Abbott RealTime

REF 2N40-80
51-608235/R1

HBV Assay Control Kit

Key to Symbols Used	
REF	List Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date
CONTROL -	Negative Control
CONTROL L	Positive Control Low
CONTROL H	Positive Control High
-10°C	Store at ≤ -10°C
	Consult instructions for use
	CAUTION: Consult accompanying documents. Handle human sourced materials as potentially infectious.
	Manufacturer

Intended Use

The Abbott RealTime HBV Controls are used to establish run validity of the Abbott RealTime HBV assay when used for the quantitation of Hepatitis B Virus (HBV) DNA in human serum and plasma (EDTA) from HBV infected individuals.

Contents

1. CONTROL - **Abbott RealTime HBV Negative Control (List No. 2G34Z) (8 vials, 1.3 mL per vial).** Negative human plasma. Negative human plasma tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by PCR methods for HIV-1 RNA, HCV RNA and HBV DNA. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. CONTROL L **Abbott RealTime HBV Low Positive Control (List No. 2G34W) (8 vials, 1.3 mL per vial).** Heat-inactivated plasma reactive for HBV DNA in negative human plasma. Negative human plasma tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by PCR methods for HIV-1 RNA, HCV RNA and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. CONTROL H **Abbott RealTime HBV High Positive Control (List No. 2G34X) (8 vials, 1.3 mL per vial).** Heat-inactivated plasma reactive for HBV DNA in negative human plasma. Negative human plasma tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by PCR methods for HIV-1 RNA, HCV RNA and HBV DNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

- Control concentrations are specified in each Abbott RealTime HBV Control Kit Card.
- The Abbott RealTime HBV Control Kit must only be used with the Abbott RealTime HBV assay (List No. 2N40-90).

Precautions

- IVD **In Vitro Diagnostic Medical Device**
- For In Vitro Diagnostic Use Only
- Do not use beyond expiration date.

CAUTION: This product contains human sourced and/or potentially infectious components. Human sourced material has been tested and found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by PCR methods for HIV-1 RNA, HCV RNA and HBV DNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. These reagents and human specimens should be handled in accordance with Biosafety In Microbiological and Biomedical Laboratories,[1] OSHA Standard on Bloodborne Pathogens,[2] CLSI Document M29-A3,[3] and other appropriate biosafety practices.[4]

The Abbott RealTime HBV Controls contain methylisothiazolines. The Abbott RealTime HBV Control Kit is classified as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:

Xi

R43	May cause sensitization by skin contact.
R36/38	Irritating to eyes and skin.
S24	Avoid contact with skin.
S25	Avoid contact with eye.
S35	This material and its container must be disposed of in a safe way.
S37	Wear suitable gloves.
S39	Wear suitable eye/face protection.
S46	If swallowed, seek medical advice immediately and show this container or label.

Consult instructions for use

-10°C **Store at ≤ -10°C**

Shipping Conditions

Ship on dry ice.

BIBLIOGRAPHY

1. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*, Fourth Edition. Washington, DC: US Government Printing Office, May 1999.
2. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens.
3. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: CLSI, 2005.
4. World Health Organization. *Laboratory Biosafety Manual*. Geneva: World Health Organization; 2004.

ProClin is a registered trademark of Rohm and Hass.

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Abbott RealTime

REF 2N40-70
51-608236/R1

HBV Assay Calibrator Kit

Key to Symbols Used	
REF	List Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
CAL A	Calibrator (A - B)
-10°C	Store at ≤ -10°C
	Consult instructions for use
	Manufacturer

Intended Use

The Abbott RealTime HBV Calibrators are for calibration of the Abbott RealTime HBV assay when used for the quantitative determination of Hepatitis B Virus (HBV) DNA in human serum and plasma (EDTA) from HBV infected individuals.

Contents

1. CAL A **Abbott RealTime HBV Calibrator A (List No. 2G34A) (12 vials, 1.3 mL per vial).** Less than 0.01% noninfectious linearized HBV DNA Plasmid in a buffer solution. Preservatives: Sodium azide and 0.15% ProClin® 950.
2. CAL B **Abbott RealTime HBV Calibrator B (List No. 2G34B) (12 vials, 1.3 mL per vial).** Less than 0.01% noninfectious linearized HBV DNA Plasmid in a buffer solution. Preservatives: Sodium azide and 0.15% ProClin 950.

- Calibrator concentrations are specified in each Abbott RealTime HBV Calibrator Kit Card.
- The Abbott RealTime HBV Calibrator Kit must only be used with the Abbott RealTime HBV assay (List No. 2N40-90).

Precautions

- IVD **In Vitro Diagnostic Medical Device**
- For In Vitro Diagnostic Use Only
- Do not use beyond expiration date.

The Abbott RealTime HBV Calibrators contain methylisothiazolines. The Abbott RealTime HBV Calibrator Kit is classified as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:

Xi

R43	May cause sensitization by skin contact.
R36/38	Irritating to eyes and skin.
S24	Avoid contact with skin.
S25	Avoid contact with eye.
S35	This material and its container must be disposed of in a safe way.
S37	Wear suitable gloves.
S39	Wear suitable eye/face protection.
S46	If swallowed, seek medical advice immediately and show this container or label.

Consult instructions for use

-10°C Store at ≤ -10°C

Shipping Conditions

Ship on dry ice.

ProClin is a registered trademark of Rohm and Hass.

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Abbott RealTime HBV

REF 2N40
51-608234/R1

Customer Service: 1-800-553-7042

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	CAL A	Calibrator A
LOT	Lot Number	CAL B	Calibrator B
IVD	In Vitro Diagnostic Medical Device	CONTROL -	Negative Control
-10°C	Store at -10°C or colder	CONTROL L	Low Positive Control
	Manufacturer	CONTROL H	High Positive Control
	Consult instructions for use	INTERNAL CONTROL	Internal Control
	Expiration Date	AMPLIFICATION REAGENT PACK	Amplification Reagent Pack
	CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. (Infection Risk)		

See REAGENTS section for a full explanation of symbols used in reagent component naming.

NAME

Abbott RealTime HBV Assay

INTENDED USE

Abbott RealTime HBV assay is an in vitro polymerase chain reaction (PCR) assay for use with the Abbott *m*2000 System$_{DNA}$ reagents and with the Abbott *m*2000*sp* and *m*2000*rt* instruments for the quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma (EDTA) from chronically HBV-infected individuals. The 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 Abbott RealTime HBV assay must be interpreted within the context of all relevant clinical and laboratory findings. Assay performance for determining the clinical stage of HBV infection has not been established. Clinical performance characteristics have been established for individuals treated with adefovir dipivoxil. This assay is not intended for use as a screening test in blood or blood products for HBV or as a diagnostic test to confirm the presence of HBV infection.

SUMMARY AND EXPLANATION OF THE TEST

HBV is a small, circular, partially double-stranded DNA virus of approximately 3,200 base pairs.[1] The virus can cause lifelong infection, cirrhosis (scarring) of the liver, liver cancer, liver failure, and death.[2] The prevalence of HBV infection and the method of transmission vary greatly around the world. In countries with a high prevalence of chronic HBV infection, the most common route of infection is from mother-to-child at birth or from child-to-child during early childhood. In areas of low prevalence, infection is usually acquired during adulthood through intravenous drug use or high-risk sexual activity.[3] The risk of developing chronic HBV infection from acute exposure ranges from 90% in newborns of HBV-infected mothers to 25-30% for children under 5 and less than 10% in adults.[1] Immunization is the most effective way to prevent HBV infection, and can offer greater than 95% protection against the development of chronic infection.[3]

Quantitation of HBV DNA is important in the evaluation and management of patients with chronic HBV infection. Current guidelines recommend HBV viral load to determine which chronic HBV patients should be treated and to monitor their response to therapy.[4,5,6] A low baseline viral load has been shown to be predictive of response to therapy.[6] Conversely, a high baseline viral load is predictive of resistance to therapy as well as relapse following therapy, and has also been found to be an independent risk factor for hepatocellular carcinoma.[7] Current treatment options include interferon, peginterferon, and antiviral drugs such as lamivudine, adefovir, and tenofovir.[5,6,8]

HBV DNA in serum or plasma can be quantitated using nucleic acid amplification signal amplification technologies.[9] The Abbott RealTime HBV assay uses PCR nology combined with homogeneous real time fluorescent detection for the quantitation of HBV DNA. The selection of a highly conserved region in the Surface gene provides for the detection of HBV genotypes A, B, C, D, E, F, G, and H. The location of the target region in the N terminal third of the Surface gene ensures that the assay is not impacted by YMDD mutants, HBsAg escape mutants, or drug-resistant mutants, as this region is essential for the assembly and secretion of subviral particles, and tolerates only minor structural changes.[10]

The assay is standardized against the World Health Organization (WHO) International Standard for Hepatitis B Virus DNA (NIBSC).[11] Results are reported in International Units per milliliter (IU/mL) or copies/mL.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTime HBV assay consists of three reagent kits:

- Abbott RealTime HBV Amplification Reagent Kit
- Abbott RealTime HBV Control Kit
- Abbott RealTime HBV Calibrator Kit

The Abbott RealTime HBV assay uses PCR to generate amplified product from the DNA genome of HBV in clinical specimens. A DNA sequence that is unrelated to the HBV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated DNA sequence is simultaneously amplified by PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. The amount of HBV target sequence that is present at each amplification cycle is measured through the use of fluorescent-labeled oligonucleotide specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is inversely proportional to the log of the HBV DNA concentration present in the original sample.

Sample Preparation

The purpose of sample preparation is to extract and concentrate nucleic acid, to make the target accessible for amplification, and to remove potential inhibitors of amplification from the extract. This process is accomplished by the *m*2000*sp*, an automated sample preparation system designed to use magnetic microparticle processes for the purification of nucleic acids from samples. The assay is suitable for use with both 0.5 mL and 0.2 mL sample input volumes.

The Abbott *m*Sample Preparation System$_{DNA}$ (4 x 24 Preps) reagents lyse the virion, capture the nucleic acids, and wash the particles to remove unbound sample components. Proteinase K is included in the lysis step to digest proteins associated with the nucleic acids.[12,13] The bound nucleic acids are eluted and transferred to a 96-deep well plate. The nucleic acids are then ready for amplification. The IC is introduced into the sample preparation procedure and is processed along with the calibrators, controls, and specimens.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m*2000*sp* combines the Abbott RealTime HBV amplification reagent components (HBV Oligonucleotide Reagent, DNA Polymerase, and Activation Reagent). The Abbott *m*2000*sp* dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate along with aliquots of the nucleic acid samples prepared by the Abbott *m*2000*sp*. After manual application of the Abbott Optical Adhesive Cover, the plate is ready for transfer to the Abbott *m*2000*rt*.

Amplification

During the amplification/detection reaction on the *m*2000*rt* instrument, the target DNA is amplified by the DNA Polymerase in the presence of deoxynucleotide triphosphates (dNTPs) and magnesium. First, the HBV and IC primers anneal to their respective targets and are extended by the polymerase. After a denaturation step in which the temperature of the reaction is raised above the melting point of the double-stranded DNA product, the newly created DNA strand is denatured from the target DNA.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature, allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the target is achieved through repeated cycling between high and lower temperatures. Amplification of both targets (HBV and IC) takes place simultaneously in the same reaction.

The target sequence for the Abbott RealTime HBV assay is in the Surface gene in the HBV genome. This region is specific for HBV and is highly conserved. The primers are designed to hybridize to this region with the fewest possible mismatches among HBV genotypes A through H.

The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant *Cucurbita pepo*, and is provided as a DNA plasmid in a buffer solution.

Detection

The presence of HBV amplification products is detected during the extension/anneal step by measuring the fluorescence of the HBV probe that binds to the target during the extension/anneal step. Similarly, the presence of IC amplification is detected during the extension/anneal step by measuring the fluorescence of the IC probe.

The HBV and IC probes are single-stranded DNA oligonucleotides consisting of a probe sequence with a fluorescent moiety that is covalently linked to the 5' end of the probe and a quenching moiety that is covalently linked to the 3' end of the probe.

In the absence of the HBV or IC target sequences, probe fluorescence is quenched. In the presence of HBV or IC target, the HBV or IC probes specifically bind to their target. During the extension/anneal step, the DNA polymerase cleaves, or nucleolytically digests, the bound probe as it moves along the template strand. This separates the fluorophore from the quencher, allowing fluorescent emission and detection.

The HBV and IC probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is inversely proportional to the log of the HBV DNA concentration present in the original sample.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- The Abbott RealTime HBV assay performs PCR amplification and oligonucleotide hybridization in a sealed 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the 96-Well Optical Reaction Plate.
- Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
- Separate dedicated areas are used to perform the Abbott RealTime HBV assay. Refer to the **SPECIAL PRECAUTIONS** section of this package insert.

REAGENTS

Abbott RealTime HBV Amplification Reagent Kit (List No. 2N40-90)

1. INTERNAL CONTROL **Abbott RealTime HBV Internal Control (List No. 2G34Y)**
   (4 vials, 0.2 mL per vial)
   - Less than 0.01% noninfectious linearized DNA plasmid in a buffer solution with carrier DNA. Preservatives: 0.085% Sodium azide and 0.15% ProClin® 950.
2. AMPLIFICATION REAGENT PACK **Abbott RealTime HBV Amplification Reagent Pack (List No. 2N40)**
   (4 packs, 24 tests/pack)
   - 1 bottle (0.078 mL) DNA Polymerase (5.4 to 5.9 Units/µL) in a buffered solution with stabilizers.
   - 1 bottle (0.917 mL) HBV Oligonucleotide Reagent. Less than 0.1% synthetic oligonucleotides (4 primers and 3 probes), and less than 0.2% dNTPs in a buffered solution with a reference dye. Preservatives: 0.085% Sodium azide and 0.15% ProClin 950.
   - 1 bottle (0.778 mL) Activation Reagent. 38 mM magnesium chloride in a buffered solution. Preservatives: 0.085% Sodium azide and 0.15% ProClin 950.

Abbott RealTime HBV Control Kit (List No. 2N40-80)

1. CONTROL − **Abbott RealTime HBV Negative Control (List No. 2G34Z)**
   (8 vials, 1.3 mL per vial)
   - Negative human plasma found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
2. CONTROL L **Abbott RealTime HBV Low Positive Control (List No. 2G34W)**
   (8 vials, 1.3 mL per vial)
   - Heat-inactivated plasma reactive for HBV DNA in negative human plasma. Negative human plasma found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.
3. CONTROL H **Abbott RealTime HBV High Positive Control (List No. 2G34X)**
   (8 vials, 1.3 mL per vial)
   - Heat-inactivated plasma reactive for HBV DNA in negative human plasma. Negative human plasma found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA. Preservatives: 0.1% ProClin 300 and 0.15% ProClin 950.

Abbott RealTime HBV Calibrator Kit (List No. 2N40-70)

1. CAL A **Abbott RealTime HBV Calibrator A (List No. 2G34A)**
   (12 vials, 1.3 mL per vial)
   - Less than 0.01% noninfectious linearized HBV DNA plasmid in a buffer solution. Preservatives: 0.085% Sodium azide and 0.15% ProClin 950.
2. CAL B **Abbott RealTime HBV Calibrator B (List No. 2G34B)**
   (12 vials, 1.3 mL per vial)
   - Less than 0.01% noninfectious linearized HBV DNA plasmid in a buffer solution. Preservatives: 0.085% Sodium azide and 0.15% ProClin 950.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

For In Vitro Diagnostic Use Only.

The Abbott RealTime HBV assay is not intended for use in the screening of blood, plasma, or tissue donors for HBV, or to be used as a diagnostic test to confirm the presence of HBV infection.

Use only USP Grade 190-200 Proof Ethanol (95-100% Ethanol) to prepare the *m*Wash2$_{DNA}$ sample preparation reagent. **Do not use ethanol that contains denaturants.**

Safety Precautions

Refer to the Hazard Sections of the Abbott *m*2000*sp* Operations Manual and the Abbott *m*2000*rt* Operations Manual for instructions on safety precautions.

⚠ **CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the **REAGENTS** section of this package insert. Negative human plasma has been found to be nonreactive by FDA licensed tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, and HBsAg. The material is also tested and found to be negative by FDA licensed PCR methods for HIV-1 RNA and HCV RNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious, using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[14] OSHA Standard on Bloodborne Pathogens,[15] CLSI Document M29-A3,[16] and other appropriate biosafety practices.[17] Therefore, all human sourced materials should be considered potentially 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.[18,19]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[20,21]

The Abbott RealTime HBV Calibrators and Controls contain methylisothiazolines as components of ProClin and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases:

R43 May cause sensitization by skin contact.
R36/38 Irritating to eyes and skin.
S24 Avoid contact with skin.
S25 Avoid contact with eyes.
S35 This material and its container must be disposed of in a safe way.
S37 Wear suitable gloves.
S39 Wear suitable eye/face protection.
S46 If swallowed, seek medical advice immediately and show this container or label.

Special Precautions

Handling Precautions

The Abbott RealTime HBV assay is only for use with human serum and plasma specimens that have been handled and stored in capped tubes as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.

During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with DNA.

Amplification reactions such as PCR are sensitive to accidental introduction of product from previous amplification reactions. False positive results could occur if either the clinical specimen or the RealTime reagents used in the amplification step become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas

Use two dedicated areas within the laboratory for performing the Abbott RealTime HBV assay.

- The **Sample Preparation Area** is dedicated to processing samples (specimens, Abbott RealTime HBV Controls and Calibrators) and to adding processed samples, controls, and calibrators to the Abbott 96-Well Optical Reaction Plate. The Abbott *m*2000*sp* combines the Abbott RealTime HBV amplification reagent components to create the amplification master mix and transfers aliquots of the master mix to the reaction plate. **All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area. Do not bring amplification product into the Sample Preparation Area.**
- The **Amplification Area** is dedicated to the amplification and detection of amplified product. **Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.**

Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.

Do not use kits or reagents beyond expiration date.

Work areas and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, negative control, positive controls, calibrators, or specimens. Refer to the Abbott *m*2000*sp* Operations Manual and the Abbott *m*2000*rt* Operations Manual for instrument cleaning procedures.

If the Abbott *m*2000*sp* instrument run is aborted, dispose of all commodities and reagents according to the Abbott *m*2000*sp* Operations Manual. If the Abbott *m*2000*sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the *m*2000*sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.

If the Abbott *m*2000*rt* instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.

Decontaminate and dispose of all specimens, reagents, and other potentially biohazardous materials in accordance with local, state, and federal regulations.[20,21] All materials should be handled in a manner that minimizes the chance of potential contamination of the work area. **Note: Autoclaving the sealed Reaction Plate will not eliminate the amplified product and may contribute to the release of the amplified product by opening of the seal. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained before and after processing.**

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only one time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m2000sp* or the Abbott *m2000rt* Operations Manuals.

Contamination and Inhibition

The following precautions should be observed to minimize the risks of DNase contamination, cross-contamination between samples, and inhibition:

- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of 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.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- Clean and disinfect spills of specimens and reagents as stated in the Abbott *m2000sp* and the Abbott *m2000rt* Operations Manuals, Hazards section.
- Replace any empty or partially used 200 µL and 1000 µL disposable tip trays with full trays before every run.
- The Abbott *m*Sample Preparation System$_{DNA}$ (4 x 24 Preps) and Abbott Proteinase K reagents are single use only. Use new reagent vessels, reaction vessels, and newly opened reagents for every new Abbott RealTime HBV assay run. At the end of each run, discard all remaining reagents from the worktable as stated in the Abbott *m2000sp* Operations Manual and the Abbott *m*Sample Preparation System$_{DNA}$ (4 x 24 Preps) and Abbott Proteinase K package inserts.

STORAGE INSTRUCTIONS

Abbott RealTime HBV Amplification Reagent Kit (List No. 2N40-90)

-10°C The Abbott RealTime HBV Amplification Reagent Pack and Internal Control vials must be stored at -10°C or colder when not in use. Care must be taken to separate the Abbott RealTime HBV Amplification Reagent Pack that is in use from direct contact with samples, calibrators, and controls.

Abbott RealTime HBV Control Kit (List No. 2N40-80)

-10°C The Abbott RealTime HBV Negative and Positive Controls must be stored at -10°C or colder.

Abbott RealTime HBV Calibrator Kit (List No. 2N40-70)

-10°C The Abbott RealTime HBV Calibrator A and Calibrator B must be stored at -10°C or colder.

SHIPPING CONDITIONS

- Abbott RealTime HBV Amplification Reagent Kit: Ship on dry ice.
- Abbott RealTime HBV Control Kit: Ship on dry ice.
- Abbott RealTime HBV Calibrator Kit: Ship on dry ice.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

When a positive or negative control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested. Assay recalibration may be necessary. Refer to the **QUALITY CONTROL PROCEDURES: Assay Calibration** section of this package insert for details.

If you receive reagents, calibrators, or controls that are in a condition contrary to label recommendation, or that are damaged, contact Abbott Molecular Technical Services.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

Human serum and plasma (EDTA) specimens may be used with the Abbott RealTime HBV assay. Follow the manufacturer's instructions for processing collection tubes.

Freshly drawn specimens (whole blood) may be held at 2 to 30°C for up to 6 hours prior to centrifugation.

After centrifugation, remove serum or plasma from cells. Serum or plasma specimens may be stored:

- At 15 to 30°C for up to 24 hours
- At 2 to 8°C for up to 3 days
- At -20°C or colder for longer term

Multiple freeze-thaw cycles should be avoided. If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if specimens are not being processed immediately, they can be stored at 2 to 8°C for up to 6 hours.

Specimen Transport

Ship specimens frozen on dry ice. For domestic and international shipments, specimens should be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical, diagnostic, or biological specimens.

INSTRUMENT PROCEDURE

The Abbott RealTime HBV application file(s) must be installed on the Abbott *m2000sp* and Abbott *m2000rt* instruments from the Abbott RealTime HBV *m*2000 System Combined Application CD-ROM prior to performing the assay. For detailed information on application file installation, refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals, Operating Instructions section.

ABBOTT REALTIME HBV ASSAY PROCEDURE

Materials Provided

- Abbott RealTime HBV Amplification Reagent Kit (List No. 2N40-90)

Materials Required But Not Provided

- Abbott RealTime HBV Control Kit (List No. 2N40-80)
- Abbott RealTime HBV Calibrator Kit (List No. 2N40-70)

Sample Preparation Area

- Abbott *m2000sp*
- Abbott *m*Sample Preparation System$_{DNA}$ (4 x 24 Preps) (List No. 06K12-24)
- Abbott Proteinase K (List No. 3L78-60)
- Abbott RealTime HBV *m*2000 System Combined Application CD-ROM (List No. 2N43)
- Sample Racks
- 5 mL Reaction Vessels
- 200 mL Reagent Vessels
- Master Mix Vial
- Abbott 96-Well Optical Reaction Plate
- Abbott 96-Deep Well Plate
- Abbott Splash-Free Support Base
- Abbott Optical Adhesive Cover
- Abbott Adhesive Cover Applicator
- Round-bottom 12.5 x 75 mm Sample Tubes
- Vortex Mixer
- 50 mL Polypropylene Centrifuge Tubes
- Centrifuge capable of 2,000*g*
- Calibrated Precision Pipettes capable of delivering 10 µL-1000 µL
- 20 µL-1000 µL Aerosol Barrier Pipette Tips for precision pipettes
- Serological Pipettes
- Graduated Cylinder, 100 mL
- USP Grade 190-200 Proof Ethanol (95-100% Ethanol). **Do not use ethanol that contains denaturants.**
- Molecular Biology Grade Water
- 1.7 mL Molecular Biology Grade Microcentrifuge Tubes (Dot Scientific, Inc. or equivalent)*
- Cotton Tip Applicators (Puritan or Equivalent)*

Note: These items are used in the procedure for Monitoring the Laboratory for the Presence of Amplification Product. Refer to the **QUALITY CONTROL PROCEDURES section of this package insert.*

Amplification Area

- Abbott *m2000rt*
- Abbott RealTime HBV *m*2000 System Combined Application CD-ROM (List No. 2N43)
- Abbott *m2000rt* Optical Calibration Kit (List No. 4J71-93)

Other Materials

- Biological safety cabinet approved for working with infectious materials.
- Sealable plastic bags

Procedural Precautions

Read the Instructions in this package insert carefully before processing samples.

The Abbott RealTime HBV Calibrators, Internal Control, Negative Control, and Low and High Positive Control vials are intended for single-use only and should be discarded after use.

Sample tubes should be inspected for air bubbles. If found, remove them with a sterile pipette tip. Reagent bubbles may interfere with proper detection of reagent levels in the reagent vessel, causing insufficient reagent aspiration, which could impact results. **Caution should be taken to avoid cross-contamination between samples by using a new sterile pipette tip for each tube.**

Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens, controls, calibrators, or Amplification Reagents. 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.

Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in this package insert.

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% sodium hypochlorite or other suitable disinfectant.

A calibration curve must be established before specimens are tested. The use of the Abbott RealTime HBV Calibrators and Controls is integral to the performance of the Abbott RealTime HBV assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for details.

ASSAY PROTOCOL

Sample Preparation Area

All specimen storage and preparation must take place in the dedicated Sample Preparation Area. Refer to the **Handling Precautions** section of this package insert for instructions before preparing samples.

For a detailed description of how to operate the Abbott *m2000sp* instrument and Abbott *m2000rt* instrument, refer to the Abbott *m2000sp* and *m2000rt* Operations Manuals, Operating Instructions section.

Laboratory personnel must be trained to operate the Abbott *m2000sp* and *m2000rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

1. A maximum of 48 samples can be processed in each run. A negative control, a low positive control, and a high positive control must be included in each run, therefore allowing a maximum of 45 specimens to be processed per run.

   NOTE: If performing a run of more than 24 samples, empty the solid waste container before the run and replace with a new biohazard bag if any waste is present.

   - Check sample volume. The Abbott RealTime HBV assay minimum sample volume and associated rack requirements on the Abbott *m2000sp* are:

		Abbott RealTime HBV Minimum Sample Volume Assay Application	
Rack	Tube Diameter*	0.2 mL	0.5 mL
13 mm	11.5 mm - 14.0 mm	0.4 mL - 0.8 mL	0.7 mL - 1.2 mL
16 mm	14.5 mm - 16.0 mm	0.4 mL - 1.0 mL	0.8 mL - 1.4 mL

   * Refers to the sample tube outer diameter.

   - Minimum sample volume varies with tube geometry and size. Refer to the *m2000sp* Operations Manual and QUICK REFERENCE GUIDE FOR SAMPLE TUBE SIZES AND VOLUMES for recommended sample input volume.
   - If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, if specimens are not being processed immediately, store at 2 to 8°C for up to 6 hours.
   - Before use, vortex specimens three times for 2 to 3 seconds. **Ensure that bubbles or foam are not created.** If found, remove them with a new sterile pipette tip for each tube. **Specimens showing particulate matter or turbidity should be clarified by centrifugation at 2,000*g* for 5 minutes prior to testing.** Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m2000sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.

2. Thaw assay controls and Internal Control (IC) at 15 to 30°C or at 2 to 8°C. Thaw calibrators at 15 to 30°C or at 2 to 8°C only if performing a calibration run; see **QUALITY CONTROL PROCEDURES** section of this package insert.
   - Once thawed, if calibrators, controls, and IC are not being processed immediately, store at 2 to 8°C for up to 24 hours.
   - Vortex each assay calibrator and each control three times for 2 to 3 seconds before use. Ensure that bubbles or foaming are not created. If found, remove them with a new sterile pipette tip for each tube. Ensure that the contents of the vials are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vials.
3. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C until required for the amplification master mix procedure. This step can be initiated before completion of the sample preparation procedure.

   Note: Do not vortex the Amplification Reagent Pack.

   - Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.
4. Open the Abbott Proteinase K reagent pack. Add 17.15 mL of Molecular Biology Grade water to a 50 mL polypropylene centrifuge tube. Pipet 2.45 mL of Proteinase K into the container of water. Mix by gentle inversion 10 to 15 times. Transfer the entire contents to a reagent vessel labeled with the Proteinase K barcode label. Place the reagent vessel in reagent carrier #1 location 2.

   NOTE: The following steps 5 through 9 pertain to the use of the *m*Sample Preparation System$_{DNA}$ kit (List No. 06K12-24).

   NOTE: Use one bottle of Proteinase K solution, one set of the *m*Sample Preparation System$_{DNA}$ reagents, one vial of IC, and one RealTime HBV Amplification Reagent Pack to support up to 24 reactions. Use a second set of reagents to support 25 to 48 reactions, with the exception of the *m*Microparticles$_{DNA}$. One bottle of mMicroparticles$_{DNA}$ will support up to 48 reactions. **Do not use more than one bottle of *m*Microparticles$_{DNA}$.**

5. Open the Abbott *m*Sample Preparation pack. If crystals are observed in any of the reagent bottles upon opening, allow the reagents to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved.
6. Prepare the *m*Wash2$_{DNA}$ by adding 70 mL of USP Grade 190-200 Proof Ethanol (95-100% Ethanol) to the *m*Wash2$_{DNA}$ bottle as described in the Abbott *m*Sample Preparation System$_{DNA}$ product information. **Do not use ethanol that contains denaturants.**
7. Vortex the IC vial(s) three times for 2 to 3 seconds before use.
8. Using a calibrated precision PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY, add 100 µL of IC to a bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming.
9. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution and pour the contents into the appropriate reagent vessels per the Abbott *m2000sp* Operations Manual, Operating Instructions.
10. Place the low and high positive controls, the negative control, the calibrators (if applicable), and the patient specimens into the *m2000sp* sample rack.
11. Place the 5 mL Reaction Vessels into the *m2000sp* 1 mL subsystem carrier.
12. Load the carrier racks containing the Abbott *m*Sample Preparation reagents and Proteinase K, and the Abbott 96-Deep Well Plate, on the Abbott *m2000sp* worktable as described in the Abbott *m2000sp* Operations Manual, Operating Instructions.
13. From the Run Sample Extraction screen, select the appropriate application file corresponding to the sample volume being tested. Initiate the sample extraction protocol as described in the *m2000sp* Operations Manual, Operating Instructions.
    - Enter calibrator (needed if a calibration curve has not been stored on the *m2000rt*) and control lot specific values in the **Sample Extraction: Assay Details** screen. Lot specific values are specified in each Abbott RealTime HBV Calibrator and Control Kit card.

      NOTE: Verify the values entered match the values on the kit cards.

    - **The Abbott *m2000sp* Master Mix Addition protocol (step 14) must be initiated within 60 minutes after completion of the Sample Extraction protocol.**

      NOTE: Change gloves before handling the amplification reagents.

14. Load the amplification reagents, the master mix vial, and the 96-well Optical Reaction Plate on the *m2000sp* worktable after sample preparation is completed.
    - Each Amplification Reagent Pack supports up to 24 reactions.
    - Ensure the amplification reagents are thoroughly thawed before use.
    - Prior to opening the amplification reagents, ensure that the contents are at the bottom of the vials by tapping the vials in an upright position on the bench.
    - Remove and discard the amplification vial caps.
    - A second Amplification Reagent Pack is required if performing 25 to 48 reactions.
15. Select the appropriate deep well plate from the Run Master Mix Addition screen that matches the corresponding sample preparation extraction. Initiate the Abbott *m2000sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m2000sp* Operations Manual, Operating Instructions section.
    - **The *m2000rt* protocol (step 20) must be started within 60 minutes of the completion of the Master Mix Addition protocol (step 15).**

Amplification Area

16. Switch on and initialize the Abbott *m2000rt* in the Amplification Area.
    - **The Abbott *m2000rt* requires 15 minutes to warm up.**

      NOTE: Remove gloves before returning to the Sample Preparation Area.

Sample Preparation Area

17. Seal the Abbott 96-Well Optical Reaction Plate after the Abbott *m2000sp* instrument has completed addition of samples and master mix according to the Abbott *m2000sp* Operations Manual, Operating Instructions section.

    NOTE: Since a maximum of 48 samples can be processed in each run, the 96-Well Optical Reaction Plate will contain empty wells.

18. Place the 96-Well Optical Reaction Plate into the Splash-Free Support Base for transfer to the Abbott *m2000rt* instrument.
19. Export the completed 96-Well Optical Reaction Plate results to a CD or Network Drive.

Amplification Area

20. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m2000rt* instrument. Import the *m2000sp* test order via CD or Network Drive per the Import Order instructions in the Abbott *m2000rt* Operations Manual, Operating Instructions section.

POST PROCESSING PROCEDURES

21. Remove the Abbott 96-Deep Well Plate from the worktable and dispose according to the Abbott *m2000sp* Operations Manual.
22. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m2000rt* Operations Manual, along with the gloves used to handle the plate.
23. Clean the Splash Free Support Base before next use, according to the Abbott *m2000rt* Operations Manual.

QUALITY CONTROL PROCEDURES

Abbott *m2000rt* Optical Calibration

Refer to the Calibration Procedures section in the Abbott *m2000rt* Operations Manual for a detailed description of how to perform an Abbott *m2000rt* Optical Calibration.

Optical calibration of the Abbott *m2000rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTime HBV assay.

The following Abbott *m2000rt* Optical Calibration Plates are used to calibrate the Abbott *m2000rt* instrument for the Abbott RealTime HBV assay:

- FAM™ Plate (Carboxyfluorescein)
- ROX™ Plate (Carboxy-X-rhodamine)
- VIC® Plate (Proprietary dye)

Assay Calibration

A calibration curve is required to quantitate HBV DNA in the specimens and controls. Two assay calibrators are run in replicates of three to generate a calibration curve (HBV concentration [log IU/mL] versus the threshold cycle [Ct] at which a reactive level of fluorescent signal is detected). The lot specific values for Calibrator A and Calibrator B are specified on each Abbott RealTime HBV Calibrator Kit Card and must be entered into the assay test order when a run is performed. The calibration curve slope and intercept are calculated and stored on the instrument. The concentration of HBV DNA in a sample is calculated from the calibration curve. Results are automatically reported on the *m2000rt* workstation.

The Low and High Positive Controls and Negative Control must be included in the calibration run.

Follow the procedure for sample extraction, reagent addition, amplification and detection protocols as stated in the Abbott *m2000sp* Operations Manual and the *m2000rt* Operations Manual. Ensure that assay control values observed in the final report are within the ranges specified on the Abbott RealTime HBV Control Kit Card.

Once an Abbott RealTime HBV calibration is accepted and stored, it may be used for 6 months. During this time, all subsequent samples may be tested without further calibration unless:

- An Abbott RealTime HBV Amplification Reagent Kit with a new lot number is used.
- An Abbott *m*Sample Preparation System$_{DNA}$ (4 x 24 Preps) with a new lot number is used.
- An Abbott RealTime HBV application specification file for a different sample volume is used.
- An updated version of the Abbott RealTime HBV application specification file is installed.

Detection of Inhibition

An IC threshold cycle [Ct] assay validity parameter is established during a calibration run.

Prior to sample preparation, a defined, consistent quantity of the IC is introduced into the lysis buffer, which is then used during the processing of each specimen, calibrator, and control, and measured on the *m2000rt* instrument to demonstrate proper sample processing and assay validity. The IC is composed of a DNA sequence unrelated to the HBV DNA sequence.

The median amplification cycle at which the IC target sequence fluorescent signal is detected in calibration samples establishes the IC Ct validity range to be met by all subsequent processed specimens using that calibration curve.

An error is displayed when a specimen or control fails to meet this specification. Refer to the Abbott *m2000rt* Operations Manual for an explanation of the corrective actions for the error code. Specimens whose IC Ct value falls outside of the established range must be retested starting with sample preparation.

Negative and Positive Controls

A negative control, a low positive control, and a high positive control are included in each run to evaluate run validity.

The lot specific values for the low positive control and high positive control are specified on each Abbott RealTime HBV Control Kit Card and must be entered into the test order when a run is performed.

An error is displayed when a control result is out of range. Refer to the Abbott *m2000rt* Operations Manual for an explanation of the corrective actions for the error code. If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

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 introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, clean the Abbott *m2000sp* and *m2000rt* instruments and repeat the sample processing for controls and specimens following the Procedural Precautions. If negative controls are persistently reactive, contact your Area Customer Support representative.

Monitoring the Laboratory for the Presence of Amplification Product

It is recommended that this test be done at least once a month to monitor laboratory surfaces and equipment for contamination by amplification product. It is very important to test all areas that may have been exposed to processed specimens and controls, calibrators, and/or amplification product. This includes routinely handled objects such as pipettes, the Abbott *m2000sp* and *m2000rt* function keys, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.8 mL Molecular Biology Grade water to a 1.7 mL DNase-free microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the Molecular Biology Grade water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
4. Swirl the cotton tip in Molecular Biology Grade water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
5. Pipette 0.5 mL of the *m*Wash 1 buffer to a clean tube using the pipette dedicated for Internal Control use.
6. Add 20 µL of the *m*Wash 1 buffer to each microcentrifuge tube.
7. Cap the microcentrifuge tube.
8. Test this sample according to the assay procedure section of this package insert.
9. Transfer liquid from microcentrifuge tube to a 5 mL Reaction Vessel.
10. Bring the volume to 1.5 mL with Molecular Biology Grade water.
11. The presence of contamination is indicated by the detection of HBV in the swab samples.
12. If HBV is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual. If HBV is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water. Note: Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol or water until chlorine residue is no longer visible.
13. Repeat testing of the contaminated area by following Steps 1 through 10.

RESULTS

Calculation

The concentration of HBV DNA in a sample or control is calculated from either a stored calibration curve, or a calibration curve created by calibrators within a calibration or sample run. The Abbott *m2000rt* instrument automatically reports the results on the *m2000rt* workstation. Assay results are reported in IU/mL or log IU/mL. Results can also be reported in copies/mL or log copies/mL using a conversion factor of 3.41 (1 IU = 3.41 copies). **Note: The assay is calibrated to the WHO International HBV DNA Standard. The 3.41 conversion factor is based on an average conversion factor across the assay dynamic range.**

The following table represents the potential *m2000rt* outputs that can be observed by the user.

Interpretation of Results

Sample Volume	Result	Interpretation
0.5 mL	Not Detected	Target not detected
	< 1.00 Log IU/mL[a]	Detected[c]
	1.00 to 9.00 Log IU/mL	[d]
	> 9.00 Log IU/mL	> ULQ[e]
0.2 mL	Not Detected	Target not detected
	< 1.18 Log IU/mL[b]	Detected[c]
	1.18 to 9.00 Log IU/mL	[d]
	> 9.00 Log IU/mL	> ULQ[e]

[a] 10 IU/mL
[b] 15 IU/mL
[c] Below LLQ (lower limit of quantitation or LLoQ); HBV DNA is not quantifiable.
[d] Calculated results are within assay linear range. If a calculated result is obtained, the interpretation field is left blank.
[e] > ULQ – above upper limit of quantitation or ULoQ; if IU/mL results are above the linear range of the assay, results are reported as ">1,000,000,000 IU/mL HBV DNA."

If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

If quantitative results are desired for those specimens reported as > ULQ (ULoQ), the original specimen should be diluted 1:50 with HBV-negative human plasma or serum (consistent with the matrix of the original specimen), and the test repeated. Multiply the reported result by the dilution factor of 50 to obtain the quantitative result. Example calculations are provided below.

Example result for diluted specimen	Calculate 10^x (x = result in log unit); round to whole number	Multiply whole number result by 50 (dilution factor) to obtain result corrected for dilution	Calculate log (y) (y = whole number result)
7.59 log IU/mL	38,904,515 IU/mL	1,945,225,750 IU/mL	9.29 log IU/mL
63,095,734 IU/mL		3,154,786,700 IU/mL	
8.80 log copies/mL	630,957,345 copies/mL	31,547,867,250 copies/mL	10.50 log copies/mL
49,905,245 copies/mL		2,495,262,250 copies/mL	

LIMITATIONS OF THE PROCEDURE

- **FOR IN VITRO DIAGNOSTIC USE ONLY.**
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this package insert).
- Human serum and plasma (EDTA) may be used with the Abbott RealTime HBV assay. The use of other anticoagulants has not been validated for use with the Abbott RealTime HBV assay.
- The use of specimens collected in serum tubes that contain Z-clot activator, or similar types of rapid clot activator, may cause inhibited results in the RealTime HBV assay. Therefore, serum collection tubes containing Z-clot activator or similar rapid clot activators should not be used.
- 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.
- In rare cases, a very low level positive result may occur from cross contamination during processing of an extremely high copy number adjacent specimen. Carryover rates in representative studies ranged from 0% to 2%. Per treatment guidelines, a 1 log increase is needed in order to impact patient management.[22,23] In addition, treatment guidelines require two consecutive elevated measurements to occur before changing patient management.[24]
- A single Entecavir mutation (rtA97V) occurs within the reverse primer. Of all known resistance mutations, it is the only one that occurs within any Abbott RealTime HBV primer or probe sequence. Software simulation predicts that this mutation (rtA97V) would not be expected to interfere with assay results when using RealTime HBV assay conditions.
- A specimen with a result of "Not Detected" cannot be presumed to be negative for HBV DNA.

- Precision was established with HBV Genotypes A and C only.
- Drug interference was evaluated using a plasma matrix, and was not evaluated in serum. The listed drugs were tested in pools, and individual drug effects were not assessed.
- The interference studies were performed with an HBV DNA concentration of 2,933 IU/mL (3.47 log IU/mL). Potential interference on HBV DNA concentrations close to the assay LLQ (LLoQ) was not assessed.
- Some of the cross-reactivity studies were performed with nucleic acids (DNA and RNA) only. For further detail, refer to the **SPECIFIC PERFORMANCE CHARACTERISTICS** section of this package insert.
- Results from the Abbott RealTime HBV assay should be interpreted in conjunction with other clinical and laboratory findings.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were determined using the RealTime HBV assay with the 0.5 mL sample preparation procedure, unless otherwise specified.

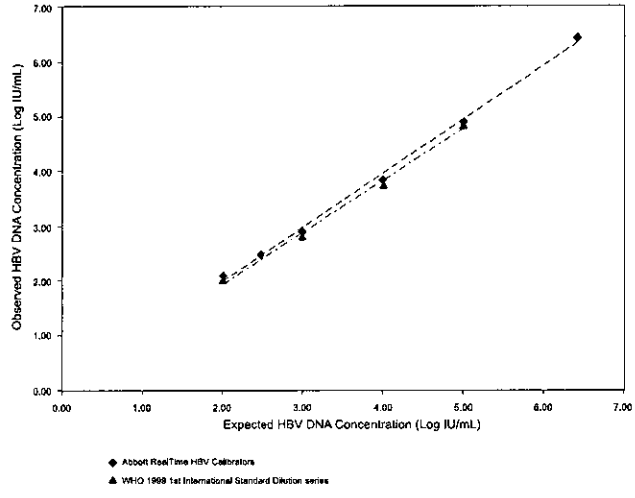
WHO STANDARDIZATION

Figure 1 demonstrates the comparison of Abbott RealTime HBV Assay Calibrators to the WHO International HBV DNA Standard. Abbott RealTime HBV Calibrators trace to the World Health Organization (WHO) International Standard for Hepatitis B Virus DNA (NIBSC) each time a lot is manufactured. Each lot of calibrator is specifically assigned a quantitation value through testing with HBV Primary Calibrators, which are directly tested against the WHO standard. The lot-specific quantitation values for each HBV calibrator are entered into the *m2000rt* software when a run is being performed.

The evaluation was conducted with the WHO 1st International HBV DNA Standard, and one lot of HBV Calibrators, and was performed on one run. The WHO standard was reconstituted to a concentration of 1 x 10^5 IU/mL and then diluted to 1 x 10^4, 1 x 10^3, and 1 x 10^2 IU/mL in negative human plasma. The highest assay calibrator, Calibrator B, which is lot-assigned at 6.42 log IU/mL, was diluted to 1 x 10^5,1 x 10^4, 1 x 10^3, and 1 x 10^2 IU/mL in Tris-EDTA (TE) buffer. The data for Calibrator B and its dilution series are presented in comparison to the WHO standard dilution series in Figure 1. The results indicate that the assay standardization process provides quantitation values for the RealTime HBV Calibrators and the WHO standard that are similar to the expected values, with deviation of not more than 0.33 log IU/mL. The maximum deviation was obtained at the assay ULQ (ULoQ).

Figure 1

Comparison of WHO 1st International HBV DNA Standard with Abbott RealTime HBV Calibrators



LINEAR RANGE

The ULQ (ULoQ) for the Abbott RealTime HBV assay is 10^9 IU/mL (9.00 log IU/mL) and the LLQ (LLoQ) is equivalent to the LoD, which is 10 IU/mL (1.00 log IU/mL) for the 0.5 mL sample preparation protocol and 15 IU/mL (1.18 log IU/mL) for the 0.2 mL sample preparation protocol.

In one study, a 13-member panel prepared by diluting an HBV-positive specimen targeted from 9.13 log IU/mL to 0.29 log IU/mL in HBV negative human plasma was tested and evaluated in accordance with methods defined in the CLSI EP6-A,[25] using the 0.5 mL sample preparation protocol. The Abbott RealTime HBV assay was shown to be linear in plasma across the range of HBV DNA concentrations tested (shown in Figure 2) with deviation from linearity of not more than 0.20 log IU/mL.

In a second study, one panel consisting of Genotype A and one panel consisting of Genotype C were tested. The two 10-member panels were prepared by diluting to concentrations targeted from 1.27 log IU/mL to 8.47 log IU/mL for Genotype A and 1.59 log IU/mL to 8.79 log IU/mL for Genotype C. The two panels were prepared with high copy HBV-positive specimens diluted in HBV serologically-negative human plasma. Least squares linear regression analysis was performed for Genotypes A and C separately. Analysis for Genotype A is shown in Figure 3 and analysis for Genotype C is shown in Figure 4. The Abbott RealTime HBV assay was shown to be linear in plasma across the range of HBV DNA concentrations tested for HBV Genotype A and HBV Genotype C.

Figure 2

Abbott RealTime HBV Linear Range Least Squares Linear Regression Analysis

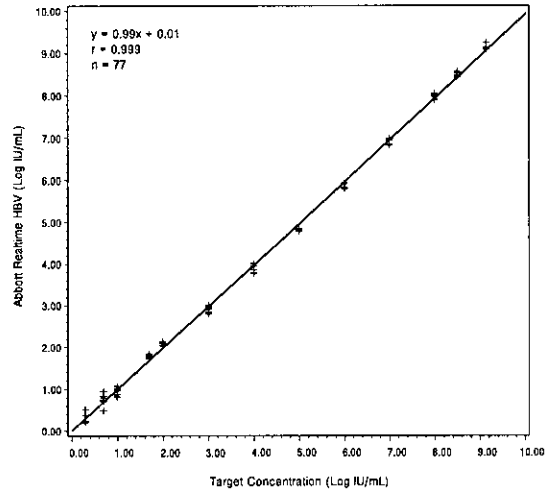


Figure 3

Abbott RealTime HBV Linear Range Least Squares Linear Regression Analysis Genotype A

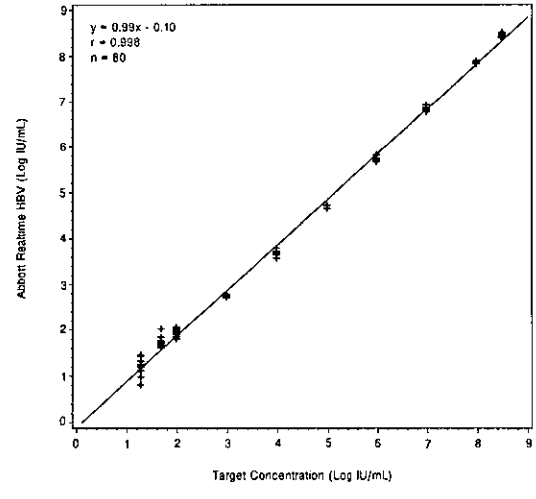
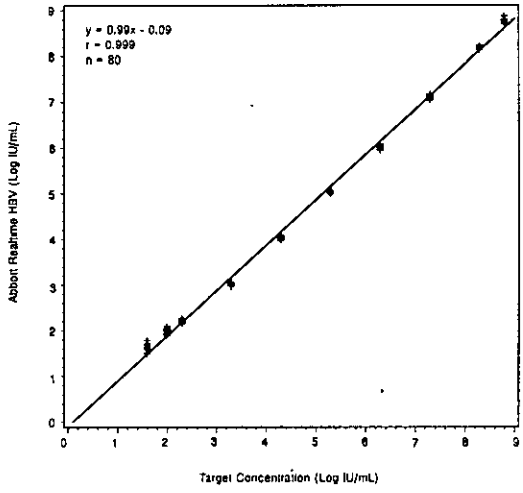


Figure 4

Abbott RealTime HBV Linear Range
Least Squares Linear Regression Analysis
Genotype C



Limit of Quantitation

The total analytical error (TAE) was calculated using estimates determined from the reproducibility studies that were conducted at three sites: two external sites and one internal site. Genotypes A and C were tested at both sample volumes and in both plasma and serum.

Presented in Table 1 are the TAE estimates for the plasma panel members that had an observed concentration at or near the assay limit of detection, for each sample input volume. Presented in Table 2 are the TAE estimates for the serum panel study. TAE was estimated by two different methods (see table footnotes).

These studies demonstrated that the Abbott RealTime HBV assay can determine with an acceptable level of accuracy the concentration of HBV DNA in EDTA plasma and serum at concentrations of 10 IU/mL (1.00 log IU/mL) for the 0.5 mL sample protocol volume and 15 IU/mL (1.18 log IU/mL) for the 0.2 mL sample protocol volume. At these concentrations, the difference between two measurements of more than 1.00 log IU/mL is statistically significant.

Table 1

Abbott RealTime HBV
Total Analytical Error (TAE) Estimates (Plasma)
(Log IU/mL)

Sample Volume (mL)	n	HBV Genotype (Panel Member)	Expected Conc.	Observed Conc.	Absolute Bias	SD[b]	TAE[c] Absolute Bias + (2 x SD)	TAE[d] SQRT (2) x 2 x SD
0.5	110	A (5)	1.04	0.90[a]	0.14	0.32	0.78[a]	0.91[a]
0.5	119	C (10)	1.14	1.13	0.01	0.29	0.59	0.82
0.2	37	A (5)	1.04	1.03[a]	0.01	0.40	0.81[a]	1.13[a]
0.2	46	C (10)	1.14	1.24	0.10	0.30	0.70	0.85

[a] Panel Member is below the assay LoD (1.00 log IU/mL for 0.5 mL and 1.18 log IU/mL for 0.2 mL). TAE is provided for information only.
[b] SD = Within-run component variability + Between-run component variability.
[c] Per section 5.1 of EP17-A CLSI guideline.[20]
[d] Based on difference between two measurements approach.

Table 2

Abbott RealTime HBV
Total Analytical Error (TAE) Estimates (Serum)
(Log IU/mL)

Sample Volume (mL)	n	HBV Genotype (Panel Member)	Expected Conc.	Observed Conc.	Absolute Bias	SD[b]	TAE[c] Absolute Bias + (2 x SD)	TAE[d] SQRT (2) x 2 x SD
0.5	88	A (6)	1.36	1.04	0.32	0.20	0.72	0.57
0.5	90	C (12)	1.48	1.29	0.19	0.20	0.59	0.57
0.5	88	C (13)	1.27	0.95[a]	0.32	0.25	0.82[a]	0.71[a]
0.2	88	A (5)	1.56	1.10[a]	0.46	0.24	0.94[a]	0.68[a]
0.2	89	C (12)	1.48	1.14	0.34	0.24	0.82	0.68

[a] Panel Member is below the assay LoD (1.00 log IU/mL for 0.5 mL and 1.18 log IU/mL for 0.2 mL). TAE is provided for information only.
[b] SD = Within-run component variability + Between-run component variability.
[c] Per section 5.1 of EP17-A CLSI guideline.[20]
[d] Based on difference between two measurements approach.

LINEARITY OF ASSAY BY HBV GENOTYPES

The ability of the RealTime HBV assay to detect and quantitate HBV genotypes was evaluated through linearity studies by diluting eight specimens, one of each genotype A through H, to target concentrations of 4.47 log IU/mL, 3.47 log IU/mL, 2.47 log IU/mL, and 1.17 log IU/mL in HBV serologically negative human plasma. Three replicates were tested at each concentration for each genotype, using the 0.5 mL sample preparation protocol. Data of the studies demonstrated that RealTime HBV assay is capable to quantitate different HBV genotypes across linear range with deviation of not more than 0.51 log IU/mL. The results are summarized in Table 3 and Figure 5.

Table 3

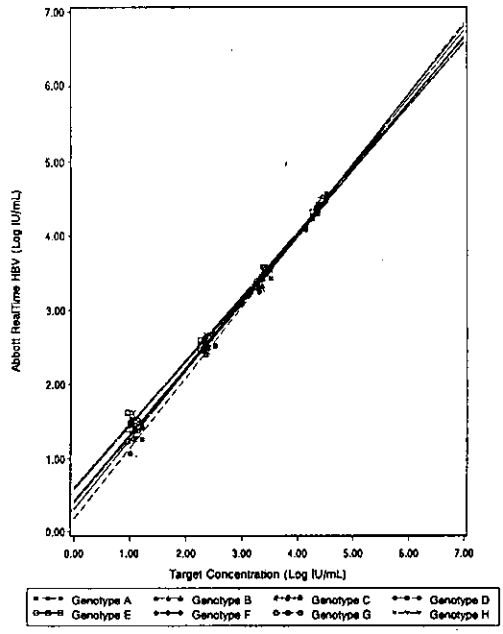
Abbott RealTime HBV
Linearity of Assay by HBV Genotypes

Genotype	Linear Equation from Linearity Study	Maximum Difference[a] Between Genotype A and Corresponding Genotype (Log IU/mL)
A	y = 0.95x + 0.19	n/a
B	y = 0.89x + 0.40	0.35
C	y = 0.93x + 0.32	0.11
D	y = 0.89x + 0.43	0.32
E	y = 0.86x + 0.58	0.44
F	y = 0.90x + 0.42	0.23
G	y = 0.85x + 0.61	0.51
H	y = 0.86x + 0.60	0.42

[a] The maximum difference was obtained at the assay ULQ (ULoQ) or LLQ (LLoQ).

Figure 5

Abbott RealTime HBV
Linearity of Assay by HBV Genotypes
(Dilutional Linearity)



WITHIN-LABORATORY PRECISION: LOT-TO-LOT

The precision of the assay was evaluated using an eight-member panel. Panel members 2, 3, 5, and 7 were prepared by diluting a high copy HBV patient sample in HBV serologically negative human serum. Panel members 1, 4, 6, and 8 were prepared by diluting the same high copy HBV patient sample into HBV serologically negative human plasma. A total of three reagent lots were used and each lot was assigned an *m2000sp* and *m2000rt* instrument pair. A total of 45 replicates were tested for each panel member across the three pairs of *m2000sp* and *m2000rt* instruments. One run was performed per day on each instrument pair for five days for a total of 15 runs. Panel members 1 through 8 were run in replicates of three. The concentration levels targeted for the precision panels spanned the linear quantitation range of the assay. The 0.5 mL sample preparation protocol was used. The between-lot/instrument component of precision was less or equal to 0.14 log IU/mL. The results are summarized in Table 4.

Table 4

Abbott RealTime HBV
Within-Laboratory Precision for the 0.5 mL Sample Preparation Protocol

Panel	Specimen Type[a]	n	Mean Conc. (Log IU/mL)	Within-Run Component SD[b]	Between-Run Component SD[b]	Between-Lot/ Instrument Component SD[b]	Total SD[b,c]
1	P	45	1.41	0.19	0.00	0.08	0.21
2	S	45	2.32	0.07	0.04	0.07	0.11
3	S	45	3.48	0.06	0.05	0.08	0.11
4	P	45	4.38	0.06	0.06	0.08	0.12
5	S	45	5.47	0.09	0.00	0.10	0.13
6	P	45	6.38	0.06	0.07	0.11	0.15
7	S	45	7.54	0.05	0.08	0.14	0.17
8	P	45	8.44	0.04	0.05	0.13	0.14

[a] P = Plasma; S = Serum
[b] Standard deviations (SD) are in log IU/mL.
[c] Total precision includes within-run, between-run and between-lot/instrument components of precision.

WITHIN-LABORATORY PRECISION: OPERATOR-TO-OPERATOR

The within-run, between-run, and between-technician (operator) precision of the Abbott RealTime HBV assay was evaluated by testing 84 replicates of each HBV panel member that span the dynamic range of the assay from approximately 1.00 log IU/mL to approximately 9.00 log IU/mL, for HBV Genotypes A and C. Panel members 1 through 5 were HBV Genotype A, and panel members 6 through 10 were HBV Genotype C. The 0.5 mL sample preparation protocol was used for this study. This same panel was also used as a part of the site-to-site reproducibility study. One lot of amplification reagents was run on one *m2000sp* and *m2000rt* instrument pair by three technicians. Each technician completed one run per day for seven days, for a total of 21 runs. Four replicates were run for each panel member. The SD for between-technician component and total SD for the Abbott RealTime HBV assay was found to be less than or equal to 0.06 log IU/mL and 0.11 log IU/mL, respectively, for all panel members greater than the assay limit of detection (1.00 log IU/mL). The results are summarized in Table 5.

Table 5

Abbott RealTime HBV
Within-Laboratory Precision (Operator-to-Operator)

Panel Member	n	Mean Concentration (Log IU/mL)	Within-Run Component SD[a]	Between-Run/Day Component SD[a]	Between-Technician Component SD[a]	Total SD[a,d]
1	84	8.87	0.06	0.03	0.05	0.08
2	84	6.77	0.05	0.03	0.01	0.06
3	84	4.53	0.10	0.00	0.05	0.11
4	84	2.72	0.06	0.02	0.02	0.07
5	73[b,c]	0.49	0.24	0.00	0.07	0.25
6	84	8.57	0.08	0.02	0.06	0.10
7	84	6.72	0.07	0.00	0.04	0.08
8	84	4.66	0.09	0.03	0.04	0.10
9	83[c]	2.69	0.07	0.03	0.05	0.09
10	84	0.78	0.19	0.00	0.04	0.19

[a] Standard Deviations (SD) are in log IU/mL.
[b] Target not detected for 10 samples.
[c] Error code "Internal Control Failed" for one sample.
[d] Total precision includes within-run, between-run/day, and between-technician components of precision.

REPRODUCIBILITY IN PLASMA

The plasma reproducibility panel was tested at three different sites by one technologist and one instrument pair at each site. Panels tested at each site consisted of a 40-member panel (10 unique panel members) that included five concentration levels of one prevalent HBV genotype and five concentration levels of a second prevalent HBV genotype, repeated four times within the panel. The concentration levels targeted for the reproducibility panels spanned the linear quantitation range of the assay. The HBV genotypes selected for the reproducibility panels were genotype A and genotype C, recognized as prevalent in the U.S. population. Each five-member panel was prepared from a high copy source sample, which was composed of at least two individual patient specimens that had a common genotype. A total of three reagent lots were used. For the 0.5 mL reproducibility, each of the three clinical sites tested two of the three lots for five days each. Site 1 used lots A and B, Site 2 used lots B and C, and Site 3 used lots A and C. The 0.2 mL reproducibility was tested at each of the three clinical sites using two lots for two days each. The SD for the between-site component was less or equal to 0.10 log IU/mL. The results are summarized in Table 6 and Table 7.

Table 6

Abbott RealTime HBV
Reproducibility in Plasma
0.5 mL Sample Preparation Protocol

Panel	Genotype	n	Mean Conc. (Log IU/mL)	Mean Conc. (IU/mL)	Within-Run Component SD[c]	Between-Run Component SD[c]	Between-Lot Component SD[c]	Between-Site Component SD[c]	Total SD[c,d]
1	A	120	8.93	872,502,276	0.07	0.00	0.03	0.08	0.11
2	A	119[a]	6.84	7,087,010	0.04	0.03	0.06	0.05	0.09
3	A	120	4.70	52,574	0.09	0.02	0.06	0.10	0.15
4	A	120	2.81	665	0.05	0.02	0.06	0.08	0.12
5	A	110[b]	0.90[e]	27[e]	0.31	0.07	0.26	0.10	0.42
6	C	119[a]	8.64	446,037,175	0.07	0.01	0.04	0.07	0.11
7	C	120	6.83	6,922,148	0.06	0.01	0.06	0.05	0.10
8	C	119[a]	4.84	72,954	0.08	0.00	0.08	0.09	0.15
9	C	120	2.84	722	0.06	0.02	0.09	0.08	0.13
10	C	119[b]	1.13	26	0.29	0.00	0.22	0.00	0.37

[a] Invalid replicate not included.
[b] Target not detected not included.
[c] Standard deviations (SD) are in log IU/mL.
[d] The total precision includes within-run, between-run, between-lot, and between-site components of precision.
[e] Concentration is below the assay LoD.

Table 7

Abbott RealTi*me* HBV
Reproducibility in Plasma
0.2 mL Sample Preparation Protocol

Panel	Genotype	n	Mean Conc. (Log IU/mL)	Mean Conc. (IU/mL)	Within-Run Component SD[b]	Between-Run Component SD[b]	Between-Lot Component SD[b]	Between-Site Component SD[b]	Total SD[b,c]
1	A	48	8.99	1,019,710,342	0.07	0.00	0.13	0.00	0.15
2	A	48	6.87	7,526,185	0.04	0.05	0.09	0.00	0.12
3	A	48	4.70	52,678	0.06	0.08	0.14	0.00	0.17
4	A	48	2.83	716	0.06	0.06	0.13	0.00	0.16
5	A	37[a]	1.03[d]	21	0.40	0.00	0.18	0.02	0.44
6	C	48	8.64	451,101,262	0.05	0.05	0.12	0.00	0.14
7	C	48	6.85	7,255,246	0.05	0.05	0.11	0.00	0.13
8	C	48	4.83	71,717	0.08	0.07	0.14	0.00	0.18
9	C	48	2.84	738	0.08	0.04	0.15	0.00	0.18
10	C	46[a]	1.24	26	0.30	0.00	0.27	0.00	0.41

[a] Target not detected not included.
[b] Standard deviations (SD) are in log IU/mL.
[c] The total precision includes within-run, between-run, between-lot, and between-site components of precision.
[d] Concentration is below the assay LoD.

REPRODUCIBILITY IN SERUM

The serum reproducibility panel tested at each site consisted of a 42-member panel (14 unique panel members) that included seven concentration levels of one prevalent HBV genotype and seven concentration levels of a second prevalent HBV genotype, repeated three times within the panel. The concentration levels targeted for the reproducibility panels spanned the linear quantitation range of the assay and also included some members below the lower limit of quantitation. The HBV genotypes selected for the serum reproducibility panels were genotypes that were recognized as prevalent in the US population. Each seven-member panel was prepared from a high copy source sample. A total of three reagent lots were used. Each of the three clinical sites tested two of the three amplification reagent lots for five days each. Site 1 used lots A and B, Site 2 used lots B and C, and Site 3 used lots A and C. Each site conducted the five day reproducibility at both the 0.2 mL volume and 0.5 mL volume for two lots of amplification reagents. The results are summarized in Table 8 and Table 9.

Table 8

Abbott RealTi*me* HBV
Reproducibility in Serum
0.5 mL Sample Preparation Protocol

Panel	Genotype	n	Mean Conc. (Log IU/mL)	Mean Conc. (IU/mL)	Within-Run Component SD[c]	Between-Run Component SD[c]	Between-Lot Component SD[c]	Between-Site Component SD[c]	Total SD[c,d]
1	A	89[a]	8.24	184,508,108	0.13	0.07	0.07	0.06	0.17
2	A	90	6.19	1,613,319	0.04	0.07	0.07	0.03	0.11
3	A	90	3.94	8,725	0.07	0.08	0.11	0.19	0.24
4	A	89[a]	1.96	105	0.12	0.07	0.07	0.20	0.25
5	A	90	1.25	22	0.20	0.10	0.12	0.20	0.32
6	A	88[b]	1.04	13	0.17	0.10	0.08	0.18	0.28
7	A	84[b]	0.74[e]	7[e]	0.22	0.12	0.21	0.12	0.35
8	C	90	7.22	17,211,265	0.05	0.05	0.08	0.05	0.12
9	C	90	6.23	1,741,264	0.05	0.06	0.08	0.02	0.12
10	C	89[a]	3.89	8,068	0.11	0.07	0.10	0.21	0.27
11	C	89[a]	1.64	55	0.18	0.12	0.06	0.28	0.36
12	C	90	1.29	23	0.18	0.09	0.12	0.15	0.27
13	C	88[b]	0.95[e]	12[e]	0.24	0.08	0.15	0.24	0.38
14	C	87[b]	0.88[e]	9[e]	0.23	0.06	0.10	0.10	0.28

[a] Invalid replicate not included.
[b] Target not detected not included.
[c] Standard deviations (SD) are in log IU/mL.
[d] The total precision includes within-run, between-run, between-lot, and between-site components of precision.
[e] Concentration is below the assay LoD.

Table 9

Abbott RealTi*me* HBV
Reproducibility in Serum
0.2 mL Sample Preparation Protocol

Panel	Genotype	n	Mean Conc. (Log IU/mL)	Mean Conc. (IU/mL)	Within-Run Component SD[c]	Between-Run Component SD[c]	Between-Lot Component SD[c]	Between-Site Component SD[c]	Total SD[c,d]
1	A	90	8.28	205,545,691	0.16	0.04	0.03	0.00	0.17
2	A	88[a]	6.21	1,638,140	0.04	0.04	0.03	0.00	0.06
3	A	89[a]	3.89	8,306	0.10	0.07	0.07	0.13	0.19
4	A	89[a]	1.90	95	0.22	0.04	0.16	0.16	0.32
5	A	88[b]	1.10[e]	16[e]	0.21	0.12	0.07	0.18	0.31
6	A	86[a,b]	0.89[e]	10[e]	0.27	0.03	0.10	0.10	0.31
7	A	82[b]	0.62[e]	7[e]	0.33	0.00	0.06	0.16	0.37
8	C	90	7.22	16,648,045	0.05	0.02	0.04	0.00	0.07
9	C	90	6.25	1,776,403	0.04	0.04	0.04	0.00	0.07
10	C	90	3.84	7,550	0.12	0.07	0.07	0.13	0.20
11	C	90	1.57	48	0.18	0.10	0.02	0.31	0.37
12	C	89[b]	1.14[e]	17[e]	0.22	0.10	0.11	0.12	0.29
13	C	87[a,b]	0.80[e]	8[e]	0.26	0.13	0.15	0.08	0.34
14	C	82[b]	0.73[e]	9[e]	0.35	0.06	0.17	0.00	0.39

[a] Invalid replicate not included.
[b] Target not detected not included.
[c] Standard deviations (SD) are in log IU/mL.
[d] The total precision includes within-run, between-run, between-lot, and between-site components of precision.
[e] Concentration is below the assay LoD.

LIMIT OF DETECTION (LoD) Using the WHO International Standard

The LoD of the Abbott RealTime HBV assay is 10 IU/mL for the 0.5 mL sample preparation protocol and 15 IU/mL for the 0.2 mL sample preparation protocol. The LoD is defined as the HBV DNA concentration detected with a probability of 95%. The LoD was determined by testing dilutions of the WHO International Standard for Hepatitis B Virus DNA (NIBSC 97/746), which were prepared in HBV negative human plasma and serum. Probit analysis of the data was used to determine the concentration of the WHO Standard detected with 95% probability. The results of the LoD study in plasma and serum at both sample volumes are summarized in Tables 10 through 13.

Table 10

Abbott RealTime HBV
Limit of Detection (LoD) in Plasma Using the WHO International Standard
0.5 mL Sample Preparation Protocol

IU/mL	Number Tested	Number Detected	Percent Detected
20.00	26	26	100
10.00	26	25	96
5.00	26	26	100
2.50	26	23	88
1.00	26	12	46
0.50	26	7	27
0.25	26	7	27
0.10	26	4	15

Probit analysis[27] of the data determined that the concentration of HBV DNA detected with 95% probability using the WHO International Standard was 6.40 IU/mL (95% CI 3.97-13.03 IU/mL).

Table 11

Abbott RealTime HBV
Limit of Detection (LoD) in Serum Using the WHO International Standard
0.5 mL Sample Preparation Protocol

IU/mL	Number Tested	Number Detected	Percent Detected
20.00	30	30	100
10.00	30	30	100
5.00	30	30	100
2.50	30	29	97
1.00	30	17	57
0.50	30	16	53
0.25	30	1	3
0.10	30	8	27

Probit analysis[27] of the data determined that the concentration of HBV DNA detected with 95% probability using the WHO International Standard was 3.82 IU/mL (95% CI 1.55-69.76 IU/mL).

Table 12

Abbott RealTime HBV
Limit of Detection (LoD) in Plasma Using the WHO International Standard
0.2 mL Sample Preparation Protocol

IU/mL	Number Tested	Number Detected	Percent Detected
40.00	27	27	100
20.00	27	27	100
10.00	27	26	96
5.00	27	23	85
2.50	27	12	44
1.00	27	11	41
0.50	27	6	22
0.20	27	0	0

Probit analysis[27] of the data determined that the concentration of HBV DNA detected with 95% probability using the WHO International Standard was 10.66 IU/mL (95% CI 7.11-19.38 IU/mL).

Table 13

Abbott RealTime HBV
Limit of Detection (LoD) in Serum Using the WHO International Standard
0.2 mL Sample Preparation Protocol

IU/mL	Number Tested	Number Detected	Percent Detected
40.00	30	30	100
20.00	30	30	100
10.00	30	30	100
5.00	29*	25	86
2.50	30	27	90
1.00	30	17	57
0.50	30	17	57
0.20	30	4	13

* One replicate was excluded due to instrument error.

Probit analysis[27] of the data determined that the concentration of HBV DNA detected with 95% probability using the WHO International Standard was 5.61 IU/mL (95% CI 3.62-10.94 IU/mL).

LIMIT OF DETECTION (LoD) BY GENOTYPE USING CLINICAL SPECIMENS

The LoD for the assay to detect HBV in clinical specimens using the 0.5 mL sample preparation protocol volume, detecting any of the eight genotypes tested, considering that assay does not differentiate between HBV genotypes, was determined to be 10 IU/mL. The LoD for the assay to detect HBV in clinical specimens using the 0.2 mL sample preparation protocol volume was determined to be 15 IU/mL.

The LoD was determined by analysis of a dilution series of patient samples representing HBV Genotypes A, B, C, D, E, F, G, and H and of the WHO International Standard. One patient sample for each HBV genotype was tested. Serial dilutions were made in HBV serologically negative human plasma and serum to create an eight-member panel with the target concentrations 0.10 IU/mL, 0.25 IU/mL, 0.50 IU/mL, 1.00 IU/mL, 2.50 IU/mL, 5.00 IU/mL, 10.0 IU/mL, and 20.0 IU/mL for the 0.5 mL sample volume and 0.20 IU/mL, 0.50 IU/mL, 1.00 IU/mL, 2.50 IU/mL, 5.00 IU/mL, 10.0 IU/mL, 20.0 IU/mL and 40.0 IU/mL for the 0.2 mL sample volume. Probit analysis of the data was used to determine the concentration of each HBV genotype detected with 95% probability. The results are summarized in Table 14 and Table 15.

Table 14

Abbott RealTime HBV
LoD by Genotype Using Clinical Specimens
0.5 mL Sample Preparation Protocol (IU/mL)

Genotype Tested	Concentration Detected (95% Confidence Interval) Plasma	Serum
WHO	3.69 (2.59, 6.19)	*
A	2.31 (1.59, 4.08)	5.49 (2.86, 19.05)
B	2.96 (2.12, 4.90)	**
C	4.53 (3.18, 7.58)	3.92 (2.09, 14.50)
D	3.23 (2.23, 5.62)	**
E	4.73 (2.11, 39.36)	3.72 (2.55, 6.47)
F	4.22 (2.80, 7.73)	**
G	2.51 (1.80, 4.21)	1.94 (1.43, 3.14)
H	8.11 (4.18, 27.97)	**

* WHO standard was not tested in serum in this study.
** Genotypes B, D, F, and H were tested in serum with 0.2 mL volume only. See Table 15.

The LoD for the assay to detect HBV in clinical specimens using the 0.5 mL sample preparation protocol volume, detecting any of the eight genotypes tested, considering that assay does not differentiate between HBV genotypes, was determined to be 10 IU/mL.

Table 15

Abbott RealTime HBV
LoD by Genotype Using Clinical Specimens
0.2 mL Sample Preparation Protocol (IU/mL)

Genotype Tested	Concentration Detected (95% Confidence Interval) Plasma	Serum
WHO	8.16 (5.63, 13.93)	*
A	5.86 (4.00, 10.22)	**
B	5.37 (3.72, 9.23)	2.40 (1.61, 4.65)
C	8.61 (5.95, 14.68)	**
D	5.34 (3.54, 9.93)	2.26 (1.55, 4.21)
E	14.57 (9.63, 26.28)	**
F	6.60 (4.41, 11.98)	7.18 (4.75, 13.20)
G	3.84 (2.61, 6.94)	**
H	10.86 (7.34, 19.10)	7.65 (5.01, 14.26)

* WHO standard was not tested in serum in this study.
** Genotypes A, C, E, and G were tested in serum with 0.5 mL volume only. See Table 14.

The LoD for the assay to detect HBV in clinical specimens using the 0.2 mL sample preparation protocol volume, detecting any of the eight genotypes tested, considering that assay does not differentiate between HBV genotypes, was determined to be 15 IU/mL.

ANALYTICAL SPECIFICITY

Potentially Interfering Substances

The susceptibility of the Abbott RealTime HBV assay to interference by elevated levels of potentially interfering substances was evaluated. HBV-negative samples and HBV-positive samples containing 2,933 IU/mL (3.47 log IU/mL) of HBV DNA were tested. Potential interference at HBV DNA concentrations close to the assay LLQ (LLoQ) was not assessed. HBV-negative and positive samples were tested in a plasma matrix and were not tested in serum.

No interference in the performance of the Abbott RealTime HBV assay was observed in the presence of the following endogenous substances for all HBV-negative and positive samples tested:

- Hemoglobin 500 mg/dL
- Triglycerides 3,000 mg/dL
- Bilirubin 20 mg/dL
- Protein 9 g/dL

Note: For hemoglobin and protein, there was a slight trend toward lowering of the values of the high level HBV specimens in the presence of interfering substances. The mean differences of the test and control conditions for hemoglobin and protein are small (-0.058 and -0.112 log IU/mL, respectively) compared to the clinically significant difference between two samples (1 log); as such, these differences are not expected to be clinically significant.

Antivirals and antibiotics at concentrations equal to or in excess of peak plasma or serum levels were tested in five pools. No interference in the performance of the Abbott RealTime HBV assay was observed in the presence of the following drug pools for all HBV-negative and positive samples tested:

Drug Pool	Drugs Tested
1	Zidovudine, Saquinavir, Ritonavir, Clarithromycin, Interferon 2a, Interferon 2b, Didanosine
2	Abacavir sulfate, Amprenavir, Peginterferon 2a, Peginterferon 2b, Ribavirin, Entecavir, Adefovir
3	Tenofovir, Lamivudine, Indinavir, Ganciclovir, Valganciclovir, Acyclovir, Paroxetine
4	Stavudine, Efavirenz, Lopinavir, Enfuvirtide, Ciprofloxacin, Fluoxetine
5	Zalcitabine, Nevirapine, Nelfinavir, Azithromycin, Valacyclovir, Sertraline

Note: A consideration was made to avoid combining specific drugs within a pool that would not be used together in a clinical setting. Because the listed drugs were tested in pools, individual drug effects were not assessed.

Cross-Reactivity Studies with Clinical Specimens

The specificity of the assay was evaluated by testing 60 patient specimens that were positive for at least one of the following DNA virus markers, RNA viruses, non-viral hepatitis, or autoimmune disease states. Specimens that were tested for DNA virus markers were in serum. Specimens that were tested for RNA virus markers were in plasma or serum. HBV DNA was not detected in any of the 60 specimens tested.

DNA and RNA Viruses	Non-viral Hepatitis and Autoimmune States
Epstein Barr Virus (EBV)	Anti-nuclear Antibody (ANA)
Herpes Simplex Virus 1 (HSV-1)	Rheumatoid Factor (RF)
Herpes Simplex Virus 2 (HSV-2)	Cirrhosis
Cytomegalovirus (CMV)	Alcoholic Hepatitis
Human Immunodeficiency Virus (HIV-1)	Non-alcoholic Steatohepatitis (NASH)
Hepatitis C Virus (HCV)	Autoimmune Hepatitis (AUH)
Hepatitis A Virus (HAV)	Hepatocellular Carcinoma

Cross-Reactivity Studies Using Nucleic Acid or Viral Lysate

The following viruses and microorganisms were evaluated for potential cross-reactivity in the Abbott RealTime HBV assay. Purified nucleic acid or viral lysate from each microorganism or virus was added at a concentration of 100,000 copies/mL to HBV DNA negative samples and HBV DNA positive samples that contained 2,933 IU/mL (3.47 log IU/mL) HBV DNA. No interference in the performance of the Abbott RealTime HBV assay was observed in the presence of the potential cross-reactant microorganisms or viruses for all the positive and negative samples tested.

Microorganism/Virus	Source
Human immunodeficiency virus 1 (HIV-1)	Viral lysate, cell culture
Human immunodeficiency virus 2 (HIV-2)	Viral lysate, cell culture
Human T-lymphotropic virus I (HTLV-I)	Viral lysate, cell culture
Hepatitis C virus (HCV)	Viral lysate, human specimen
Hepatitis A virus (HAV)	Purified nucleic acid
Epstein-Barr virus (EBV)	Purified nucleic acid
Herpes simplex virus 1 (HSV-1)	Purified nucleic acid
Herpes simplex virus 2 (HSV-2)	Purified nucleic acid
Cytomegalovirus (CMV)	Purified nucleic acid
Human herpesvirus 6B (HHV-6B)	Purified nucleic acid
Human herpesvirus 8 (HHV-8)	Purified nucleic acid
Varicella-zoster virus (VZV)	Purified nucleic acid
Vaccinia virus (VACV)	Purified nucleic acid
BK human polyomavirus	Purified nucleic acid
Human papilloma virus 16 (HPV-16)	Purified nucleic acid
Human papilloma virus 18 (HPV-18)	Purified nucleic acid
Neisseria gonorrhoeae	Purified nucleic acid
Chlamydia trachomatis	Purified nucleic acid
Candida albicans	Purified nucleic acid
Staphylococcus aureus	Purified nucleic acid
Staphylococcus epidermidis	Purified nucleic acid
Mycobacterium gordonae	Purified nucleic acid
Mycobacterium smegmatis	Purified nucleic acid

Performance of the Assay with HBV-Negative Specimens

Performance of the Abbott RealTime HBV assay was evaluated by testing 124 HBV serologically-negative serum and 125 HBV serologically-negative plasma specimens from blood donors. The specimens were tested on one *m*2000 Instrument system with one lot of amplification reagents. HBV DNA was not detected for all 249 specimens, resulting in 100% correct results: 100% (124/124) with 95% CI: 97.0% to 100% for serum samples and 100% (125/125) with 95% CI: 97.0% to 100% for plasma samples.

ANALYTICAL CARRYOVER

Potential carryover was determined by performing three studies in which high copy HBV-positive samples were interspersed with negative samples in a checkerboard pattern. For these studies, the targeted level for the high copy HBV-positive samples was greater than 8 log IU/mL. The carryover rate in these representative studies ranged from 0% to 2%. For results greater or equal to LoD, there was an overall carryover rate of 0.63% (95% CI 0.08%-2.24%). Results from the three studies are summarized in Table 16.

Table 16

Abbott RealTime HBV
Analytical Carryover

Study	Number of Runs	Number of Negatives Tested	Number Detected	Percent Detected	Number Detected (> LoD)	Percent Detected (> LoD)	95% CI of Percent Detected
1	5	100	1	1.00	0	0.00	(0.00, 3.62)
2	5	100	2	2.00	2	2.00	(0.24, 7.04)
3	6	120	2	1.67	0	0.00	(0.00, 3.03)
Overall	**16**	**320**	**5**	**1.56**	**2**	**0.63**	**(0.08, 2.24)**

COMPARISON OF 0.2 mL VS. 0.5 mL SAMPLE PREPARATION PROTOCOLS

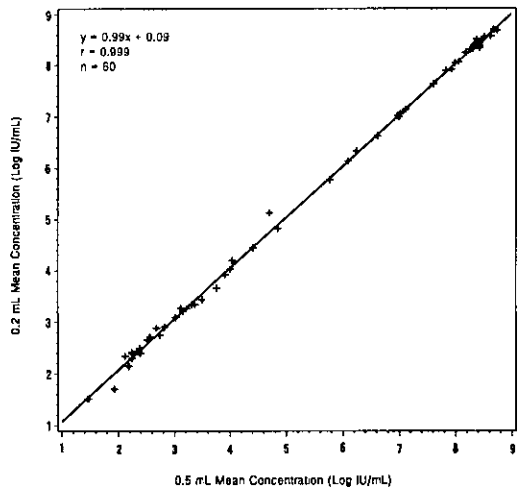
This study used the Abbott RealTime HBV assay to quantitate HBV-positive patient specimens. Sixty HBV-positive EDTA plasma specimens were tested in duplicate with both the 0.2 mL and 0.5 mL sample preparation protocols. Each duplicate pair was tested in the same run. The data showed a slope of 0.99 and an intercept of 0.09. The study was designed to cover the dynamic range of the Abbott RealTime HBV assay with actual patient samples representing genotypes (A, B, C, and D) commonly encountered within the US.

The observed lowest value in the specimen population for the 0.2 mL sample volume was 1.33 log IU/mL (mean value of the duplicate pair was 1.51 log IU/mL). For the 0.5 mL sample volume, the same specimen had an observed lowest value of 1.40 log IU/mL (mean value of the duplicate pair was 1.46 log IU/mL). The results are summarized in Figure 6.

Figure 6

Abbott RealTime HBV

Comparison of 0.2 mL vs. 0.5 mL Sample Preparation Protocols



SERUM VS. PLASMA ACROSS THE LINEAR RANGE

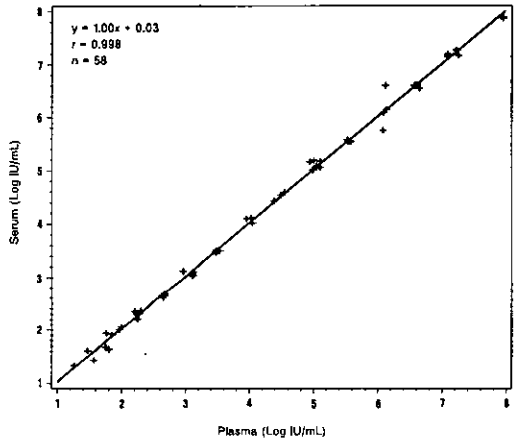
This study was conducted with specimens from 30 individual HBV serologically-negative donors. The specimens from each donor were collected as matched sets in serum and in EDTA-plasma tubes. Each pair of serum and plasma specimens was spiked with HBV-positive material at two targeted concentration levels throughout the dynamic range of the Abbott RealTime HBV Assay. Specimen types at each targeted concentration were tested once using the 0.5 mL sample preparation protocol. Two plasma-serum pairs had quantitation values below the assay dynamic range and were therefore excluded from the analysis.

Using a sample size of 58, linear regression analysis demonstrated a slope of 1.00 (95% CI 0.98 to 1.01) and an intercept of 0.03 (95% CI -0.04 to 0.10). The mean difference between serum and plasma specimens was -0.02 log IU/mL (95% CI -0.05 to 0.01). The linear regression is shown in Figure 7.

Figure 7

Abbott RealTime HBV

Serum vs. Plasma Across the Linear Range



STABILITY

Specimen Stability

Human serum or plasma specimens may be stored at 15 to 30°C for up to 24 hours or at 2 to 8°C for up to three days. Freshly drawn whole blood (plasma or serum) specimens may be held for up to 6 hours at 2 to 30 °C prior to centrifugation. Freeze/thaw effect was tested in both serum and plasma for up to eight cycles. Frozen specimens may be thawed at 15 to 30°C or 2 to 8°C. Thawed specimens may be stored at 2 to 8°C for up to 6 hours, if not processed immediately. Serum and plasma specimens may be stored at -20°C or colder for longer term storage. Stability testing results are summarized in Table 17.

Table 17

Abbott RealTime HBV

Specimen Stability (Log IU/mL)

Sample Type	Test Condition	Test Condition Mean	Baseline Condition Mean	Mean Difference
Plasma	24-26 hours at 28-32°C	3.781	3.722	0.059
	72-74 hours at 2-8°C	3.777	3.722	0.055
Serum	24-26 hours at 28-32°C	3.871	3.844	0.027
	72-74 hours at 2-8°C	3.870	3.844	0.026
Plasma (Whole Blood)	6-8 hours at 28-32°C	3.863	3.866	-0.003
	6-8 hours at 2-8°C	3.862	3.866	-0.004
Serum (Whole Blood)	6-8 hours at 28-32°C	3.823	3.628	0.195
	6-8 hours at 2-8°C	3.730	3.628	0.102
Plasma Freeze/Thaw	8 freeze/thaw cycles (frozen at -20°C or colder for a minimum of 8 hours; thawed at 15°C to 30°C for a maximum of 24 hours)	2.704	2.693	0.011
Serum Freeze/Thaw		2.722	2.714	0.007

CLINICAL STUDIES

Study Population

The clinical performance of the Abbott RealTime HBV Assay for use with the *m*2000 System was evaluated by assessing the antiviral therapy response in chronic HBV-infected subjects undergoing treatment with adefovir dipivoxil. The HBV DNA data were obtained from testing patient samples previously collected under two study protocols, one of which evaluated patients with chronic HBeAg-positive HBV infection and compensated liver function[28] and one that evaluated patients with HBeAg-negative HBV infection with compensated liver function.[29] The relationship between HBV DNA viral levels at various time points to histologic, biochemical, and serological responses to treatment was determined in this study.

The study population consisted of chronic HBV-infected patients enrolled in double-blind, randomized, placebo-controlled studies of adefovir dipivoxil that spanned 240 weeks. In the HBeAg-positive protocol, patients were randomized to 10 mg adefovir dipivoxil, 30 mg adefovir dipivoxil, or placebo for the first 48 weeks. Only the 10 mg adefovir dipivoxil treated patients (169 out of 171 total) and placebo patients (60 randomly selected from 167 total) were included in this study. Viral load testing was performed at baseline and at Weeks 12, 24, and 48. The viral load results were evaluated against histologic, biochemical, and serological response at 48 weeks. In addition, patients that remained on the 10 mg adefovir dipivoxil treatment were also tested at Weeks 144, 192, 240, as available.

In the HBeAg-negative protocol, patients were randomized to either 10 mg adefovir dipivoxil or placebo for the first 48 weeks. The adefovir dipivoxil treated patients (123 out of 123 total) and placebo patients (61 out of 61 total) were tested at baseline and at Weeks 12, 24, and 48. The viral load results were evaluated against histologic and biochemical response at 48 weeks. In addition, patients that remained on the 10 mg adefovir dipivoxil treatment were also tested at Weeks 96, 144, 192, and 240, as available.

Demographic data, HBV genotype, HBeAg, anti-HBe, and HBsAg seroconversion results, and baseline (pretreatment) and post-treatment liver biopsy results were available. Table 18 summarizes the subject demographics.

Table 18

Subject Demographics

Characteristic	Category	Summary Statistics	HBeAg+	HBeAg-	Total
Total Number of Subjects	-	N	229	184	413
• Placebo	-	n (%)	60[a] (26.20)	61 (33.15)	121
• 10 mg adefovir dipivoxil	-	n (%)	169[a] (73.80)	123 (66.85)	292
Total Number of Subjects with Demographic Information	-	n	220	184	404
Age (yr)	-	Median (Min, Max)	34 (16, 65)	46 (18, 65)	40 (16, 65)
Weight (kg)	-	Median (Min, Max)	71 (43, 117.73)	74.55 (46, 135)	72.5 (43, 135)
Sex	Male	n (%)	164 (74.55)	152 (82.61)	316 (78.22)
	Female	n (%)	56 (25.45)	32 (17.39)	88 (21.78)
Race	White	n (%)	80 (36.36)	122 (66.30)	202 (50.00)
	Asian	n (%)	129 (58.64)	56 (30.43)	185 (45.79)
	Other	n (%)	11 (5.00)	6 (3.26)	17 (4.21)
Genotype	A	n (%)	64 (29.09)	11 (5.98)	75 (18.56)
	B	n (%)	41 (18.64)	31 (16.85)	72 (17.82)
	C	n (%)	82 (37.27)	24 (13.04)	106 (26.24)
	D	n (%)	27 (12.27)	114 (61.96)	141 (34.90)
	Other	n (%)	6 (2.73)	4 (2.17)	10 (2.48)
Total Number of Subjects with Knodell Score	-	n	210	175	385
Total	-	Mean (SD)	9.38 (3.29)	9.35 (3.34)	9.37 (3.31)
Necroinflammatory	-	Mean (SD)	7.70 (2.71)	7.50 (2.75)	7.61 (2.73)
Fibrosis	-	Mean (SD)	1.67 (1.09)	1.86 (1.15)	1.76 (1.12)

[a] Demographic data were not provided for three placebo and six treatment subjects.

The HBeAg-positive subjects were primarily Asian and HBV Genotypes A and C, while the HBeAg-negative subjects were primarily White and HBV Genotype D. Patients included in the clinical performance analysis received either the standard 10 mg adefovir dipivoxil dosing or placebo. Table 19 summarizes subjects by treatment arm and available specimens.

Table 19

Summary of Subjects by Treatment Arm

Population	Total No. of Subjects	No. of Subjects - Placebo	No. of Subjects - 10 mg Adefovir	No. of Specimens per Subject[a]	Total No. of Specimens Tested
Chronic HBeAg+	229	60	169	2 to 7	1,036
Chronic HBeAg-	184	61	123	2 to 8	939
Total Number of Subjects Tested	413	121	292	2 to 8	1,975

[a] This number is reported as a range because the number of specimens varied for each subject.

CLINICAL STUDY RESULTS AND STATISTICAL ANALYSES

Statistical analysis of clinical data was used to assess whether viral response to treatment measured with Abbott RealTime HBV Assay for use with the *m*2000 System is informative for determining the response to treatment in HBeAg-positive and HBeAg-negative patients with chronic hepatitis B. Observing changes in viral load in individual patients over time may help the clinician in the assessment of a patient's response to therapy.

Within-Subject Variability in Absence of Treatment

The objective of this analysis was to assess the change in viral load (in log IU/mL units) between two successive measurements of placebo patients. There were 55 patients in the placebo arm of the HBeAg-positive group and 57 patients in the HBeAg-negative group that had available results for both Weeks 0 and 12. These results were used to estimate within-subject variability, which includes biological variability as well as total assay variability.

The within-subject variability (SD) based on these results was estimated to be 0.79 log IU/mL for HBeAg-positive patients and 0.86 log IU/mL for HBeAg-negative patients. Biological within-subject variability was similar to the estimated within-subject variability since the assay analytical variability was negligible. The median change (Week 12 – Week 0) of viral load within a subject was estimated to be 0.00 log IU/mL for HBeAg-positive patients and -0.28 log IU/mL for HBeAg-negative patients. Approximately 89% of the HBeAg-positive patients' and 81% of HBeAg-negative patients' change of viral load was less than 2.00 log IU/mL.

HBeAg-Positive Patients

Characterization of Viral Load

Table 20 and Figure 8 illustrate the efficacy, based on HBV viral load testing, of treating HBeAg-positive patients with 10 mg adefovir dipivoxil compared to placebo based on HBV viral load testing results using Abbott RealTime HBV assay for use with the *m*2000 System. At Week 48, 22.92% (33/144) of HBeAg-positive patients on treatment versus 0% (0/55) on placebo had achieved very low viral loads below 100 IU/mL. In addition, only 23.61% (34/144) of patients on treatment versus 81.82% (45/55) on placebo had viral loads greater than or equal to 10^8 IU/mL.

Table 20

Distribution of HBV Viral Load at Week 48 for HBeAg-Positive Patients

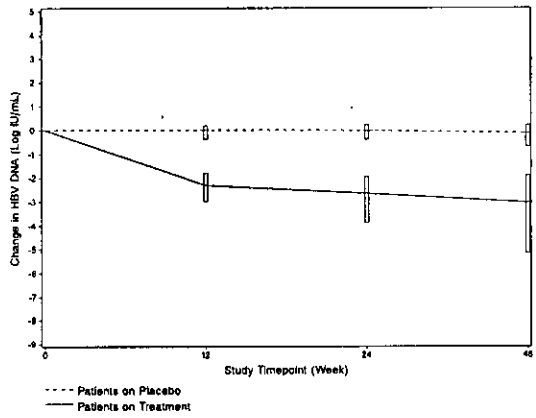
Viral Load (IU/mL)	Adefovir Dipivoxil			Placebo		
	n	%	Cumulative %	n	%	Cumulative %
TND[a]	4	2.78	2.78	0	0.0	0.0
< 15	13	9.03	11.81	0	0.0	0.0
15 - <100	16	11.11	22.92	0	0.0	0.0
100 - < 10^3	21	14.58	37.50	2	3.64	3.64
10^3 - < 10^4	18	12.50	50.00	1	1.82	5.46
10^4 - < 10^5	14	9.72	59.72	4	7.27	12.73
10^5 - < 10^6	24	16.67	76.39	3	5.45	18.18
10^6 - < 10^8	32	22.22	98.61	43	78.18	96.36
≥ 10^8	2	1.39	100.00	2	3.64	100.00
Total	144	100.00		55	100.00	

[a] TND = Target Not Detected

Figure 8 demonstrates the median viral load change and inter-quartile range of change from baseline for HBeAg-positve subjects on treatment compared to placebo. This shows the impact of adefovir dipivoxil treatment on the viral load of HBeAg-positive patients with chronic hepatitis B.

Figure 8

Median and Inter-Quartile Range of Change in HBV DNA from Baseline: HBeAg-Positive Subjects



The effect of therapy for patients with chronic HBV infection can be assessed by measuring the HBV DNA (expected reduction to low or undetectable levels), and monitoring for viral rebound that could be associated with resistance. Results in Table 21 show that 70.41% (119/169) of the treated subjects achieved a nadir, or lowest concentration, viral load level by Week 48. Of the 49 subjects that achieved a nadir by Week 24, 13.79% (4/29) of the subjects had a greater than or equal to one log IU/mL increase by Week 48 (20 of these subjects did not have a Week 48 result).

Table 21

Distribution of HBeAg-Positive Subjects by Week on Treatment and the Viral Load at Which the Nadir was Reached

Nadir Viral Load (IU/mL)	Number (%) of Patients With the Nadir Viral Load Achieved by Week						Total By Viral Load	Cumulative By Viral Load
	12	24	48	144	192	240		
TND[a]	0 (0.00)	0 (0.00)	4 (2.37)	1 (0.59)	1 (0.59)	4 (2.37)	10 (5.92)	10 (5.92)
< 15	0 (0.00)	1 (0.59)	13 (7.69)	1 (0.59)	6 (3.55)	1 (0.59)	22 (13.02)	32 (18.94)
15 - < 100	0 (0.00)	0 (0.00)	12 (7.10)	3 (1.78)	4 (2.37)	2 (1.18)	21 (12.43)	53 (31.37)
100 - < 10^3	1 (0.59)	3 (1.78)	15 (8.88)	0 (0.00)	1 (0.59)	7 (4.14)	27 (15.98)	80 (47.35)
10^3 - < 10^4	3 (1.78)	8 (4.73)	5 (2.96)	1 (0.59)	1 (0.59)	2 (1.18)	20 (11.83)	100 (59.18)
10^4 - < 10^5	3 (1.78)	5 (2.96)	7 (4.14)	3 (1.78)	1 (0.59)	2 (1.18)	21 (12.43)	121 (71.61)
10^5 - < 10^6	3 (1.78)	5 (2.96)	8 (4.73)	1 (0.59)	3 (1.78)	2 (1.18)	22 (13.02)	143 (84.63)
10^6 - < 10^9	8 (4.73)	9 (5.33)	6 (3.55)	1 (0.59)	0 (0.00)	2 (1.18)	26 (15.38)	169 (100.00)
Total By Week	18 (10.65)	31 (18.34)	70 (41.42)	11 (6.51)	17 (10.06)	22 (13.02)		
Cumulative By Week	18 (10.65)	49 (28.99)	119 (70.41)	130 (76.92)	147 (86.98)	169 (100.00)		

[a] TND = Target Not Detected

Two patients out of 169 achieved HBsAg seroconversion. One patient had results showing HBsAg serconversion at both Weeks 192 and 240. The other patient achieved seroconversion at Week 240. These two patients were white males, HBV genotype A, and > 30 years of age. A summary of these results is provided in Table 22.

Table 22

HBeAg-Positive Subjects with HBsAg Seroconversion

	Concentration (Log IU/mL)						
	Week 0	Week 12	Week 24	Week 48	Week 144	Week 192	Week 240
Subject 1	6.99	4.98	2.08	1.50	TND[a]	TND[a]	TND[a]
Subject 2	8.59	6.57	6.85	6.72	5.68	1.45	*

[a] TND = Target Not Detected

* The Abbott RealTime HBV result for the Week 240 time point was excluded due to technician error.

Summaries of the effect of baseline covariates for the HBeAg-positive population are provided in Table 23 through Table 25.

Table 23

Association Between Responses to Treatment at Week 48 and Baseline Covariates for HBeAg-Positive Patients

Response to Treatment	Covariate	Category	N	No. of Patients with Response	Proportion (%) of Patients with Response	Unadjusted Odds Ratio (95% CI)
Histological	Race	Asian	75	47	62.67	1.44 (0.66, 3.14)
		Other	52	28	53.85	
	Sex	Male	97	58	59.79	1.14 (0.45, 2.81)
		Female	30	17	56.67	
	Age	≤ 30	52	34	65.38	1.57 (0.71, 3.49)
		> 30	75	41	54.67	
	Genotype	B,C	72	47	65.28	1.81 (0.83, 3.95)
		Non-B,C	55	28	50.91	
Biochemical	Race	Asian	82	47	57.32	1.77 (0.82, 3.82)
		Other	51	22	43.14	
	Sex	Male	102	53	51.96	1.01 (0.42, 2.45)
		Female	31	16	51.61	
	Age	≤ 30	56	33	58.93	1.63 (0.77, 3.48)
		> 30	77	36	46.75	
	Genotype	B,C	79	45	56.96	1.65 (0.78, 3.53)
		Non-B,C	54	24	44.44	
HBeAg Loss	Race	Asian	84	21	25.00	0.89 (0.38, 2.09)
		Other	55	15	27.27	
	Sex	Male	105	26	24.76	0.79 (0.31, 2.11)
		Female	34	10	29.41	
	Age	≤ 30	59	13	22.03	0.70 (0.29, 1.63)
		> 30	80	23	28.75	
	Genotype	B,C	80	19	23.75	0.77 (0.34, 1.78)
		Non-B,C	59	17	28.81	
HBeAg Sero-conversion	Race	Asian	84	8	9.52	0.47 (0.15, 1.45)
		Other	55	10	18.18	
	Sex	Male	105	14	13.33	1.15 (0.33, 5.18)
		Female	34	4	11.76	
	Age	≤ 30	59	7	11.86	0.84 (0.26, 2.58)
		> 30	80	11	13.75	
	Genotype	B,C	80	6	7.50	0.32 (0.09, 1.00)
		Non-B,C	59	12	20.34	

The statistical significance of the associations of the Race, Sex, Age and Genotype covariates with viral response was studied, and the results are summarized in Tables 24 and Table 25. All lower limits of the 95% confidence intervals in Table 24 are smaller than 1, except for Race and Genotype at Weeks 12 and 24 (when viral response is defined as < 2,000 IU/mL). When response is defined as < 2,000 IU/mL, logistic regression analyses resulted in no statistically significant associations between the four covariates and viral load. All lower limits of the 95% confidence intervals in Table 25 are smaller than 1 (when viral response is defined as ≥ 2 log decrease). When response is defined as ≥ 2 log decrease, logistic regression analyses resulted in only gender at Week 12 showing a borderline statistically significant association (p = 0.043) 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.

Table 24

Odds Ratios for the Association Between Viral Response (< 2,000 IU/mL) and Covariates, by Week, for an HBeAg-Positive Population

Covariate	Category	Week	N	No. Below 2,000 IU/mL	Proportion (%) Below 2,000 IU/mL	Unadjusted Odds Ratio (95% CI)
Race	Asian	12	87	21	24.14	2.92 (1.04, 9.41)
	Other		61	6	9.84	
	Asian	24	81	30	37.04	3.20 (1.30, 8.42)
	Other		58	9	15.52	
	Asian	48	79	34	43.04	1.56 (0.71, 3.47)
	Other		52	17	32.69	
Sex	Male	12	112	20	17.86	0.90 (0.32, 2.78)
	Female		36	7	19.44	
	Male	24	104	27	25.96	0.67 (0.28, 1.70)
	Female		35	12	34.29	
	Male	48	100	37	37.00	0.71 (0.29, 1.76)
	Female		31	14	45.16	
Age	≤ 30	12	63	10	15.87	0.75 (0.28, 1.92)
	> 30		85	17	20.00	
	≤ 30	24	61	18	29.51	1.14 (0.50, 2.55)
	> 30		78	21	26.92	
	≤ 30	48	55	21	38.18	0.95 (0.44, 2.05)
	> 30		76	30	39.47	
Genotype	B,C	12	81	20	24.69	2.81 (1.04, 8.41)
	Non-B,C		67	7	10.45	
	B,C	24	76	29	38.16	3.27 (1.36, 8.29)
	Non-B,C		63	10	15.87	
	B,C	48	74	32	43.24	1.52 (0.70, 3.34)
	Non-B,C		57	19	33.33	

Table 25

Odds Ratios for the Association Between Viral Response (≥ 2 Log Decrease From Baseline Result) and Covariates, by Week, for an HBeAg-Positive Population

Covariate	Category	Week	N	No. with ≥ 2 Log Decrease	Proportion (%) with ≥ 2 Log Decrease	Unadjusted Odds Ratio (95% CI)
Race	Asian	12	87	54	62.07	0.86 (0.41, 1.79)
	Other		61	40	65.57	
	Asian	24	81	62	76.54	1.24 (0.53, 2.88)
	Other		58	42	72.41	
	Asian	48	79	56	70.89	0.81 (0.33, 1.91)
	Other		52	39	75.00	
Sex	Male	12	112	66	58.93	0.41 (0.15, 1.03)
	Female		36	28	77.78	
	Male	24	104	74	71.15	0.41 (0.11, 1.22)
	Female		35	30	85.71	
	Male	48	100	71	71.00	0.71 (0.23, 1.96)
	Female		31	24	77.42	
Age	≤ 30	12	63	41	65.08	1.13 (0.54, 2.36)
	> 30		85	53	62.35	
	≤ 30	24	61	44	72.13	0.78 (0.34, 1.81)
	> 30		78	60	76.92	
	≤ 30	48	55	40	72.73	1.02 (0.44, 2.41)
	> 30		76	55	72.37	
Genotype	B,C	12	81	51	62.96	0.95 (0.46, 1.96)
	Non-B,C		67	43	64.18	
	B,C	24	76	58	76.32	1.19 (0.51, 2.75)
	Non-B,C		63	46	73.02	
	B,C	48	74	52	70.27	0.77 (0.32, 1.80)
	Non-B,C		57	43	75.44	

Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Odds Ratio (OR) Analysis in a HBeAg-Positive Population

For each patient, the clinical responses: Histologic, Biochemical, HBeAg Loss, Anti-HBe Gain, and Seroconversion were measured at various times on treatment. These clinical responses were defined as follows:

- 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

Additionally, HBsAg seroconversion data were collected. Two patients out of 169 achieved HBsAg seroconversion. One patient had results showing HBsAg serconversion at both Weeks 192 and 240. The other patient achieved seroconversion at Week 240. These two patients were white males, HBV genotype A, and > 30 years of age. A summary of these results is provided in Table 22.

Viral load response was defined as either HBV DNA less than 2,000 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, 144, 192, or 240 and a viral load response at Weeks 12, 24, or 48 of treatment. Statistical analysis (NPV) was performed to evaluate whether there is an association between the clinical non-responses at Weeks 48, 144, 192, or 240 and a viral load non-response at Weeks 12, 24, or 48 of treatment.

Viral Response < 2,000 IU/mL

As shown in Table 26, early viral response (Weeks 12, 24, 48) is informative in predicting clinical responses at Week 48. 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).

Viral response at Weeks 12, 24, and 48 is informative in predicting biochemical, HBeAg loss, anti-HBe gain, and seroconversion at Week 48 (i.e., the lower 95% CI limits for the odds ratio exceed 1.0). Viral response at Week 24 is also informative in predicting histologic improvement at Week 48. Viral response at Week 24 is informative in predicting HBeAg loss at Week 144 and viral response at Week 48 is also informative in predicting anti-HBe gain and seroconversion at Week 240 of treatment.

Table 26

PPV, NPV, and Odds Ratio for Individual Clinical Responses During Treatment Predicted by Early Viral Response (< 2,000 IU/mL) in HBeAg-Positive Subjects

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	Histologic	79.2 (19/24)	(57.3, 92.1)	43.3 (39/90)	(33.1, 54.2)	2.91 (0.93, 10.76)
		Biochemical	84.0 (21/25)	(63.1, 94.7)	55.3 (52/94)	(44.7, 65.5)	
		HBeAg Loss	64.0 (16/25)	(42.6, 81.3)	82.8 (82/99)	(73.6, 89.4)	
		Anti-HBe Gain	36.0 (9/25)	(18.7, 57.4)	91.9 (91/99)	(84.2, 96.2)	
		Seroconversion[b]	36.0 (9/25)	(18.7, 57.4)	91.9 (91/99)	(84.2, 96.2)	
24	48	Histologic	80.0 (28/35)	(62.5, 90.9)	49.3 (37/75)	(37.7, 61.0)	
		Biochemical	83.8 (31/37)	(67.3, 93.2)	59.7 (46/77)	(47.9, 70.6)	
		HBeAg Loss	62.2 (23/37)	(44.8, 77.1)	90.2 (74/82)	(81.2, 95.4)	
		Anti-HBe Gain	24.3 (9/37)	(12.4, 41.6)	92.7 (76/82)	(84.2, 97.0)	
		Seroconversion	24.3 (9/37)	(12.4, 41.6)	92.7 (76/82)	(84.2, 97.0)	
48[c]	48	Histologic	74.0 (37/50)	(59.4, 84.9)	49.3 (35/71)	(37.3, 61.3)	
		Biochemical	78.0 (39/50)	(63.7, 88.0)	63.2 (48/76)	(51.3, 73.7)	
		HBeAg Loss	62.7 (32/51)	(48.1, 75.5)	97.5 (78/80)	(90.4, 99.6)	
		Anti-HBe Gain	33.3 (17/51)	(21.1, 48.0)	100.0 (80/80)	(94.3, 100.0)	
		Seroconversion	33.3 (17/51)	(21.1, 48.0)	100.0 (80/80)	(94.3, 100.0)	
12	144	Histologic			not available		
		Biochemical	66.7 (4/6)	(24.1, 94.0)	55.6 (25/45)	(40.1, 70.0)	2.50 (0.31, 29.77)
		HBeAg Loss	50.0 (3/6)	(13.9, 86.1)	84.4 (38/45)	(69.9, 93.0)	5.43 (0.58, 47.41)
		Anti-HBe Gain	33.3 (2/6)	(6.0, 75.9)	86.7 (39/45)	(72.5, 94.5)	3.25 (0.24, 28.53)
		Seroconversion	33.3 (2/6)	(6.0, 75.9)	86.7 (39/45)	(72.5, 94.5)	3.25 (0.24, 28.53)
24	144	Histologic			not available		
		Biochemical	70.0 (7/10)	(35.4, 91.9)	57.5 (23/40)	(41.0, 72.6)	3.16 (0.60, 21.20)
		HBeAg Loss	50.0 (5/10)	(20.1, 79.9)	87.5 (35/40)	(72.4, 95.3)	
		Anti-HBe Gain	30.0 (3/10)	(8.1, 64.6)	87.5 (35/40)	(72.4, 95.3)	3.00 (0.37, 19.68)
		Seroconversion	30.0 (3/10)	(8.1, 64.6)	87.5 (35/40)	(72.4, 95.3)	3.00 (0.37, 19.68)
48	144	Histologic			not available		
		Biochemical	63.6 (7/11)	(31.6, 87.6)	58.3 (21/36)	(40.9, 74.0)	2.45 (0.50, 13.33)
		HBeAg Loss	36.4 (4/11)	(12.4, 68.4)	86.1 (31/36)	(69.7, 94.8)	3.54 (0.54, 21.21)
		Anti-HBe Gain	27.3 (3/11)	(7.3, 60.7)	88.9 (32/36)	(73.0, 96.4)	3.00 (0.36, 21.45)
		Seroconversion	27.3 (3/11)	(7.3, 60.7)	88.9 (32/36)	(73.0, 96.4)	3.00 (0.36, 21.45)
12	192	Histologic			not available		
		Biochemical	60.0 (3/5)	(17.0, 92.7)	39.5 (15/38)	(24.5, 56.6)	0.98 (0.10, 13.02)
		HBeAg Loss	40.0 (2/5)	(7.3, 83.0)	57.9 (22/38)	(40.9, 73.3)	0.92 (0.07, 9.02)
		Anti-HBe Gain	40.0 (2/5)	(7.3, 83.0)	76.3 (29/38)	(59.4, 88.0)	2.15 (0.15, 21.62)
		Seroconversion	40.0 (2/5)	(7.3, 83.0)	76.3 (29/38)	(59.4, 88.0)	2.15 (0.15, 21.62)
24	192	Histologic			not available		
		Biochemical	66.7 (6/9)	(30.9, 91.0)	40.6 (13/32)	(24.2, 59.2)	1.37 (0.24, 9.92)
		HBeAg Loss	44.4 (4/9)	(15.3, 77.3)	62.5 (20/32)	(43.7, 78.3)	1.33 (0.22, 7.58)
		Anti-HBe Gain	33.3 (3/9)	(9.0, 69.1)	78.1 (25/32)	(59.6, 90.1)	1.79 (0.23, 11.22)
		Seroconversion	33.3 (3/9)	(9.0, 69.1)	78.1 (25/32)	(59.6, 90.1)	1.79 (0.23, 11.22)
48	192	Histologic			not available		
		Biochemical	77.8 (7/9)	(40.2, 96.1)	37.5 (12/32)	(21.7, 56.3)	2.10 (0.32, 23.57)
		HBeAg Loss	66.7 (6/9)	(30.9, 91.0)	62.5 (20/32)	(43.7, 78.3)	3.33 (0.56, 23.78)
		Anti-HBe Gain	55.6 (5/9)	(22.7, 84.7)	81.3 (26/32)	(63.0, 92.1)	5.42 (0.83, 35.32)
		Seroconversion	55.6 (5/9)	(22.7, 84.7)	81.3 (26/32)	(63.0, 92.1)	5.42 (0.83, 35.32)
12	240	Histologic	* (0/0)	*	33.3 (3/9)	(9.0, 69.1)	*
		Biochemical	66.7 (2/3)	(12.5, 98.2)	29.0 (9/31)	(14.9, 48.2)	0.82 (0.04, 53.57)
		HBeAg Loss	66.7 (2/3)	(12.5, 98.2)	58.1 (18/31)	(39.3, 74.9)	2.77 (0.13, 172.28)
		Anti-HBe Gain	33.3 (1/3)	(1.8, 87.5)	86.7 (26/30)	(68.4, 95.6)	3.25 (0.04, 73.98)
		Seroconversion	33.3 (1/3)	(1.8, 87.5)	87.1 (27/31)	(69.2, 95.8)	3.38 (0.05, 76.68)
24	240	Histologic	0.0 (0/1)	(0.0, 94.5)	37.5 (3/8)	(10.2, 74.1)	0.00 (0.00, 15.20)
		Biochemical	60.0 (3/5)	(17.0, 92.7)	29.6 (8/27)	(14.5, 50.3)	0.63 (0.06, 9.06)
		HBeAg Loss	60.0 (3/5)	(17.0, 92.7)	63.0 (17/27)	(42.5, 79.9)	2.55(0.24, 34.44)
		Anti-HBe Gain	20.0 (1/5)	(1.1, 70.1)	84.6 (22/26)	(64.3, 95.0)	1.38 (0.02, 20.07)
		Seroconversion	20.0 (1/5)	(1.1, 70.1)	85.2 (23/27)	(65.4, 95.1)	1.44 (0.02, 20.91)
48	240	Histologic	100.0 (1/1)	(5.5, 100.0)	57.1 (4/7)	(20.2, 88.2)	*
		Biochemical	83.3 (5/6)	(36.5, 99.1)	30.8 (8/26)	(15.1, 51.9)	2.22 (0.19, 118.05)
		HBeAg Loss	83.3 (5/6)	(36.5, 99.1)	65.4 (17/26)	(44.4, 82.1)	9.44 (0.81, 472.23)
		Anti-HBe Gain	50.0 (3/6)	(13.9, 86.1)	96.0 (24/25)	(77.7, 99.8)	
		Seroconversion	50.0 (3/6)	(13.9, 86.1)	96.2 (25/26)	(78.4, 99.8)	

* Undefined (division by zero)

[a] Shading indicates statistical significance.

[b] Seroconversion - HBeAg undetectable and antibody against HBeAg detected

[c] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

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

Tables 27 and 28 demonstrate that the NPV is very high (greater than 89% for Week 48 of clinical response) for the association of early viral response with the combination of all three responses (histologic, biochemical, and serological - HBeAg loss or seroconversion). These data indicate that HBeAg-positive subjects without an early viral response (defined as < 2,000 IU/mL decrease) are very unlikely to achieve all three clinical responses by Week 48, as a result of treatment. The number of subjects at Week 240 was small, and therefore available data is inadequate to draw conclusions about the association of the early viral response with clinical responses at later weeks.

Table 27

PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and HBeAg Loss Responses During Treatment Predicted by an Early Viral Response (< 2,000 IU/mL) in HBeAg-Positive Subjects

Week of Viral Response	Week of Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	41.7 (10/24)	(22.8, 63.1)	89.9 (80/89)	(81.2, 95.0)	[redacted]
24	48	42.9 (15/35)	(26.8, 60.5)	97.3 (72/74)	(89.7, 99.5)	
48[b]	48	36.7 (18/49)	(23.8, 51.7)	98.6 (70/71)	(91.3, 99.9)	
12	240	* (0/0)	*	55.6 (5/9)	(22.7, 84.7)	*
24	240	0.0 (0/1)	(0.0, 94.5)	62.5 (5/8)	(25.9, 89.8)	0.00 (0.00, 38.00)
48	240	100.0 (1/1)	(5.5, 100.0)	71.4 (5/7)	(30.3, 94.9)	*

* Undefined (division by zero)

[redacted] Shading indicates statistical significance.

[b] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

Table 28

PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and Seroconversion Responses During Treatment Predicted by an Early Viral Response (< 2,000 IU/mL) in HBeAg-Positive Subjects

Week of Viral Response	Week of Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	25.0 (6/24)	(10.6, 47.1)	97.8 (87/89)	(91.4, 99.6)	[redacted]
24	48	17.1 (6/35)	(7.2, 34.3)	98.6 (73/74)	(91.7, 99.9)	
48[b]	48	16.3 (8/49)	(7.8, 30.2)	100.0 (71/71)	(93.6, 100.0)	
12	240	* (0/0)	*	77.8 (7/9)	(40.2, 96.1)	*
24	240	0.0 (0/1)	(0.0, 94.5)	75.0 (6/8)	(35.6, 95.5)	0.00 (0.00, 66.50)
48	240	100.0 (1/1)	(5.5, 100.0)	100.0 (7/7)	(56.1, 100.0)	*

* Undefined (division by zero)

[redacted] Shading indicates statistical significance.

[b] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

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

Viral Response ≥ 2 Log IU/mL Decrease

As shown in Table 29, early viral response (Weeks 12, 24, 48) is informative in predicting clinical responses at Week 48. High NPV (>90.9%) is observed for the association of viral response and the serological responses (HBeAg loss, anti-HBe Gain, and seroconversion). The significance of a viral response at Weeks 12, 24, and 48 in predicting histologic, biochemical, HBeAg loss, anti-HBe gain, and seroconversion at Week 48 and later time points is assessed by the lower 95% CI limit for the odds ratio exceeding 1.0.

Table 29

PPV, NPV, and Odds Ratio for Individual Clinical Responses During Treatment Predicted by Early Viral Response (≥ 2 Log IU/mL Decrease) In HBeAg-Positive Subjects

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	Histologic	67.5 (52/77)	(55.8, 77.5)	51.4 (19/37)	(34.7, 67.8)	2.20 (0.91, 5.28)
		Biochemical	58.2 (46/79)	(46.6, 69.1)	57.5 (23/40)	(41.0, 72.6)	1.89 (0.82, 4.39)
		HBeAg Loss	36.3 (29/80)	(26.0, 47.8)	90.9 (40/44)	(77.4, 97.0)	
		Anti-HBe Gain	18.8 (15/80)	(11.2, 29.4)	95.5 (42/44)	(83.3, 99.2)	
		Seroconversion[b]	18.8 (15/80)	(11.2, 29.4)	95.5 (42/44)	(83.3, 99.2)	
24	48	Histologic	62.7 (52/83)	(51.3, 72.8)	48.1 (13/27)	(29.2, 67.6)	1.56 (0.59, 4.09)
		Biochemical	59.3 (51/86)	(48.2, 69.6)	60.7 (17/28)	(40.7, 77.9)	2.25 (0.87, 5.98)
		HBeAg Loss	33.3 (30/90)	(24.0, 44.1)	96.6 (28/29)	(80.4, 99.8)	
		Anti-HBe Gain	16.7 (15/90)	(9.9, 26.3)	100.0 (29/29)	(85.4, 100.0)	>5.60[d]
		Seroconversion	16.7 (15/90)	(9.9, 26.3)	100.0 (29/29)	(85.4, 100.0)	>5.60[d]
48[c]	48	Histologic	66.7 (60/90)	(55.9, 76.0)	58.1 (18/31)	(39.3, 74.9)	
		Biochemical	63.4 (59/93)	(52.8, 73.0)	75.8 (25/33)	(57.4, 88.3)	
		HBeAg Loss	35.8 (34/95)	(26.4, 46.3)	100.0 (36/36)	(88.0,100.0)	
		Anti-HBe Gain	17.9 (17/95)	(11.1, 27.4)	100.0 (36/36)	(88.0,100.0)	
		Seroconversion	17.9 (17/95)	(11.1, 27.4)	100.0 (36/36)	(88.0,100.0)	
12	144	Histologic	not available				
		Biochemical	43.3 (13/30)	(26.0, 62.3)	47.6 (10/21)	(26.4, 69.7)	0.70 (0.20, 2.46)
		HBeAg Loss	23.3 (7/30)	(10.6, 42.7)	85.7 (18/21)	(62.6, 96.2)	1.83 (0.35, 12.35)
		Anti-HBe Gain	16.7 (5/30)	(6.3, 35.5)	85.7 (18/21)	(62.6, 96.2)	1.20 (0.20, 8.70)
		Seroconversion	16.7 (5/30)	(6.3, 35.5)	85.7 (18/21)	(62.6, 96.2)	1.20 (0.20, 8.70)
24	144	Histologic	not available				
		Biochemical	44.7 (17/38)	(29.0, 61.5)	41.7 (5/12)	(16.5, 71.4)	0.58 (0.12, 2.59)
		HBeAg Loss	26.3 (10/38)	(14.0, 43.4)	100.0 (12/12)	(69.9, 100.0)	>3.93[d]
		Anti-HBe Gain	21.1 (8/38)	(10.1, 37.8)	100.0 (12/12)	(69.9, 100.0)	>2.93[d]
		Seroconversion	21.1 (8/38)	(10.1, 37.8)	100.0 (12/12)	(69.9, 100.0)	>2.93[d]
48	144	Histologic	not available				
		Biochemical	48.3 (14/29)	(29.9, 67.1)	55.6 (10/18)	(31.3, 77.6)	1.17 (0.31, 4.49)
		HBeAg Loss	20.7 (6/29)	(8.7, 40.3)	83.3 (15/18)	(57.7, 95.6)	1.30 (0.23, 9.25)
		Anti-HBe Gain	17.2 (5/29)	(6.5, 36.5)	88.9 (16/18)	(63.9, 98.1)	1.67 (0.23, 19.34)
		Seroconversion	17.2 (5/29)	(6.5, 36.5)	88.9 (16/18)	(63.9, 98.1)	1.67 (0.23, 19.34)
12	192	Histologic	not available				
		Biochemical	60.0 (15/25)	(38.9, 78.2)	38.9 (7/18)	(18.3, 63.9)	0.95 (0.23, 3.89)
		HBeAg Loss	48.0 (12/25)	(28.3, 68.2)	66.7 (12/18)	(41.2, 85.6)	1.85 (0.45, 7.96)
		Anti-HBe Gain	28.0 (7/25)	(12.9, 49.6)	77.8 (14/18)	(51.9, 92.6)	1.36 (0.27, 7.62)
		Seroconversion	28.0 (7/25)	(12.9, 49.6)	77.8 (14/18)	(51.9, 92.6)	1.36 (0.27, 7.62)
24	192	Histologic	not available				
		Biochemical	56.7 (17/30)	(37.7, 74.0)	27.3 (3/11)	(7.3, 60.7)	0.49 (0.07, 2.64)
		HBeAg Loss	43.3 (13/30)	(26.0, 62.3)	72.7 (8/11)	(39.3, 92.7)	2.04 (0.38, 14.07)
		Anti-HBe Gain	33.3 (10/30)	(17.9, 52.9)	100.0 (11/11)	(67.9, 100.0)	
		Seroconversion	33.3 (10/30)	(17.9, 52.9)	100.0 (11/11)	(67.9, 100.0)	
48	192	Histologic	not available				
		Biochemical	76.9 (20/26)	(55.9, 90.2)	53.3 (8/15)	(27.4, 77.7)	3.81 (0.80, 18.49)
		HBeAg Loss	46.2 (12/26)	(27.1, 66.3)	60.0 (9/15)	(32.9, 82.5)	1.29 (0.30, 5.77)
		Anti-HBe Gain	34.6 (9/26)	(17.9, 55.6)	86.7 (13/15)	(58.4, 97.7)	3.44 (0.55, 37.07)
		Seroconversion	34.6 (9/26)	(17.9, 55.6)	86.7 (13/15)	(58.4, 97.7)	3.44 (0.55, 37.07)
12	240	Histologic	100.0 (5/5)	(46.3, 100.0)	75.0 (3/4)	(21.9, 98.7)	*
		Biochemical	73.7 (14/19)	(48.6, 89.9)	33.3 (5/15)	(13.0, 61.3)	1.40 (0.25, 7.92)
		HBeAg Loss	57.9 (11/19)	(34.0, 78.9)	73.3 (11/15)	(44.8, 91.1)	3.78 (0.72, 21.84)
		Anti-HBe Gain	27.8 (5/18)	(10.7, 53.6)	100.0 (15/15)	(74.7, 100.0)	>5.38[d]
		Seroconversion	26.3 (5/19)	(10.1, 51.4)	100.0 (15/15)	(74.7, 100.0)	>5.00[d]
24	240	Histologic	71.4 (5/7)	(30.3, 94.9)	100.0 (2/2)	(19.8, 100.0)	*
		Biochemical	69.6 (16/23)	(47.0, 85.9)	33.3 (3/9)	(9.0, 69.1)	1.14 (0.14, 7.48)
		HBeAg Loss	52.2 (12/23)	(31.1, 72.6)	88.9 (8/9)	(50.7, 99.4)	8.73 (0.86, 418.80)
		Anti-HBe Gain	22.7 (5/22)	(8.7, 45.8)	100.0 (9/9)	(62.9, 100.0)	>2.35[d]
		Seroconversion	21.7 (5/23)	(8.3, 44.2)	100.0 (9/9)	(62.9, 100.0)	>2.22[d]
48	240	Histologic	66.7 (4/6)	(24.1, 94.0)	100.0 (2/2)	(19.8, 100.0)	*
		Biochemical	77.3 (17/22)	(54.2, 91.3)	40.0 (4/10)	(13.7, 72.6)	2.27 (0.32, 14.76)
		HBeAg Loss	54.5 (12/22)	(32.7, 74.9)	80.0 (8/10)	(44.2, 96.5)	4.80 (0.69, 53.92)
		Anti-HBe Gain	19.0 (4/21)	(6.3, 42.6)	100.0 (10/10)	(65.5, 100.0)	>2.12[d]
		Seroconversion	18.2 (4/22)	(6.0, 41.0)	100.0 (10/10)	(65.5, 100.0)	>2.00[d]

[a] Undefined (division by zero)

[b] Shading Indicates statistical significance.

[b] Seroconversion - HBeAg undetectable and antibody against HBeAg detected.

[c] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

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

Tables 30 and 31 demonstrate that the NPV is high (greater than or equal to 97% for response at Week 48) for the association of early viral response with the combination of all three responses (histologic, biochemical, and serological - HBeAg loss or seroconversion). These data indicate that HBeAg-positive subjects without an early viral response (defined as ≥ 2 log IU/mL decrease) are unlikely to achieve all three clinical responses by Week 48, as a result of treatment. The number of subjects at Week 240 was small, and therefore available data is inadequate to draw conclusions about the association of the early viral response with clinical responses at this time point.

Table 30

PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and HBeAg Loss Responses During Treatment Predicted by an Early Viral Response (≥ 2 Log IU/mL Decrease) in HBeAg-Positive Subjects

Week of Viral Response	Week of Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	23.7 (18/76)	(15.0, 35.1)	97.3 (36/37)	(84.2, 99.9)	
24	48	20.7 (17/82)	(12.9, 31.4)	100.0 (27/27)	(84.5, 100.0)	
48[b]	48	21.3 (19/89)	(13.7, 31.6)	100.0 (31/31)	(86.3, 100.0)	
12	240	60.0 (3/5)	(17.0, 92.7)	75.0 (3/4)	(21.9, 98.7)	4.50 (0.15, 313.49)
24	240	42.9 (3/7)	(11.8, 79.8)	100.0 (2/2)	(19.8, 100.0)	*
48	240	50.0 (3/6)	(13.9, 86.1)	100.0 (2/2)	(19.8, 100.0)	*

* Undefined (division by zero)

[a] Shading indicates statistical significance.

[b] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

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

Table 31

PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic, Biochemical, and Seroconversion Responses During Treatment Predicted by an Early Viral Response (≥ 2 Log IU/mL Decrease) in HBeAg-Positive Subjects

Week of Viral Response	Week of Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
12	48	10.5 (8/76)	(5.0, 20.2)	100.0 (37/37)	(88.3, 100.0)	>4.24[b]
24	48	8.5 (7/82)	(3.8, 17.3)	100.0 (27/27)	(84.5, 100.0)	>2.43[b]
48[a]	48	9.0 (8/89)	(4.2, 17.4)	100.0 (31/31)	(86.3, 100.0)	>2.96[b]
12	240	40.0 (2/5)	(7.3, 83.0)	100.0 (4/4)	(39.6, 100.0)	*
24	240	28.6 (2/7)	(5.1, 69.7)	100.0 (2/2)	(19.8, 100.0)	*
48	240	16.7 (1/6)	(0.9, 63.5)	100.0 (2/2)	(19.8, 100.0)	*

* Undefined (division by zero)

[a] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

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

HBeAg-Negative Patients

Characterization of Viral Load

Table 32 below demonstrates the efficacy, based on HBV viral load testing, of treating HBeAg-negative patients with 10 mg adefovir dipivoxil compared to placebo. At Week 48, 48.72% (57/117) of HBeAg-negative patients on treatment versus 0% (0/55) on placebo had achieved very low viral loads below 100 IU/mL. Furthermore, 3.42% (4/117) of patients on treatment versus 30.91 % (17/55) on placebo had viral loads greater than or equal to 10^5 IU/mL.

Table 32

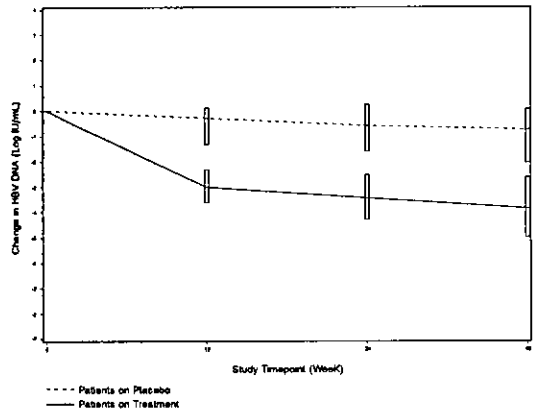
Distribution of HBV Viral Load at Week 48 for HBeAg-Negative Patients

Viral Load (IU/mL)	Adefovir Dipivoxil			Placebo		
	N	%	Cumulative %	N	%	Cumulative %
TND[a]	9	7.69	7.69	0	0.00	0.00
< 15	20	17.09	24.79	0	0.00	0.00
15 - < 100	28	23.93	48.72	0	0.00	0.00
100 - < 10^3	33	28.21	76.92	7	12.73	12.73
10^3 - < 10^4	9	7.69	84.62	5	9.09	21.82
10^4 - < 10^5	6	5.13	89.74	11	20.00	41.82
10^5 - < 10^6	8	6.84	96.58	15	27.27	69.09
10^6 - < 10^9	4	3.42	100.00	17	30.91	100.00
≥ 10^9	0	0.00	100.00	0	0.00	100.00
Total	117	100.00		55	100.00	

[a] TND = Target Not Detected

Figure 9 below demonstrates the median viral load change and inter-quartile range of change from baseline to Week 48 for HBeAg-negative subjects on treatment compared to placebo. This shows the impact of adefovir dipivoxil treatment on the viral load of HBeAg-negative patients with chronic hepatitis B.

Figure 9
Median and Inter-Quartile Range of Change in HBV DNA from Baseline: HBeAg-Negative Subjects



The effect of therapy for patients with chronic HBV infection can be assessed by measuring the HBV DNA (expected reduction to low or undetectable levels) and monitoring for viral rebound that could be associated with resistance. Results in Table 33 show that 56.91% (70/123) of the treated patients achieved a nadir, or lowest concentration, viral load level by Week 48. Of the 30 subjects that achieved a nadir by Week 24, 26.92% (7/26) subjects had a greater than or equal to one log IU/mL increase by Week 48 (four of these subjects did not have a Week 48 result).

Table 33
Distribution of the HBeAg-Negative Subjects by Week on Treatment and the Viral Load at Which the Nadir was Reached

Nadir Viral Load (IU/mL)	Number (%) of Patients With the Nadir Viral Load Achieved by Week							Total By Viral Load	Cumulative By Viral Load
	12	24	48	96	144	192	240		
TND*	1 (0.81)	3 (2.44)	9 (7.32)	4 (3.25)	11 (8.94)	2 (1.63)	0 (0.00)	30 (24.39)	30 (24.39)
< 15	1 (0.81)	5 (4.07)	6 (4.88)	3 (2.44)	8 (6.50)	8 (6.50)	2 (1.63)	33 (26.83)	63 (51.22)
15 - < 100	2 (1.63)	4 (3.25)	10 (8.13)	1 (0.81)	3 (2.44)	1 (0.81)	2 (1.63)	23 (18.70)	86 (69.92)
100 - < 10^3	2 (1.63)	3 (2.44)	12 (9.76)	3 (2.44)	1 (0.81)	0 (0.00)	1 (0.81)	22 (17.89)	108 (87.80)
10^3 - < 10^4	2 (1.63)	0 (0.00)	1 (0.81)	0 (0.00)	0 (0.00)	1 (0.81)	1 (0.81)	5 (4.07)	113 (91.87)
10^4 - < 10^5	0 (0.00)	2 (1.63)	1 (0.81)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (2.44)	116 (94.31)
10^5 - < 10^6	2 (1.63)	1 (0.81)	1 (0.81)	0 (0.00)	1 (0.81)	0 (0.00)	0 (0.00)	5 (4.07)	121 (98.37)
10^6 - < 10^9	1 (0.81)	1 (0.81)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.63)	123 (100.00)
Total By Week	11 (8.94)	19 (15.45)	40 (32.52)	11 (8.94)	24 (19.51)	12 (9.76)	6 (4.88)		
Cumulative By Week	11 (8.94)	30 (24.39)	70 (56.91)	81 (65.85)	105 (85.37)	117 (95.12)	123 (100.00)		

* TND = Target Not Detected

Two patients out of 123 achieved HBsAg seroconversion. One patient was a white female, HBV genotype D, > age 30, and seroconverted at Week 96. The other patient was a white male, HBV genotype D, > age 30, and seroconverted at Week 240. A summary of these results is provided in Table 34.

Table 34
HBeAg-Negative Subjects with HBsAg Seroconversion

	Concentration (Log IU/mL)							
	Week 0	Week 12	Week 24	Week 48	Week 96	Week 144	Week 192	Week 240
Subject 1	6.86	3.83	3.25	< 1.18	TND*	n/a**	n/a**	n/a**
Subject 2	6.36	4.53	4.56	5.04	5.09	< 1.00	TND*	TND*

* TND = Target Not Detected
** Aliquot not available for testing.

Summaries of the effect of baseline covariates for the HBeAg-negative population are provided in Table 35 through Table 37.

Table 35
Association Between Responses to Treatment at Week 48 and Baseline Covariates for HBeAg-Negative Patients

Response to Treatment	Covariate	Category	N	No. of Patients with Response	Proportion (%) of Patients with Response	Unadjusted Odds Ratio (95% CI)
Histological	Race	Asian	32	19	59.38	0.52 (0.20, 1.38)
		Other	76	56	73.68	
	Sex	Male	90	63	70.00	1.17 (0.32, 3.79)
		Female	18	12	66.67	
	Age	≤ 30	7	6	85.71	2.78 (0.31, 131.93)
		> 30	101	69	68.32	
	Genotype	B,C	32	19	59.38	0.52 (0.20, 1.38)
		Non-B,C	76	56	73.68	
Biochemical	Race	Asian	30	21	70.00	0.68 (0.24, 1.99)
		Other	80	62	77.50	
	Sex	Male	93	67	72.04	0.16 (0.00, 1.16)
		Female	17	16	94.12	
	Age	≤ 30	7	6	85.71	2.03 (0.23, 96.72)
		> 30	103	77	74.76	
	Genotype	B,C	30	21	70.00	0.68 (0.24, 1.99)
		Non-B,C	80	62	77.50	

The statistical significance of the associations of the Race, Sex, Age and Genotype covariates with viral response was studied by calculating odds ratios and their exact 95% confidence intervals for both definitions of viral response and summarized in Table 36 and Table 37. 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.

Table 36
Odds Ratios for the Association Between Viral Response (< 2,000 IU/mL) and Covariates, by Week, for an HBeAg-Negative Population

Covariate	Category	Week	N	No. Below 2,000 IU/mL	Proportion (%) Below 2,000 IU/mL	Unadjusted Odds Ratio (95% CI)
Race	Asian	12	33	25	75.76	2.36 (0.89, 6.79)
	Other		79	45	56.96	
	Asian	24	34	28	76.47	1.03 (0.37, 3.08)
	Other		79	60	75.95	
	Asian	48	33	29	87.88	2.34 (0.69, 10.20)
	Other		82	62	75.61	
Sex	Male	12	94	59	62.77	1.07 (0.32, 3.36)
	Female		18	11	61.11	
	Male	24	93	72	77.42	1.47 (0.41, 4.71)
	Female		20	14	70.00	
	Male	48	95	76	80.00	1.33 (0.34, 4.51)
	Female		20	15	75.00	
Age	≤ 30	12	7	4	57.14	0.79 (0.13, 5.67)
	> 30		105	66	62.86	
	≤ 30	24	7	5	71.43	0.77 (0.12, 8.59)
	> 30		106	81	76.42	
	≤ 30	48	6	4	66.67	0.51 (0.07, 5.97)
	> 30		109	87	79.82	
Genotype	B,C	12	33	25	75.76	2.36 (0.89, 6.79)
	Non-B,C		79	45	56.96	
	B,C	24	34	26	76.47	1.03 (0.37, 3.08)
	Non-B,C		79	60	75.95	
	B,C	48	33	29	87.88	2.34 (0.69, 10.20)
	Non-B,C		82	62	75.61	

Table 37

Odds Ratios for the Association Between Viral Response (≥ 2 Log Decrease From Baseline Result) and Covariates, by Week, for an HBeAg-Negative Population

Covariate	Category	Week	N	No. with ≥ 2 Log Decrease	Proportion (%) with ≥ 2 Log Decrease	Unadjusted Odds Ratio (95% CI)
Race	Asian	12	33	25	75.76	0.86 (0.30, 2.60)
	Other		79	62	78.48	
	Asian	24	34	31	91.18	1.50 (0.35, 9.02)
	Other		79	69	87.34	
	Asian	48	33	31	93.94	2.15 (0.42, 21.22)
	Other		82	72	87.80	
Sex	Male	12	94	72	76.60	0.65 (0.11, 2.64)
	Female		18	15	83.33	
	Male	24	93	82	88.17	0.83 (0.08, 4.32)
	Female		20	18	90.00	
	Male	48	95	83	87.37	0.00 (0.00, 1.28)
	Female		20	20	100.00	
Age	≤ 30	12	7	5	71.43	0.70 (0.11, 7.84)
	> 30		105	82	78.10	
	≤ 30	24	7	6	85.71	0.77 (0.08, 38.13)
	> 30		106	94	88.68	
	≤ 30	48	6	5	83.33	0.56 (0.06, 28.93)
	> 30		109	98	89.91	
Genotype	B,C	12	33	25	75.76	0.86 (0.30, 2.60)
	Non-B,C		79	62	78.48	
	B,C	24	34	31	91.18	1.50 (0.35, 9.02)
	Non-B,C		79	69	87.34	
	B,C	48	33	31	93.94	2.15 (0.42, 21.22)
	Non-B,C		82	72	87.80	

Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Odds Ratio (OR) Analysis in an HBeAg-Negative Population

For each patient, two responses: Histologic and Biochemical - were measured at various times during treatment.

- Histologic response - improvement of histologic status by at least 2 units of the Knodell necroinflammatory 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 the baseline

Additionally, HBsAg seroconversion data were collected. Two patients out of 123 achieved HBsAg seroconversion. One patient was a white female, HBV genotype D, > age 30, and seroconverted at Week 96. The other patient was a white male, HBV genotype D, > age 30, and seroconverted at Week 240. A summary of these results is provided in Table 34.

Viral load response was defined as either HBV DNA less than 2,000 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, 96, 144, 192, or 240 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, 96, 144, 192, or 240 and a viral load non-response at Weeks 12, 24, or 48.

Viral Response < 2,000 IU/mL

As shown in Table 38, viral response at Weeks 24 and 48 (when defined as < 2,000 IU/mL) appears informative (i.e., lower 95% CI limit for the odds ratio exceeding 1.0) in predicting biochemical response at 48 and 96 weeks on treatment. The PPV for the association of viral response and histologic response increased throughout the study and was ≥ 68.3% at Week 48 and ≥ 85.7% at the end of the study. The PPV for the association of viral response and biochemical response was ≥ 78.3% at Week 48 and remained consistent through the end of the study.

Table 38

PPV, NPV, and Odds Ratio for Individual Clinical Responses During Treatment Predicted by Early Viral Response (< 2,000 IU/mL) in HBeAg-Negative Subjects

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV % (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	Histologic	68.3 (41/60)	(54.9, 79.4)	29.7 (11/37)	(16.4, 47.2)	0.91 (0.34, 2.41)
		Biochemical	78.3 (47/60)	(65.5, 87.5)	27.5 (11/40)	(15.1, 44.1)	1.37 (0.48, 3.82)
24	48	Histologic	71.4 (55/77)	(59.8, 80.9)	30.4 (7/23)	(14.1, 53.0)	1.09 (0.33, 3.30)
		Biochemical	81.6 (62/76)	(70.7, 89.2)	44.0 (11/25)	(25.0, 64.7)	
48[b]	48	Histologic	69.8 (60/86)	(58.8, 79.0)	35.0 (7/20)	(16.3, 59.1)	1.24 (0.37, 3.82)
		Biochemical	83.3 (70/84)	(73.3, 90.3)	45.8 (11/24)	(26.2, 66.8)	
12	96	Histologic	88.9 (8/9)	(50.7, 99.4)	16.7 (1/6)	(0.9, 63.5)	1.60 (0.02, 141.06)
		Biochemical	83.3 (25/30)	(64.5, 93.7)	32.1 (9/28)	(16.6, 52.4)	2.37 (0.59, 10.41)
24	96	Histologic	84.6 (11/13)	(53.7, 97.3)	33.3 (1/3)	(1.8, 87.5)	2.75 (0.03, 78.72)
		Biochemical	87.8 (36/41)	(73.0, 95.4)	56.3 (9/16)	(30.6, 79.2)	
48	96	Histologic	85.7 (12/14)	(56.2, 97.5)	25.0 (1/4)	(1.3, 78.1)	2.00 (0.03, 50.57)
		Biochemical	82.6 (38/46)	(68.0, 91.7)	50.0 (7/14)	(24.0, 76.0)	
12	144	Histologic	not available				
		Biochemical	72.7 (24/33)	(54.2, 86.1)	30.4 (7/23)	(14.1, 53.0)	1.17 (0.30, 4.37)
24	144	Histologic	not available				
		Biochemical	76.7 (33/43)	(61.0, 87.7)	46.2 (6/13)	(20.4, 73.9)	2.83 (0.62, 12.41)
48	144	Histologic	not available				
		Biochemical	74.5 (35/47)	(59.4, 85.6)	41.7 (5/12)	(16.5, 71.4)	2.08 (0.43, 9.30)
12	192	Histologic	not available				
		Biochemical	82.8 (24/29)	(63.5, 93.5)	10.0 (2/20)	(1.8, 33.1)	0.53 (0.05, 3.78)
24	192	Histologic	not available				
		Biochemical	84.6 (33/39)	(68.8, 93.6)	22.2 (2/9)	(3.9, 59.8)	1.57 (0.13, 11.50)
48	192	Histologic	not available				
		Biochemical	86.0 (37/43)	(71.4, 94.2)	25.0 (2/8)	(4.5, 64.4)	2.06 (0.16, 15.55)
12	240	Histologic	85.7 (6/7)	(42.0, 99.2)	25.0 (3/12)	(6.7, 57.2)	2.00 (0.12, 122.23)
		Biochemical	78.3 (18/23)	(55.8, 91.7)	6.3 (1/16)	(0.3, 32.3)	0.24 (0.00, 2.58)
24	240	Histologic	100.0 (12/12)	(69.9, 100.0)	42.9 (3/7)	(11.8, 79.8)	>8.25[c]
		Biochemical	80.0 (24/30)	(60.9, 91.6)	11.1 (1/9)	(0.6, 49.3)	0.50 (0.01, 5.31)
48	240	Histologic	100.0 (12/12)	(69.9, 100.0)	42.9 (3/7)	(11.8, 79.8)	>8.25[c]
		Biochemical	80.6 (25/31)	(61.9, 91.9)	11.1 (1/9)	(0.6, 49.3)	0.52 (0.01, 5.51)

[a] Shading indicates statistical significance.
[b] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.
[c] The odds ratio calculations are undefined when NPV is 100% or PPV is 100% or missing. Where the denominators for both the NPV and PPV are greater than five, a "minimum" odds ratio was determined by subtracting one specimen from the numerator of the 100% parameter estimate (NPV or PPV).

Table 39 shows PPV, NPV, and OR results for a combination of both histologic and biochemical responses. PPV for the association of viral response at 12, 24, and 48 Weeks (when defined as < 2,000 IU/mL) with the combination of both responses (histologic and biochemical) at Week 240 ranges from 85% to 100% (however, 95% CI is wide and OR is not significant with the available population).

Table 39

PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic and Biochemical Responses During Treatment Predicted by an Early Viral Response (< 2,000 IU/mL) in HBeAg-Negative Subjects

Week of Viral Response	Week of Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	60.0 (33/55)	(45.9, 72.7)	51.4 (19/37)	(34.7, 67.8)	1.58 (0.63, 3.99)
24	48	64.8 (46/71)	(52.5, 75.5)	65.2 (15/23)	(42.8, 82.8)	
48[b]	48	62.5 (50/80)	(50.9, 72.9)	70.0 (14/20)	(45.7, 87.2)	
12	96	55.6 (5/9)	(22.7, 84.7)	50.0 (3/6)	(13.9, 86.1)	1.25 (0.10, 15.38)
24	96	61.5 (8/13)	(32.3, 84.9)	100.0 (3/3)	(31.0, 100.0)	*
48	96	64.3 (9/14)	(35.6, 86.0)	75.0 (3/4)	(21.9, 98.7)	5.40 (0.30, 314.24)
12	240	85.7 (6/7)	(42.0, 99.2)	25.0 (3/12)	(6.7, 57.2)	2.00 (0.12, 122.23)
24	240	100.0 (12/12)	(69.9, 100.0)	42.9 (3/7)	(11.8, 79.8)	>8.25[c]
48	240	100.0 (12/12)	(69.9 100.0)	42.9 (3/7)	(11.8, 79.8)	>8.25[c]

* Undefined (division by zero)
[a] Shading indicates statistical significance.
[b] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.
[c] The odds ratio calculations are undefined when NPV is 100% or PPV is 100% or missing. Where the denominators for both the NPV and PPV are greater than five, a "minimum" odds ratio was determined by subtracting one specimen from the numerator of the 100% parameter estimate (NPV or PPV).

Viral Response ≥ 2 Log IU/mL Decrease

As shown in Table 40, viral response at Weeks 12, 24, and 48, when defined as ≥ 2 log decrease in value from the baseline viral load result, is informative (i.e., lower limits of the 95% CIs exceed 1) in predicting biochemical response at Week 48 of treatment. Viral response at Weeks 12 and 48 is also informative for predicting biochemical response at Week 96 on treatment. The PPV for the association of viral response at Weeks 12, 24 and 48 and histologic response at the end of the study (240 weeks) was 76.9 %, 86.7%, and 82.4%, respectively. The PPV for the association of viral response and biochemical response was greater than or equal to 78.8% for biochemical response at Week 48 and remained consistent through the end of the study.

Table 40

PPV, NPV, and Odds Ratio for Individual Clinical Responses During Treatment Predicted by Early Viral Response (≥ 2 Log IU/mL Decrease) in HBeAg-Negative Subjects

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	Histologic	71.2 (52/73)	(59.3, 80.9)	37.5 (9/24)	(19.5, 59.2)	1.49 (0.49, 4.30)
		Biochemical	83.1 (64/77)	(72.5, 90.4)	47.8 (11/23)	(27.4, 68.9)	
24	48	Histologic	70.8 (63/89)	(60.0, 79.7)	27.3 (3/11)	(7.3, 60.7)	0.91 (0.14, 4.18)
		Biochemical	78.9 (71/90)	(68.8, 86.5)	54.5 (6/11)	(24.6, 81.9)	
48[b]	48	Histologic	68.0 (66/97)	(57.7, 76.9)	22.2 (2/9)	(3.9, 59.8)	0.61 (0.06, 3.46)
		Biochemical	83.3 (80/96)	(74.0, 89.9)	75.0 (9/12)	(42.8, 93.3)	
12	96	Histologic	83.3 (10/12)	(50.9, 97.1)	0.0 (0/3)	(0.0, 69.0)	0.0 (0.00, 15.31)
		Biochemical	84.1 (37/44)	(69.3, 92.8)	50.0 (7/14)	(24.0, 76.0)	
24	96	Histologic	84.6 (11/13)	(53.7, 97.3)	33.3 (1/3)	(1.8, 87.5)	2.75 (0.03, 78.72)
		Biochemical	81.3 (39/48)	(66.9, 90.6)	55.6 (5/9)	(22.7, 84.7)	5.42 (0.92, 32.22)
48	96	Histologic	82.4 (14/17)	(55.8, 95.3)	0.0 (0/1)	(0.0, 94.5)	0.00 (0.00, 95.00)
		Biochemical	80.0 (44/55)	(66.6, 89.1)	80.0 (4/5)	(29.9, 98.9)	
12	144	Histologic	not available				
		Biochemical	75.0 (33/44)	(59.4, 86.3)	41.7 (5/12)	(16.5, 71.4)	2.14 (0.44, 9.72)
24	144	Histologic	not available				
		Biochemical	72.9 (35/48)	(57.9, 84.3)	37.5 (3/8)	(10.2, 74.1)	1.62 (0.22, 9.67)
48	144	Histologic	not available				
		Biochemical	72.7 (40/55)	(58.8, 83.5)	50.0 (2/4)	(9.2, 90.8)	2.67 (0.18, 39.14)
12	192	Histologic	not available				
		Biochemical	89.7 (35/39)	(74.8, 96.7)	30.0 (3/10)	(8.1, 64.6)	3.75 (0.44, 27.27)
24	192	Histologic	not available				
		Biochemical	85.4 (35/41)	(70.1, 93.9)	28.6 (2/7)	(5.1, 69.7)	2.33 (0.18, 18.75)
48	192	Histologic	not available				
		Biochemical	87.5 (42/48)	(74.1, 94.8)	66.7 (2/3)	(12.5, 98.2)	14.00 (0.59, 844.47)
12	240	Histologic	76.9 (10/13)	(46.0, 93.8)	16.7 (1/6)	(0.9, 63.5)	0.67 (0.01, 11.40)
		Biochemical	82.8 (24/29)	(63.5, 93.5)	10.0 (1/10)	(0.5, 45.9)	0.53 (0.01, 5.90)
24	240	Histologic	86.7 (13/15)	(58.4, 97.7)	25.0 (1/4)	(1.3, 78.1)	2.17 (0.03, 54.35)
		Biochemical	78.8 (26/33)	(60.6, 90.4)	0.0 (0/6)	(0.0, 48.3)	0.00 (0.00, 2.99)
48	240	Histologic	82.4 (14/17)	(55.8, 95.3)	0.0 (0/2)	(0.0, 80.2)	0.00 (0.00, 20.71)
		Biochemical	81.6 (31/38)	(65.1, 91.7)	0.0 (0/2)	(0.0, 80.2)	0.00 (0.00, 17.04)

[a] Shading indicates statistical significance.

[b] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

Table 41 shows PPV, NPV, and OR results for a combination of both histologic and biochemical responses. PPV for the association of viral response at 12, 24, and 48 weeks (when defined as ≥ 2 log IU/mL decrease in value from the baseline viral load result) with the combination of both responses (histologic and biochemical) at Week 240 ranges from 76.9% to 86.7% (however, 95% CI is wide, and OR is not significant with the available population).

Table 41

PPV, NPV, and Odds Ratio (OR) for a Combination of Histologic and Biochemical Responses During Treatment Predicted by an Early Viral Response (≥ 2 Log IU/mL Decrese) in HBeAg-Negative Subjects

Week of Viral Response	Week of Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)[a]
12	48	61.4 (43/70)	(49.0, 72.6)	63.6 (14/22)	(40.8, 82.0)	2.79 (0.93, 8.68)
24	48	59.5 (50/84)	(48.2, 69.9)	60.0 (6/10)	(27.4, 86.3)	2.21 (0.48, 11.36)
48[b]	48	60.4 (55/91)	(49.6, 70.4)	88.9 (8/9)	(50.7, 99.4)	
12	96	58.3 (7/12)	(28.6, 83.5)	66.7 (2/3)	(12.5, 98.2)	2.80 (0.11, 188.36)
24	96	53.8 (7/13)	(26.1, 79.6)	66.7 (2/3)	(12.5, 98.2)	2.33 (0.09, 157.00)
48	96	58.8 (10/17)	(33.5, 80.6)	100.0 (1/1)	(5.5, 100.0)	*
12	240	76.9 (10/13)	(46.0, 93.8)	16.7 (1/6)	(0.9, 63.5)	0.67 (0.01, 11.40)
24	240	86.7 (13/15)	(58.4, 97.7)	25.0 (1/4)	(1.3, 78.1)	2.17 (0.03, 54.35)
48	240	82.4 (14/17)	(55.8, 95.3)	0.0 (0/2)	(0.0, 80.2)	0.00 (0.00, 20.71)

[a] Shading indicates statistical significance.

[b] An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

* Undefined (division by zero)

Conclusions Drawn from the Studies

Based on the results of the nonclinical and clinical laboratory studies, the Abbott RealTi*me* HBV assay, when used according to the provided directions and in conjunction with other serological and clinical information, is found to be useful in quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma from chronically HBV-infected individuals, for aiding in assessing response to treatment and the management of patients with chronic HBV infection undergoing anti-viral therapy by measuring HBV DNA levels at baseline and during treatment.

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