTRL Kit
- 09N12-001 Alinity m Sample Prep Kit 2
- 09N20-001 Alinity m Lysis Solution
- 09N20-003 Alinity m Diluent Solution
- 09N20-004 Alinity m Vapor Barrier Solution
- 09N50-001 Alinity m Specimen Dilution Kit I[a]
- Alinity m HBV Application Specification File
- Vortex mixer
- Centrifuge capable of 2000*g*
- 09N49-001 Alinity m LRV Tube[a]
- Calibrated pipettes capable of delivering 10 to 1000 µL[a]
- Aerosol barrier pipette tips for 10 to 1000 µL pipettes[a]
- Plate adapter for 384 well plates (e.g., Eppendorf Catalog No. 022638955)
- Centrifuge with swing plate rotor capable of accommodating the plate adapter and capable of ≥ 100*g*
- 09N49-010 Alinity m Transport Tube Pierceable Capped
- 09N49-011 Alinity m Transport Tube
- 09N49-012 Alinity m Pierceable Cap
- 09N49-013 Alinity m Aliquot Tube

[a] These items are used in the Specimen Dilution Procedure if dilution is required.

For information on materials required for operation of the instrument, refer to the Alinity m System Operations Manual, Section 1.

For general operating procedures, refer to the Alinity m System Operations Manual, Section 5.

For optimal performance, it is important to perform routine maintenance as described in the Alinity m System Operations Manual, Section 9.

Procedural Precautions

- Read the instructions in this package insert carefully before processing samples.
- Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Work area and instrument platforms must be considered potential sources of contamination.
- Ensure the Alinity m HBV AMP TRAY 1 is tapped prior to loading on the Alinity m System per instructions in the **Assay Procedure** section.

- Ensure the Alinity m HBV ACT TRAY 2 is centrifuged prior to loading on the Alinity m System per instructions in the **Assay Procedure** section.
- Monitoring procedures for the presence of amplification product can be found in the Alinity m System Operations Manual, Section 9.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% (v/v) sodium hypochlorite or other suitable disinfectant.
- To prevent contamination, change to new gloves before handling the Alinity m Sample Prep Kit 2, assay trays, system solutions, Integrated Reaction Unit (IRU) sleeves, and pipette tips. Also change to new gloves whenever they are contaminated by a specimen, a calibrator, a control, or a reagent. Always use powder-free gloves.
- The use of the Alinity m HBV CAL and CTRL Kits is integral to the performance of Alinity m HBV. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details. Refer to the Alinity m HBV CAL Kit package insert and/or Alinity m HBV CTRL Kit package insert for preparation and usage.
- The Alinity m HBV calibrator and control reagents are contained in single-use tubes with pierceable caps. Avoid contamination or damage to the caps after removal from their original packaging. Discard tubes after use.

Assay Procedure

Prior to loading on the Alinity m System, hold the AMP TRAY 1 by the edges with the label facing up and tap 3 times on the bench.

Prior to loading on the Alinity m System, the ACT TRAY 2 must be centrifuged as follows:

1. Load the ACT TRAY 2 onto the plate adapter (e.g., Eppendorf Catalog No. 022638955).
2. Load the plate adapter (with the ACT TRAY 2) on a swing plate centrifuge capable of accommodating the plate adapter. Spin at 100 to 800*g* for 1 to 5 minutes to remove potential bubbles.
3. Immediately following centrifugation, carefully transfer the ACT TRAY 2 to the Alinity m Assay Tray Carriers. Take care to minimize disturbance to the ACT TRAY 2. Load the tray carriers per the Alinity m System Operations Manual, Section 5.
4. If disturbance occurs during transfer that could potentially introduce bubbles (e.g., dropping, bumping, inversion of the ACT TRAY 2), re-centrifuge the ACT TRAY 2.
5. Proceed with the **Reagent and sample management** procedure per the Alinity m System Operations Manual, Section 5.

For a detailed description of how to run an assay, refer to the Alinity m System Operations Manual, Section 5. Prior to testing specimens, check the calibration and control status. If recalibration or control testing is required, refer to the **QUALITY CONTROL PROCEDURES** section. Calibrators and/or controls may be tested separately or with specimens.

From the Create Order screen, select the assay (HBV) being tested.

The Alinity m System will track the onboard storage time of amplification reagents, calibrators, controls, and specimens while on the instrument. The Alinity m System will not allow the use of amplification reagents, calibrators, controls, or process specimens that have exceeded the allowable onboard storage time.

Specimen tubes need to meet the requirements below for minimum sample volume and the use of caps when loaded on the Alinity m System. Blood collection tubes with separated plasma or serum and specimen aliquot tubes may be placed on the Alinity m Universal Sample Rack (sample rack) onboard the system for up to 4 hours prior to processing.

Tube Type[a]	List No.	Minimum Plasma/Serum Volume Required	Cap Requirement on Instrument
Blood Collection Tube (Primary Tube)			
Blood collection tubes with minimum inner diameter 10.0 mm	NA	7.0 mm[b] above the gel, clot, or blood cells	Uncapped
Specimen Aliquot Tube (Secondary Tube)			
Alinity m Aliquot Tube	09N49-013	0.45 mL	Capped[c] or uncapped
Alinity m Transport Tube	09N49-011	1.0 mL	Uncapped
		0.45 mL	Capped[c]
Alinity m Transport Tube Pierceable Capped	09N49-010	1.0 mL	Uncapped
		0.45 mL	Capped
Other aliquot tubes with minimum inner diameter 10.0 mm	NA	0.65 mL for tubes with 10.6 mm or less inner diameter. 0.95 mL for tubes with 13.2 mm or less inner diameter.	Uncapped

[a] Refer to the Alinity m System Operations Manual, Section 4, for sample tube specifications and requirements and Section 5 for sample rack loading instructions.
[b] Represents requirement for minimum column height of plasma or serum above the gel/clot/blood cells in the primary tube. The minimum volume in milliliters can be calculated using the inner diameter (ID in mm) of the tube in the formula: Minimum Volume = $0.00550 \times ID^2$.
[c] Alinity m Pierceable Cap, List No. 09N49-012, is the only type of cap that can be used when loaded on the Alinity m System.

Prior to loading the specimen tubes on to the Alinity m System:

- Ensure individual specimen tubes are labeled correctly with specimen ID barcodes.
- Inspect serum and plasma specimens for bubbles and foam. Specimens should be free of bubbles and foam. If found, remove them with a new sterile pipette tip for each tube to prevent cross-contamination.

Specimen Dilution Procedure (Optional)

Specimens may be diluted manually for testing on the Alinity m System using the Alinity m Specimen Dilution Kit I in the following manner:

Low volume specimens with 140 to 449 µL volume available for Alinity m HBV testing may only be diluted 1:2.5. Specimens with 50 to 139 µL volume available for Alinity m HBV testing may only be diluted 1:50. High-titer specimens with a result of > ULoQ, (i.e., above the upper limit of quantitation; see result interpretation below) may only be diluted 1:50 before testing, if an exact viral load is desired.

Specimen Dilution Scenario	Available Specimen Volume	Dilution Factor
Low volume	140 to 449 µL	1:2.5
	50 to 139 µL	1:50
> ULoQ result	≥ 50 µL	1:50

Refer to the **Specimen Dilution Procedure Scheme** section.

The operator must select the dilution factor in the Specimen tab of the Create Order screen of the Alinity m System software. The system will use the selected dilution factor to automatically calculate and report the result of the neat specimen.

NOTE: Upon dilution, the specimen must be loaded onto the system within 2 hours. The Alinity m Specimen Diluent Tubes are single use and may not be reused.

Specimens are diluted with a dilution factor of 2.5, using Specimen Dilution Kit I as follows:

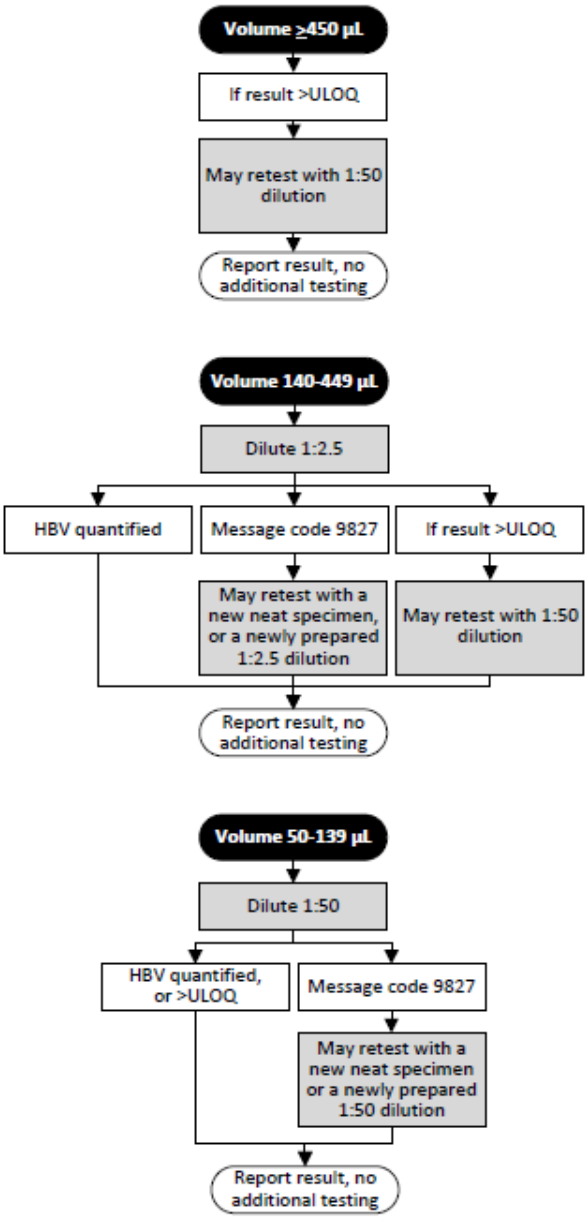
1. Apply a barcode label for the designated specimen ID to an Alinity m LRV Tube.
2. Open a fresh Alinity m Specimen Diluent Tube and transfer 210 µL of Specimen Diluent into the Alinity m LRV Tube.
3. Add 140 µL of the patient specimen into the Alinity m LRV Tube.
4. Cap the tube, vortex 3 times for 2 to 3 seconds, and tap upright on the bench to bring liquid to the bottom of the tube.
5. Remove the cap from the Alinity m LRV Tube. Inspect the fluid in the tube and remove any bubbles if found.
6. Place the Alinity m LRV Tube in the sample rack.

Specimens are diluted with a dilution factor of 50, using Specimen Dilution Kit I as follows:

1. Apply a barcode label for the designated specimen ID to an unused Alinity m Specimen Diluent Tube. Remove the cap from the Alinity m Specimen Diluent Tube. Save the cap for later use.
2. Add 50 µL of the patient specimen to the Alinity m Specimen Diluent Tube.
3. Cap the tube, vortex 3 times for 2 to 3 seconds, and tap upright on the bench to bring liquid to the bottom of the tube.
4. Load the tube directly onto the sample rack. The cap may remain on the tube.

NOTE: Do not use an Alinity m Specimen Diluent Tube that has crystals or liquid on the outside of the tube because this may be evidence of leakage.

Specimen Dilution Procedure Scheme



QUALITY CONTROL PROCEDURES

Assay Calibration

For instructions on performing an assay calibration, refer to the Alinity m System Operations Manual, Section 6.

Lot-specific concentration values for assay calibrators and controls are available via: Abbott Mail, the Abbott Molecular customer portal www.molecular.abbott/portal, and from your Abbott Representative.

When an assay calibration is being performed:

- Lot-specific concentration values can be automatically imported to the Alinity m System via Abbott Mail upon scanning the calibrator (HBV CAL A and HBV CAL B) or control (HBV NEG CTRL, HBV LOW POS CTRL, and HBV HIGH POS CTRL) tube barcodes.
- Lot-specific concentration values can also be obtained from the Abbott Molecular customer portal or provided by your Abbott Representative and imported via a USB drive.

For instructions on creating a test order for calibration and loading calibrators on the instrument, refer to the Alinity m System Operations Manual, Section 5.

A calibration curve is required to quantitate the HBV DNA concentration. At a minimum, 1 Alinity m HBV CAL A tube and 1 Alinity m HBV CAL B tube from the Alinity m HBV CAL Kit are required for performing an assay calibration on the Alinity m System. The Alinity m System will process 3 replicates from each calibrator tube. The output data of the 2 calibrators will be used to generate a calibration curve (lot-specific HBV concentration versus the threshold cycle [C_t] at which a reactive level of fluorescent signal is detected). The calibration curve slope and intercept are calculated and stored on the instrument.

Once an assay calibration is accepted and stored, all subsequent samples may be tested without further calibration unless any of the following situations occur:

- An Alinity m HBV AMP Kit with a new lot number is used.
- An Alinity m Sample Prep Kit 2 or Alinity m Lysis Solution with a new lot number is used.
- The assay calibration has expired.
- A new version of the Alinity m HBV Application Specification File is installed.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed. Contact your Abbott Representative for further instructions.

Detection of Inhibition

An IC C_t assay validity parameter is established during a calibration run. A defined, consistent quantity of IC is introduced into each specimen, calibrator, and control at the beginning of sample preparation and measured on the Alinity m System to demonstrate proper sample processing and assay validity.

The median IC C_t value from calibrator samples establishes an IC C_t validity range for subsequently processed specimens and controls.

A Message Code is assigned to a specimen or control when its IC Ct value is outside of the IC C_t validity range. When the IC C_t value exceeds the upper limit of the IC C_t validity range, abnormal assay conditions, such as inhibition, are indicated.

Refer to the Alinity m System Operations Manual, Section 10, for an explanation of the corrective actions for Message Codes.

Negative and Positive Controls

An Alinity m HBV Negative CTRL, Low Positive CTRL, and High Positive CTRL are recommended to be tested, at or above the minimum frequency of once every 24 hours, to monitor the performance of the assay and Alinity m System. Valid results for all control levels must be obtained before specimen results are reported. The assay controls are

also tested following calibrators and valid results for controls are required to establish a new calibration curve.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

A flag is displayed for specimens when a control result is invalid. All of the specimens processed following an invalid assay control must be retested.

If control results are invalid, refer to the Alinity m System Operations Manual, Section 5 for a description of quality control flags and Section 10 for troubleshooting information.

The presence of HBV must not be detected in the negative control. HBV detected in the negative control is indicative of contamination by other samples or by amplified product. To avoid contamination, clean the Alinity m System and repeat sample processing for controls and specimens following the Procedural Precautions in this package insert. Monitoring procedures for the presence of amplification product can be found in the Alinity m System Operations Manual, Section 9.

If negative controls are persistently reactive, contact your Abbott Representative.

When a set of assay controls are being processed, the lot-specific concentration values of the Alinity m HBV low-positive control and Alinity m HBV high-positive control can be:

- Automatically imported to the Alinity m System via Abbott Mail upon scanning the barcode labels on control tubes (HBV LOW POS CTRL and HBV HIGH POS CTRL), or
- Obtained from the Abbott Molecular customer portal or provided by your Abbott Representative and imported to the Alinity m System via a USB drive.

RESULTS

Calculation

Quantitative viral load results are reported for patient specimens with HBV viral concentrations within the assay's quantitation range. The concentration of HBV DNA in a specimen is calculated from the calibration curve by the system software. The Alinity m System reports the results in International Units as IU/mL or Log [IU/mL].

Refer to the Alinity m System Operations Manual for configuration of result units.

For specimens tested with the Specimen Dilution Procedure, the Alinity m System calculates and reports the neat concentration (i.e., prior to dilution), by using the dilution factor selected by the user.

Interpretation of Results

Undiluted Specimens

The Alinity m System will report a Result and an Interpretation for each specimen. If applicable, message codes or flags will also be displayed. The interpretation can be performed by the user, based on the Result, according to the table below.

Diluted Specimens

For specimens diluted 1:2.5 or 1:50, the Alinity m System reports a result, interpretation (if applicable), and a DIL flag indicating that the specimen has been diluted. The quantitative results represent the HBV DNA concentration in the specimen prior to dilution.

For diluted specimens with analyte concentration below the detection limit, no result is reported, and a message code (9827) is displayed. These specimens cannot be interpreted as target not detected and may be retested with a new neat specimen or a newly prepared dilution (refer to Specimen Dilution Procedure Scheme).

Note: The LLoQ of Alinity m HBV is 10 IU/mL (1.00 Log IU/mL) for specimens tested without dilution. Therefore, the lowest HBV DNA concentration that can be reported for a specimen that is tested diluted is 25 IU/mL (1.40 Log IU/mL) for the 1:2.5 dilution procedure, and 500 IU/mL (2.70 Log IU/mL) for the 1:50 dilution procedure.

The ULoQ of Alinity m HBV is 1,000,000,000 IU/mL (9.00 Log IU/mL) for specimens tested without dilution. Therefore, the HBV DNA concentration of a specimen that is tested diluted and returns a result of >ULoQ is >2,500,000,000 IU/mL (9.40 Log IU/mL) for the 1:2.5 dilution procedure; and >50,000,000,000 IU/mL (10.70 Log IU/mL) for the 1:50 dilution procedure. Refer to the following table for neat and dilution testing schemes.

Result and Interpretation

Alinity m System Reported		Interpretation Additional Information
Result	**Interpretation**	
Not Detected	HBV DNA not detected.	
< LLoQ	HBV DNA detected, but not quantified.	HBV DNA concentration is below the Lower Limit of Quantitation (LLoQ) of the assay.
LLoQ to ≤ ULoQ	HBV DNA detected and quantified.	Calculated HBV DNA concentration is within the linear range of the assay (≥ LLoQ and ≤ ULoQ).
> ULoQ	HBV DNA detected.	HBV DNA concentration is above the Upper Limit of Quantitation (ULoQ) of the assay.

Flags, Result Codes, and Message Codes

Some results may contain information in the Flags and Codes fields. For a description of the flags and result codes that may appear in these fields, refer to the Alinity m System Operations Manual, Section 5.

For a description of message codes refer to the Alinity m System Operations Manual, Section 10.

LIMITATIONS OF THE PROCEDURE

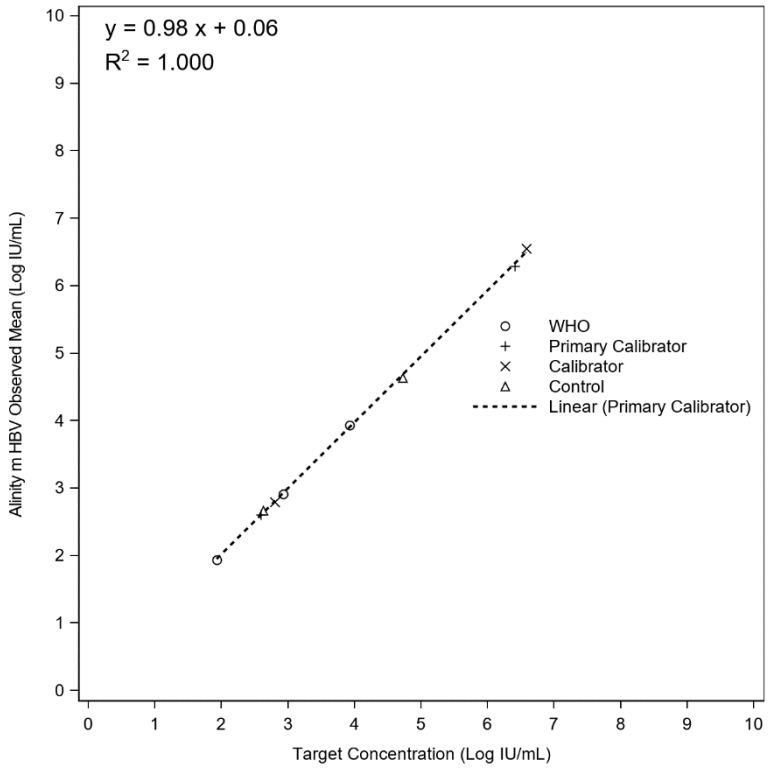
- Optimal performance of this test requires appropriate specimen collection and handling (refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert).
- Human serum (including serum separator tubes and rapid-clot tubes) and plasma (ACD, K_2 EDTA, K_3 EDTA, and PPT) specimens may be used with the Alinity m HBV assay. The use of other plasma and serum tubes have not been evaluated.
- Diluted specimens must be tested within 2 hours after dilution and should not be frozen.
- Debris within serum and plasma specimens (e.g., clots, fibrin strands) may interfere with sample processing.
- Assay performance for determining the clinical stage of HBV infection has not been established.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the calibrators, positive controls, or specimens must be controlled by good laboratory practice and careful adherence to the procedures specified in this package insert.

SPECIFIC PERFORMANCE CHARACTERISTICS

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 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, as presented in **Figure 1**.

Figure 1. Traceability to the WHO Standard



Limit of Detection

The limit of detection (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. Testing for each HBV DNA concentration was performed with 4 lots of amplification reagents across multiple days. The results, representative of the

analytical sensitivity performance of Alinity m HBV assay in plasma and serum, are included in **Table 1** and **Table 2**.

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) **(Table 1**).

Table 1. Alinity m HBV Limit of Detection (LOD) in Plasma

HBV DNA (IU/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
15.00	111	111	100.0
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

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) (**Table 2**).

Table 2. Alinity m HBV Limit of Detection (LOD) in Serum

HBV DNA (IU/mL)	No. of Valid Replicates	No. of Detected Replicates	Detection Rate (%)
15.00	113	112	99.1
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

The LOD of Alinity m HBV is 10 IU/mL (1.00 Log IU/mL) in plasma and serum.

Limit of Detection for Genotypes B, C, D, E, F, G, H, and I

HBV clinical specimens for genotypes B, C, D, E, F, G, H, and I were diluted to 3 different concentrations in HBV negative human plasma and serum. Testing across multiple days was performed using the lot of amplification reagents with the highest LOD estimate by Probit for each matrix. The results, representative of the analytical sensitivity performance of Alinity m HBV for genotypes B, C, D, E, F, G, H, and I are summarized in **Table 3** and **Table 4.** In this study, Alinity m HBV detected 95% or greater HBV samples at and above 10 IU/mL (1.00 Log IU/mL) in plasma and serum. These results demonstrate the ability of Alinity m HBV to detect genotypes B, C, D, E, F, G, H, and I at the claimed LOD.

Table 3. Alinity m HBV 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 4. Alinity m HBV 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

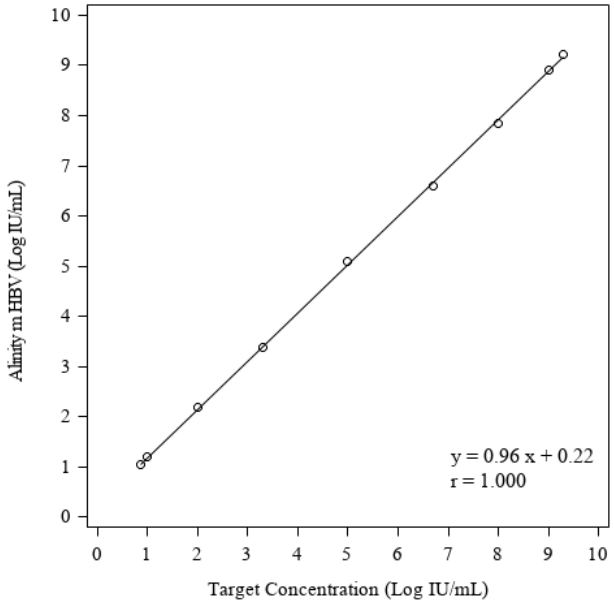
Linear Range

Linearity of Alinity m HBV 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). 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 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).

Alinity m HBV 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).

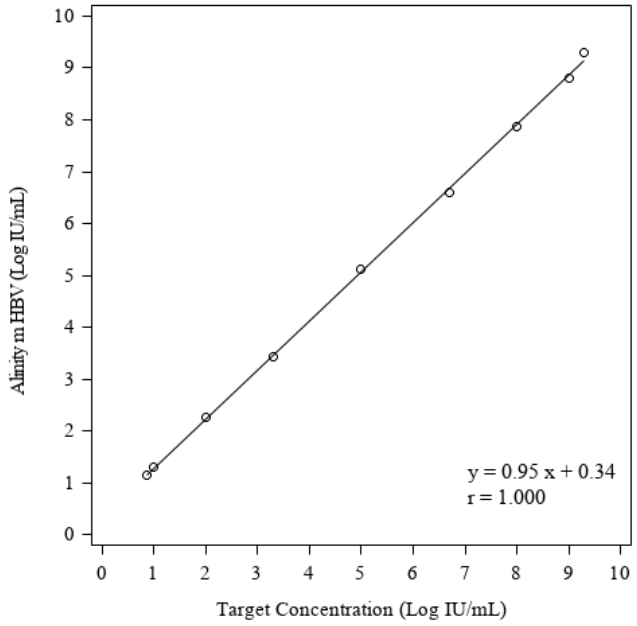
The representative results for Alinity m HBV linearity performance are shown in **Figure 2** and **Figure 3.**

Figure 2. Linearity in Plasma[a]



[a]The markers in the plot represent the mean Alinity m HBV concentration (in Log IU/mL) for each panel member.

Figure 3. Linearity in Serum[a]



[a]The markers in the plot represent the mean Alinity m HBV concentration (in Log IU/mL) for each panel member.

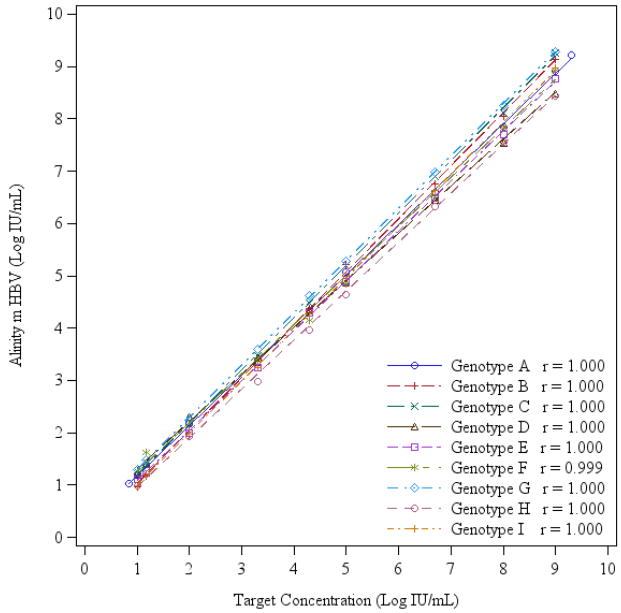
Linearity Across HBV Genotypes

Linearity of Alinity m HBV for genotypes B, C, D, E, F, G, H, and I was confirmed by testing a dilution series in negative human plasma and serum, each consisting of 9 panel levels spanning from 10 IU/mL to 1,000,000,000 IU/mL (1.00 to 9.00 Log IU/mL). For each genotype, the panel levels with lower concentrations were prepared using either an HBV positive sample or synthetic DNA, while the panel levels with higher concentrations were prepared using synthetic DNA.

Alinity m HBV 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 B, C, D, E, F, G, H, and I.

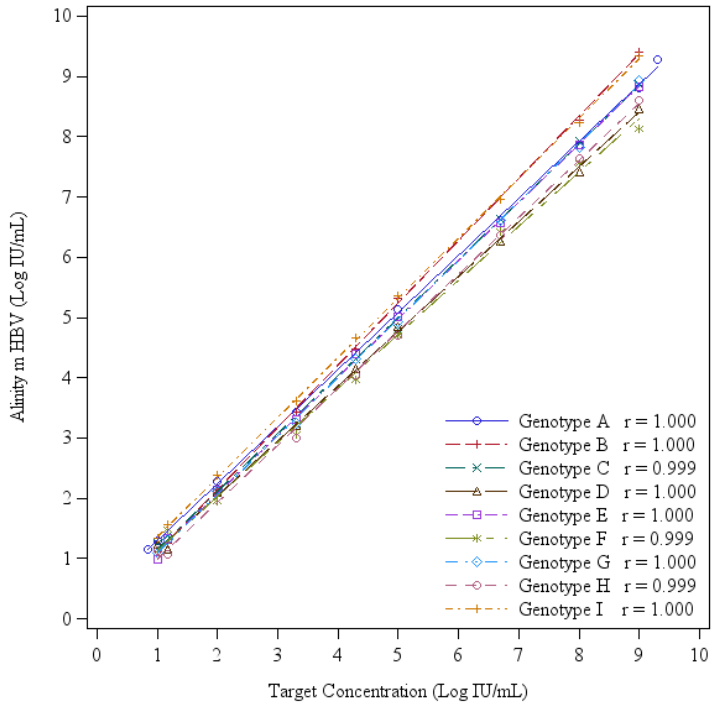
The representative results for Alinity m HBV linearity performance for genotypes A, B, C, D, E, F, G, H, and I are shown in **Figure 4** and **Figure 5**.

Figure 4. Linearity Across HBV Genotypes in Plasma[a]



[a] The markers in the plot represent the mean Alinity m HBV concentration (in Log IU/mL) for each panel member.

Figure 5. Linearity Across HBV Genotypes in Serum[a]



[a] The markers in the plot represent the mean Alinity m HBV concentration (in Log IU/mL) for each panel member.

Precision

Precision of Alinity m HBV was determined by analyzing an 8-member plasma panel and an 8-member serum panel. Panel members with concentrations 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 operated by 3 operators (one operator per instrument), for a total of 288 replicates per panel member.
The results, representative of the precision of Alinity m HBV in plasma and serum, (**Table 5** and **Table 6**, respectively) demonstrated that the assay 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 (i.e., concentrations from 1.00 to 1.48 Log IU/mL or 10 to 30 IU/mL).

Table 5. 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
8	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
7	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
6	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
5	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
4	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
3	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
2	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
1	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 6. 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
8	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
7	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
6	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
5	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
4	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
3	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
2	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
1	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.

Lower Limit of Quantitation

The lower limit of quantitation (LLoQ) is defined as the lowest concentration at which HBV DNA is reliably quantitated within an acceptable total error. Total error was estimated for detected samples from each study 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 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 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.

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.

Performance with HBV Negative Specimens

The specificity of Alinity m HBV was determined by testing 250 HBV negative plasma specimens and 250 HBV negative serum specimens 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%).

Analytical Specificity – Potential Cross-Reactants

The analytical specificity of Alinity m HBV was evaluated with a panel of microorganisms (**Table 9**) in HBV negative plasma, positive plasma containing 30 IU/mL HBV DNA and positive plasma containing 2,000 IU/mL HBV DNA. Organisms were tested by adding either the microorganism or its nucleic acids at a final concentration of 10^5 Units/mL for viruses, protozoans and yeast or 10^6 Units/mL for bacteria. No cross-reactivity or interference in the performance of Alinity m HBV was observed in the presence of the tested microorganisms.

Table 9. Microorganisms

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

Analytical Specificity - Potentially Interfering Substances

The effects of endogenous substances, the presence of non-HBV related diseases, and the presence of high levels of therapeutic drugs commonly prescribed for the treatment of HBV and related diseases were evaluated. Potential interference on Alinity m HBV performance 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, i.e., 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.

No interference was observed in the presence of drug compounds tested in pools that are listed in **Table 10**, at a concentration of 3 times the reported C_{max} or higher.

Table 10. 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

Carryover

The carryover rate for Alinity m HBV 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%).

Matrix Equivalency

Matrix equivalency in Alinity m HBV results for plasma and serum 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 represented in the positive samples.

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 Alinity m HBV quantitation in plasma demonstrated a slope of 0.99, intercept of 0.07, r = 0.999, and mean bias of 0.03 Log IU/mL when compared to the results in serum.

Alinity m HBV Testing Using Dilution Procedure

The 1:2.5 and 1:50 dilution procedures were evaluated by comparing quantitation of neat samples and samples tested using the Alinity m HBV dilution procedures. Ten plasma panel members and 10 serum panel members, consisting of HBV concentrations ranging from 75 to 2,000,000,000 IU/mL, were tested for each of the dilution procedures. Each panel member was tested, neat or using the dilution procedures, in multiple replicates. For the 10 plasma panel members, the differences in mean (i.e., diluted minus neat) 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 in mean (i.e., diluted minus neat) 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.

Precision of Alinity m HBV Using Dilution Procedures

Precision of Alinity m HBV, using the dilution procedures, was determined by analyzing 3 panel members prepared by spiking HBV clinical specimen (panel member 1) or synthetic DNA (panel member 2 and 3) in HBV negative human plasma. 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 results, representative of the precision of Alinity m HBV using dilution procedures, are summarized in **Table 11**.

Table 11. Precision of Alinity m HBV Using Dilution Procedures

Panel Member	Dilution Factor	N[a]	Mean Conc. (Log IU/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.

[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.

Confirmation of the LLoQ Using Dilution Procedures

LLoQ for Alinity m HBV 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. Total error was estimated by TAE and TE, as shown in **Table 12**.

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

Table 12. Total Error for Dilution Procedures

Specimen Type	Panel Member	Dilution Factor	Target Conc. in Specimen Diluent (Log IU/mL)	Target Conc. Neat (Log IU/mL)	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	1.44	0.04	0.29	0.62	0.83
	2	2.5	1.18	1.57	1.55	-0.02	0.20	0.42	0.57
	3	50	1.00	2.70	2.67	-0.03	0.24	0.50	0.67
	4	50	1.18	2.88	2.69	-0.19	0.18	0.55	0.51
Serum	1	2.5	1.00	1.40	1.48	0.08	0.31	0.71	0.89
	2	2.5	1.18	1.57	1.55	-0.02	0.27	0.56	0.77
	3	50	1.00	2.70	2.74	0.04	0.21	0.47	0.61
	4	50	1.18	2.88	2.91	0.03	0.14	0.31	0.40

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

CLINICAL PERFORMANCE

Clinical Utility Study

The study was designed to determine whether viral response as measured by Alinity m HBV is informative for determining the response to treatment in HBeAg-positive and HBeAg-negative subjects with chronic hepatitis B. The de-identified, remnant specimens tested in this study were previously collected from subjects treated with adefovir dipivoxil or placebo. Testing with Alinity m HBV was performed across 3 Alinity m Systems and 3 sites, using a total of 4 Alinity m HBV AMP Kit lots. A total of 412 subjects were included in the study. **Table 13** summarizes the demographics and baseline clinical characteristics of the included subjects. **Table 14** summarizes the numbers of subjects and associated specimens.

Table 13. Summary of Subject Demographics and Baseline Characteristics

Characteristics	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%)
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)

Table 14. Summary of Subjects and Specimens

Population	Number of 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

For each study population (HBeAg-Positive and HBeAg-Negative), the viral load measurements at two successive timepoints (baseline and Week 12) of placebo subjects were assessed by Alinity m HBV. The within-subject variability (SD) was estimated to be 0.72 Log IU/mL for HBeAg-positive subjects and 0.74 Log IU/mL for HBeAg-negative subjects. The median difference of viral load (Week 12 minus baseline) within a subject was estimated to be -0.01 Log IU/mL for HBeAg-positive subjects and -0.17 Log IU/mL for HBeAg-negative subjects. 90.2% of the HBeAg-positive subjects and 88.2% of HBeAg-negative subjects had change of viral load less than 2.00 Log IU/mL.

Determination of Response to Antiviral Treatment

Alinity m HBV testing was performed for specimens from baseline and 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.

Definitions

Viral Response

- HBV DNA <2000 IU/mL
- HBV DNA ≥2 Log IU/mL decrease from baseline

Clinical Response

- 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

Predictive Value and Odds Ratios

PPV = True Positive / (True Positive + False Positive) or the probability of clinical response given the presence of viral response

NPV = True Negative / (False Negative + True Negative) or the probability of absence of clinical response given the absence of viral response

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

HBeAg-Positive Subjects

Association Between Viral Response (<2000 IU/mL) and Clinical Responses to Treatment at Week 48

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 15**. 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 15. 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

		PPV (%)		NPV (%)		OR
Week of Viral Response	**Clinical Response**	**Estimate (95% CI)**	**n/N**	**Estimate (95% CI)**	**n/N**	**Estimate (95% CI)[a]**
12	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	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	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 associations between the combined clinical responses based on positive histologic, positive biochemical and positive HBeAg loss responses at Week 48 and viral response (<2000 IU/mL) at Weeks 12, 24, and 48 are summarized in **Table 16**.

Table 16. 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

	PPV (%)		NPV (%)		OR
Week of Viral Response	Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	35.0 (16.3, 59.1)	7/20	86.2 (76.8, 92.4)	75/87	3.37 (0.93, 11.37)
24	43.3 (26.0, 62.3)	13/30	94.6 (86.0, 98.3)	70/74	**13.38 (3.46, 61.47)**
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).

The associations between the combined clinical responses based on positive histologic, positive biochemical, and positive seroconversion responses at Week 48 and viral response (< 2000 IU/mL) at Weeks 12, 24 and 48 are summarized in **Table 17**.

Table 17. 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

	PPV (%)		NPV (%)		OR
Week of Viral Response	Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	25.0 (9.6, 49.4)	5/20	96.6 (89.5, 99.1)	84/87	**9.33 (1.57, 64.43)**
24	20.0 (8.4, 39.1)	6/30	98.6 (91.7, 99.9)	73/74	**18.25 (2.00, 850.47)**
48	15.6 (7.0, 30.1)	7/45	98.6 (91.2, 99.9)	69/70	**12.71 (1.51, 580.90)**

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

Association Between Viral Response (≥2 Log Decrease) and Clinical Responses to Treatment at Week 48

The associations between individual clinical responses at Week 48 and viral response (≥2 Log decrease) at Weeks 12, 24 and 48 are summarized in **Table 18**. 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 18. PPV, NPV, and Odds Ratio (OR) for Individual Clinical Responses During Treatment at Week 48, Associated with Viral Response (≥2 Log Decrease) in HBeAg-Positive Subjects

		PPV (%)		NPV (%)		OR
Week of Viral Response	**Clinical Response**	**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 associations between the combined clinical responses based on positive histologic, positive biochemical and positive HBeAg loss responses at Week 48 and viral response (≥2 Log decrease) at Weeks 12, 24, and 48 are summarized in **Table 19**.

Table 19. 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 Decrease) in HBeAg-Positive Subjects

	PPV (%)		NPV (%)		OR
Week of Viral Response	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 20**.

Table 20. 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 Decrease) in HBeAg-Positive Subjects

	PPV (%)		NPV (%)		OR
Week of Viral Response	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

Association Between Viral Response (<2000 IU/mL) and Clinical Responses to Treatment at Week 48

The associations between individual clinical responses (histologic and biochemical) at Week 48 and viral response (<2000 IU/mL) at Weeks 12, 24, and 48 are summarized in **Table 21**. 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 21. 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

		PPV (%)		NPV (%)		OR
Week of Viral Response	Clinical Response	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 22**.

Table 22. 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

	PPV (%)		NPV (%)		OR
Week of Viral Response	Estimate (95% CI)	n/N	Estimate (95% CI)	n/N	Estimate (95% CI)[a]
12	67.6 (49.4, 82.0)	23/34	44.7 (29.0, 61.5)	17/38	1.69 (0.58, 4.98)
24	68.0 (53.2, 80.1)	34/50	56.5 (34.9, 76.1)	13/23	2.76 (0.89, 8.65)
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).

Association Between Viral Response (≥2 Log Decrease) and Clinical Responses to Treatment at Week 48

The associations 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 23**. 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 23. 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

		PPV (%)		NPV (%)		OR
Week of Viral Response	**Clinical Response**	**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 positive biochemical responses at Week 48 and viral response (≥2 Log decrease) at Weeks 12, 24 and 48 are summarized in **Table 24**.

Table 24. 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

	PPV (%)		NPV (%)		OR
Week of Viral Response	**Estimate (95% CI)**	**n/N**	**Estimate (95% CI)**	**n/N**	**Estimate (95% CI)[a]**
12	72.2 (58.1, 83.1)	39/54	72.2 (46.4, 89.3)	13/18	**6.76 (1.81, 27.79)**
24	65.0 (51.5, 76.6)	39/60	61.5 (32.3, 84.9)	8/13	2.97 (0.74, 12.91)
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).

Conclusions

Alinity m HBV can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. The results of this study demonstrate the clinical utility of Alinity m HBV for determining on-treatment responses to therapy in the management of patients with chronic HBV infection.

Reproducibility

Nine unique panel members for Genotype A and 9 unique panel members for Genotype C covering the quantitation range of the assay were formulated in plasma to make an 18-member panel. Each panel member was repeated 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 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 different lot of Alinity m HBV CAL Kit, Alinity m HBV CTRL Kit, and Alinity m Sample Prep Kit 2. The reproducibility results are summarized in **Table 25**.

Table 25. Reproducibility of Alinity m HBV

Genotype	N[a]	Mean Conc (Log IU/mL)	Within-Run/Day Component		Between-Run/Day Component		Within-Laboratory[b]		Between-Lot Component		Between-Site Component		Total[c]	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%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
C	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.

BIBLIOGRAPHY

1. Liang TJ, Hepatitis B: the virus and disease. *Hepatology* 2009;49(5):S13-21.
2. Lok AS, McMahon BJ, Practice Guidelines Committee American Association for the Study of Liver Diseases. Chronic hepatitis B. *Hepatology* 2001;34(6):1225-41.
3. Hepatitis and Liver Cancer: A National Strategy for Prevention and Control of Hepatitis B and C. Colvin AM, Mitchell AE, eds. Washington, DC: National Academies Press; 2010.
4. World Health Organization: Newsroom; Hepatitis B Fact Sheet. https://www.who.int/en/news-room/fact-sheets/detail/hepatitis-b. Updated 27 July 2020. Accessed 10 August 2020.
5. Gentile I, Borgia G. Vertical transmission of hepatitis B virus: challenges and solutions. *Int J Womens Health* 2014;6:605-11.
6. Srivastava A, Mathias A, Yachla SK, et al. Need for immunization against hepatotropic viruses in children with chronic liver disease. *J Pediatr Gastroenterol Nutr* 2014;59(3):393-7.
7. Yeung LT, Roberts EA. Current issues in the management of paediatric viral hepatitis. *Liver Int* 2010;30(1):5-18.
8. Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* 2015;386(10003):1546-55.
9. Terrault NA, Lok ASF, McMahon BJ, et al. Update on Prevention, Diagnosis, and Treatment of Chronic Hepatitis B: AASLD 2018 Hepatitis B Guidance. *Hepatology* 2018;67(4):1560-99.
10. Laoi BN, Crowley B. Molecular characterization of hepatitis B virus (HBV) isolates, including identification of a novel recombinant, in patients with acute HBV infection attending an Irish hospital. *J Med Virol* 2008;80(9):1554-64.
11. Kramvis A. Genotypes and genetic variability of hepatitis B virus. *Intervirology* 2014;57(3-4):141-50.
12. Ramachandran S, Purdy MA, Xia, GL, et al. Recent population expansions of hepatitis B virus in the United States. *J Virol* 2014;88(24):13971-80.
13. Hollinger FB. Hepatitis B virus genetic diversity and its impact on diagnostic assays. *J Viral Hepat* 2007;14:11-5.
14. Kamitsukasa H, Iri M, Tanaka A, et al. Spontaneous reactivation of hepatitis B virus (HBV) infection in patients with resolved or occult HBV infection. *J Med Virol* 2015;87(4):589-600.
15. Charuworn P, Hengen PN, Aguilar Schall R, et al. Baseline interpatient hepatitis B viral diversity differentiates HBsAg outcomes in patients treated with tenofovir disoproxil fumarate. *J Hepatol* 2015;62(5):1033-9.
16. Wang HY, Chien MH, Huang HP, et al. Distinct hepatitis B virus dynamics in the immunotolerant and early immunoclearance phases. *J Virol* 2010;84(7):3454-63.
17. Hannoun C, Norder H, Lindh M. An aberrant genotype revealed in recombinant hepatitis B virus strains from Vietnam. *J Gen Virol*, 2000;81(9):2267-72.
18. Yu H, Yuan Q, Ge SX, et al. Molecular and Phylogenetic Analyses Suggest an Additional Hepatitis B Virus Genotype "I." *PLoS One* 2010;5(2):e9297.
19. Delwart E, Slikas E, Stramer SL, et al. Genetic diversity of recently acquired and

prevalent HIV, hepatitis B virus, and hepatitis C virus infections in US blood donors. *J Infect Dis* 2012;205(6):875-85.
20. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. Type> www.cdc.gov, search>BMBL>look up sections III and IV.].
21. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, *Bloodborne pathogens*.
22. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
23. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.

KEY TO SYMBOLS

REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
For In Vitro Diagnostic Use	For In Vitro Diagnostic Use
AMP TRAY	AMP TRAY
ACT TRAY	ACT TRAY
UNIT	Unit
	For Prescription Use Only
	Systemic Health Effects
	Warning
	Consult Instructions for Use
	Temperature Limitation
	Contains sufficient for <*n*> tests
	Use By
	Manufacturer

TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 (within the US) or +49-6122-580 (outside the US), or visit the Abbott Molecular website at www.molecular.abbott/portal.

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