SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: In vitro polymerase chain reaction (PCR) based assay for HBV viral load detection.

Device Trade Name: Abbott RealTi*m*e HBV Assay, Abbott RealTi*m*e HBV Amplification Reagent Kit, Abbott RealTi*m*e HBV Calibrator Kit, Abbott RealTi*m*e HBV Control Kit.

Applicant's Name and Address: Abbott Molecular Inc., 1300 E. Touhy Avenue, Des Plaines, IL 60018

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P080026

Date of FDA Notice of Approval: August 13, 2010

Expedited: Not applicable

II. INDICATIONS FOR 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 RealTi*m*e 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.

III. CONTRAINDICATIONS

None known

IV. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only
The warnings and precautions for the Abbott RealTi*me* HBV assay are stated in the respective product labeling.

V. DEVICE DESCRIPTION

The Abbott RealTi*me* HBV assay is an in vitro polymerase chain reaction (PCR) assay for the quantitation of HBV DNA in human plasma (EDTA) or serum from HBV-infected individuals. The Abbott RealTi*me* HBV assay uses PCR to generate amplified product from the DNA genome of HBV in clinical specimens. The Abbott RealTi*me* HBV assay uses the Abbott *m2000sp* instrument for processing samples and the Abbott *m2000rt* instrument for amplification and detection. The *m2000sp* and *m2000rt* instruments, as a part of the *m*2000 System, were approved in the Abbott RealTi*me* HIV-1 PMA BP060002, May 11, 2007, and subsequently cleared in the Abbott RealTime CT/NG 510(k) k080739 on 7/10/2008.
The Abbott *m2000sp* uses the Abbott *m*Sample Preparation System$_{DNA}$ reagents to extract DNA for amplification and detection. The *m*Sample Preparation reagents include lysis reagent, magnetic particle reagent for capturing nucleic acids, wash, and elution reagents. Proteinase K is included in the lysis step to digest proteins associated with the nucleic acids. The Abbott *m2000sp* provides automated sample eluate transfer to an Abbott 96-Deep Well Plate, and reaction assembly in the Abbott 96-Well Optical Reaction Plate. The 96-Well Optical Reaction Plate is manually sealed and transferred to the *m2000rt* to perform the amplification and real-time fluorescence detection reaction. Results are automatically reported on the *m2000rt* workstation. An internal control nucleic acid is introduced into each sample during the sample preparation process and measured on the *m2000rt* to demonstrate that the process was completed correctly for each sample and control. The Abbott RealTi*me* HBV assay contains sufficient reagents to process approximately 96 tests. A total 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 in each run.
Application parameters specific to the Abbott RealTi*me* HBV assay are contained on an assay specific application file, housed on a CD-ROM and loaded onto the *m2000sp* and *m2000rt* instruments.
The Abbott RealTi*me* HBV assay consists of three kits:

Abbott RealTi*me* HBV Amplification Reagent Kit (List No. 2N40-90)
Abbott RealTi*me* HBV Control Kit (List No. 2N40-80)
Abbott RealTi*me* HBV Calibrator Kit (List No. 2N40-70)

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 *m2000sp*, an automated sample preparation system designed to use magnetic microparticle processes for the purification of nucleic acids from samples.
The Abbott *m*Sample Preparation System$_{DNA}$ 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 The bound nucleic acids are eluted and transferred to a 96-deep well plate. The nucleic acids are then ready for amplification. The Internal Control (IC) is introduced into the Sample Preparation Protocol and is processed along with the calibrators, controls, and specimens.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m2000sp* combines the Abbott RealTi*me* HBV amplification reagent components (HBV Oligonucleotide Reagent, DNA Polymerase, and Activation Reagent). The Abbott *m2000sp* 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 *m2000sp*. After manual application of the Abbott Optical Adhesive Cover, the plate is ready for transfer to the Abbott m2000rt.

Amplification

During the amplification/detection reaction on the *m2000rt* instrument, the target DNA is amplified by the DNA polymerase in the presence of deoxynucleotide triphosphates (dNTPs) and magnesium. First, the HBV and Internal Control 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 RealTi*me* 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 target sequence for the Internal Control is derived from the hydroxypyruvate reductase gene from the pumpkin plant *Cucurbita pepo*, and is provided as a DNA plasmid in a buffered 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 the 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 the polymerase moves along the template strand. This

separates the fluorophore from the quencher, allowing fluorescent emission and detection.
The HBV and IC specific 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 related to the log of the HBV DNA concentration present in the original sample.

Abbott RealTi*me* HBV Amplification Reagent Kit Description
The Abbott RealTi*me* HBV Amplification Reagent Kit consists of:

- Abbott RealTi*me* HBV Internal Control (List No. 2G34Y)
- Abbott RealTi*me* HBV Amplification Reagent Pack (List No. 2N40), which contains:
  - DNA Polymerase (Part 33794)
  - HBV Oligonucleotide Reagent (List No. 2G34L)
  - Activation Reagent (Part 51-503200)

The Abbott RealTi*me* HBV Amplification Reagent Kit contains four vials of Abbott RealTi*me* HBV Internal Control and four Abbott RealTi*me* HBV Amplification Reagent Packs.

Abbott RealTi*me* HBV Internal Control (List No. 2G34Y)
The Abbott RealTi*me* HBV Internal Control consists of noninfectious linearized DNA plasmid in a buffer solution with carrier DNA and contains the preservatives sodium azide and ProClin® 950.
Prior to sample preparation, the Internal Control is introduced into the lysis buffer, which is then used during the processing of each specimen, calibrator and control and measured on the *m*2000*rt* instrument to demonstrate proper sample processing and assay validity

Abbott RealTi*me* HBV Amplification Reagent Pack (List No. 2N40)
The Abbott RealTi*me* HBV Amplification Reagent Pack consists of the DNA polymerase the HBV Oligonucleotide Reagent, and the Activation Reagent.

DNA Polymerase (Part No. 33794)
Each vial contains DNA polymerase in a buffered solution with stabilizers. The DNA polymerase functions as an enzyme in PCR amplification.

HBV Oligonucleotide Reagent (List No. 2G34L)
Each vial of HBV Oligonucleotide Reagent contains two pairs of oligonucleotide primers and three probes; one primer pair and two probes are specific for amplifying and detecting HBV DNA, and the other primer pair and remaining probe are specific for amplifying and detecting Internal Control DNA. The reagent also contains dNTPs and ROX™ passive reference dye. The reagent is formulated in a TRIS-potassium chloride buffer with the preservatives sodium azide and ProClin 950.

Activation Reagent (Part No. 51-503200)
Each vial of Activation Reagent contains a 38 mM magnesium chloride solution and the preservatives sodium azide and ProClin 950.

Abbott RealTi*me* HBV Control Kit (List No. 2N40-80)

The Abbott RealTi*me* HBV Control Kit consists of:

Abbott RealTi*me* HBV Negative Control (List No. 2G34Z)
Abbott RealTi*me* HBV Low Positive Control (List No. 2G34W)
Abbott RealTi*me* HBV High Positive Control (List No. 2G34X)

The Abbott RealTi*me* HBV Control Kit contains three controls (eight vials of Abbott RealTi*me* HBV Negative Control, eight vials of Abbott RealTi*me* HBV Low Positive Control, and eight vials of Abbott RealTi*me* HBV High Positive Control) that are used to establish the run validity of the Abbott RealTi*me* HBV assay.

The Abbott RealTi*me* HBV Negative Control contains negative human plasma that is 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 FDA licensed PCR methods for HIV-1 RNA and HCV RNA, and contains the antimicrobials ProClin 300 and ProClin 950.

The Abbott RealTi*me* HBV Low Positive Control and High Positive Control contain heat-inactivated plasma reactive for HBV DNA in negative human plasma. The negative human plasma used in the Abbott RealTi*me* HBV Low Positive Control and High Positive Control is 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 FDA licensed PCR methods for HIV-1 RNA and HCV RNA, and contains the preservatives ProClin 300 and ProClin 950.

2.10.7 Abbott RealTi*me* HBV Calibrator Kit (List No. 2N40-70)

The Abbott RealTi*me* HBV Calibrator Kit consists of:

Abbott RealTi*me* HBV Calibrator A (List No. 2G34A)
Abbott RealTi*me* HBV Calibrator B (List No. 2G34B)

The Abbott RealTi*me* HBV Calibrator Kit contains two calibrators (12 vials of Abbott RealTi*me* HBV Calibrator A and 12 vials of Abbott RealTi*me* HBV Calibrator B) that are used to generate a calibration curve for the quantitative determination of HBV in human plasma.

The Abbott RealTi*me* HBV Calibrator A and Calibrator B contain noninfectious linearized HBV DNA plasmid in a buffer solution and contains the antimicrobials sodium azide and ProClin 950.

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 RealTi*me* 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 the HBV DNA in a sample is calculated from the calibration curve. Results are automatically reported on

the *m*2000*rt* workstation. The Low and High Positive Controls and Negative Control must be included in the calibration run.
Once an Abbott RealTi*me* HBV calibration is accepted and stored, it may be used for six months. During this time, all subsequent samples may be tested without further calibration unless a new lot of the Abbott RealTi*me* HBV Amplification Reagent Kit or new lot of an Abbott *m*Sample Preparation System$_{DNA}$ is used; an Abbott RealTi*me* HBV application specification file for a different sample volume is used; or an updated version of the Abbott RealTi*me* HBV application specification file is installed.

Quality Control Procedures:
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 *m*2000*rt* instrument to demonstrate proper sample processing and assay validity. The IC is comprised of a DNA sequence unrelated to the HBV target sequence. The median amplification cycle at which the IC target sequence fluorescent signal is detected in calibration samples establishes an IC Ct validity range to be met by all subsequent processed specimens. 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 RealTi*me* HBV Control Kit Card and must be entered into the assay test order when a run is performed. 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.

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 *m*2000*rt* instrument automatically reports the results on the *m*2000*rt* 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 Standard for HBV. The 3.41 conversion factor is an approximation based on an average conversion factor across the assay dynamic range. The following table represents the potential *m*2000*rt* outputs.
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 – below 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 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, 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.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Currently, methods for following the progress of antiviral therapy include immunoassay (serological tests, enzyme immunoassay), biochemical (alanine aminotransferase), and histological (liver biopsy - fibrosis, inflammation). A molecular method commercially available to follow HBV DNA response to antiviral therapy during the course of treatment is the Roche Cobas® TaqMan® HBV Test.

VII. MARKETING HISTORY

The Abbott RealTime HBV assay received CE certification and was launched in May 2007 outside of the United States, under the list number of 2G34. The following countries receive the Abbott RealTi*me* HBV assay: Australia, Austria, Belgium, Central Africa, Croatia, Czech Republic, Finland, France, Germany, Ireland, Italy, Netherlands, Norway, West Africa, Poland, Portugal, Romania, Saudi Arabia, South Africa, Spain, Sweden, Switzerland, Turkey, and United Kingdom. In Asia, it is currently being marketed in Korea.
This product has not been withdrawn from the market from any country related to safety or effectiveness, or for any other reasons.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

To aid in the management of patients with chronic HBV infection undergoing anti-viral therapy, the results from the Abbott RealTi*m*e HBV assay must be interpreted within the context of all relevant clinical and laboratory findings. Failure of the Abbott RealTi*m*e HBV assay to perform as indicated or human error in use of the product may result in an erroneous test result that is too low or too high. An erroneous low test result may lead to inappropriate treatment, or instill a false sense of security in a patient. An erroneous high test result may contribute to unnecessary treatment or create anxiety in the patient.
The assay is not intended for use as a screening test for blood or blood products for the presence of HBV or as a diagnostic test to confirm the presence of HBV infection. Assay performance characteristics have been established for individuals treated with adefovir dipivoxil. Assay performance for determining the state of HBV infection has not been established.

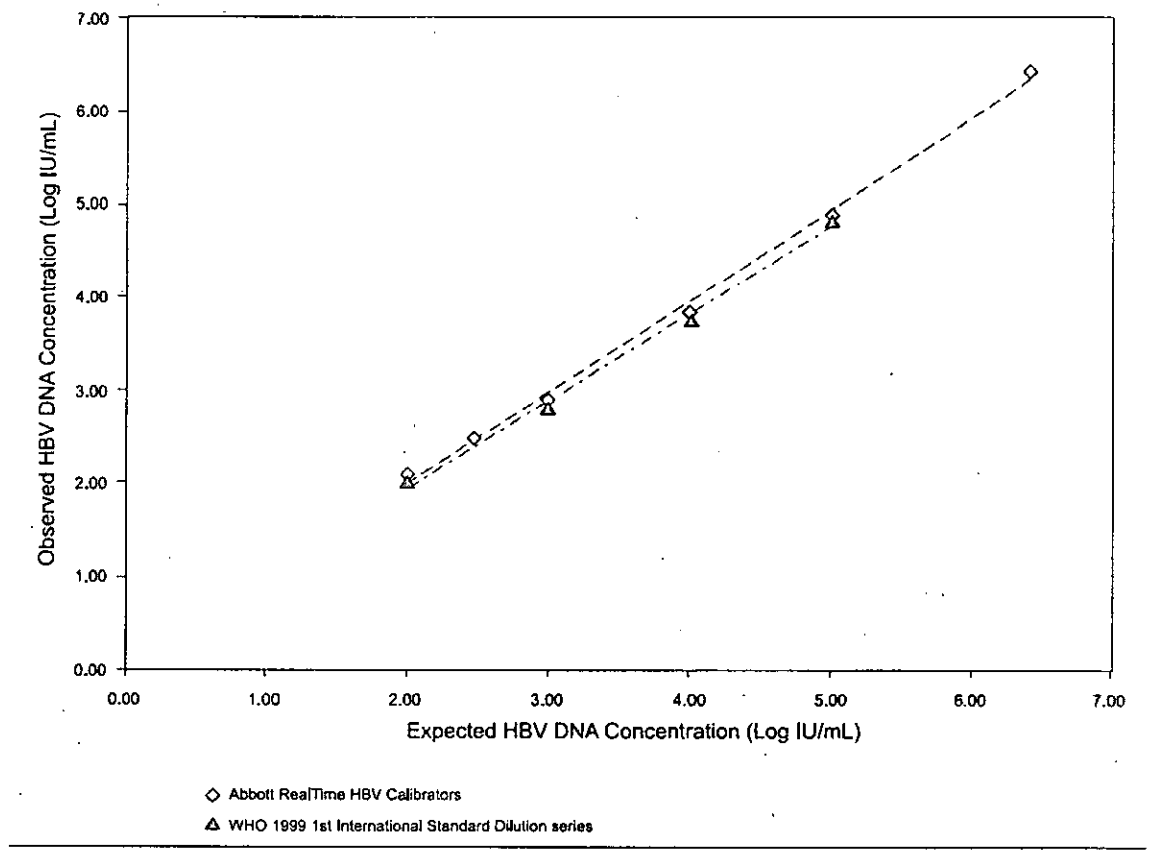
IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

Traceability to the WHO Standard

The figure below demonstrates the comparison of Abbott RealTi*m*e HBV Assay Calibrators to the WHO International HBV DNA Standard. Abbott RealTi*m*e HBV Calibrators trace to the World Health Organization (WHO) International Standard for Hepatitis B Virus DNA (NIBSC Code 97/746) 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 *m*2000*rt* 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 International HBV DNA Standard was reconstituted to a concentration of 1×10^5 IU/mL and then diluted to 1×10^4, 1×10^3, and 1×10^2 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 1×10^5, 1×10^4, 1×10^3, and 1×10^2 IU/mL in Tris-EDTA (TE) buffer. The data for Calibrator B and its dilution series are presented in comparison to the WHO International HBV DNA Standard dilution series in the figure below. The results indicate that the assay standardization process provides quantitation values for the RealTi*m*e HBV Calibrators and the WHO International HBV DNA 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.

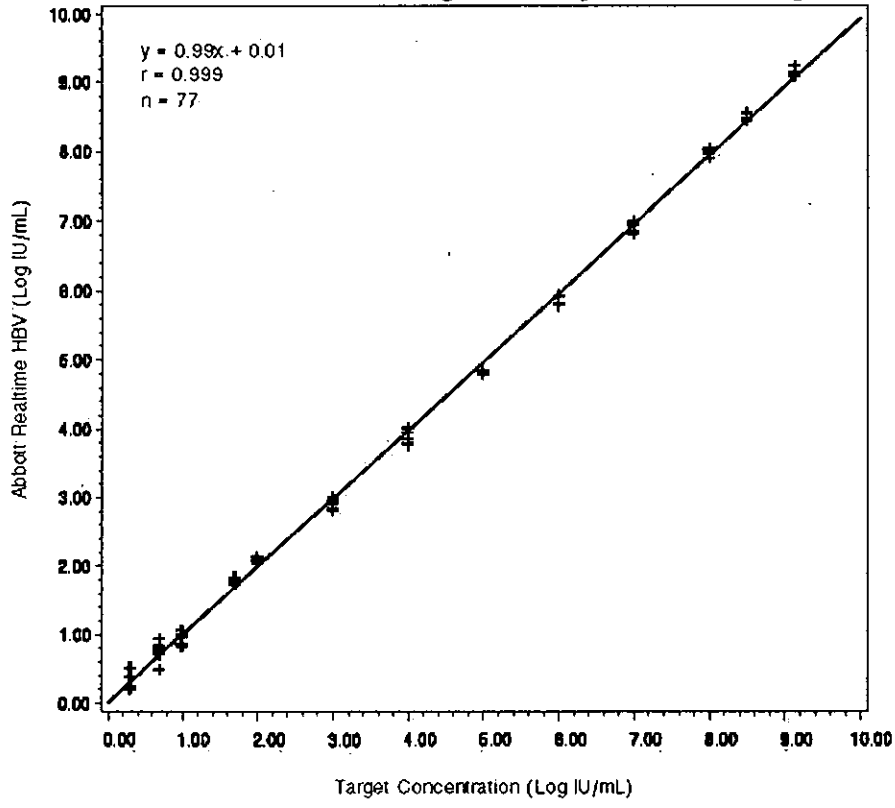
Comparison of WHO 1999 1st International Standard with Abbott RealTi*m*e HBV Calibrators:



Linear Range

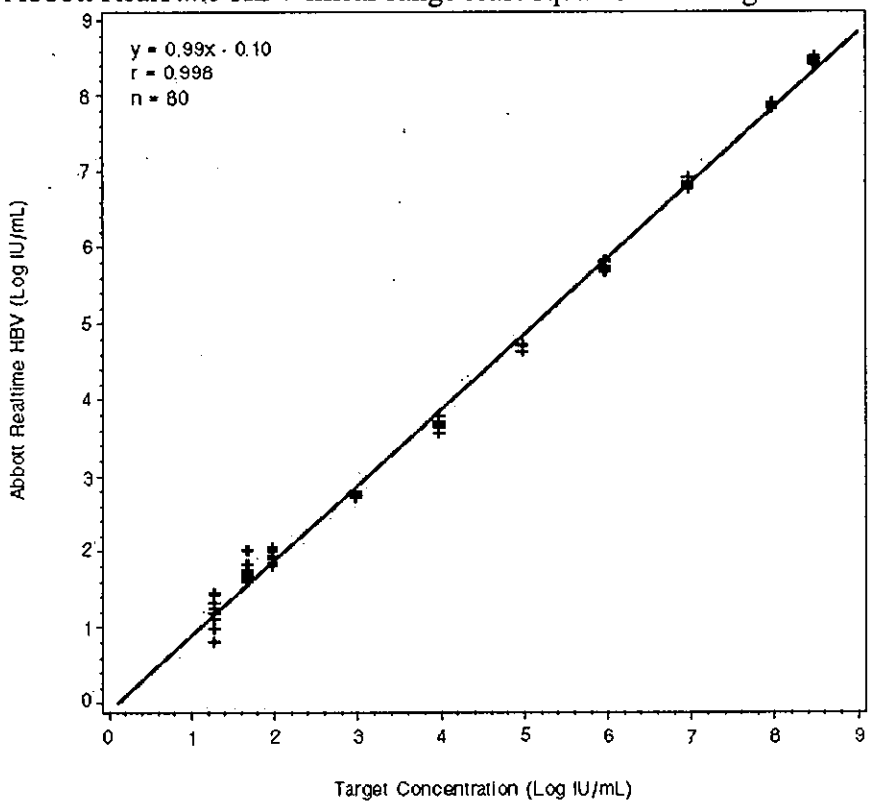
In one study, a 13-member panel prepared by diluting an HBV-positive specimen targeted from 9.13 log IU/mL to 0.29 log IU/mL in HBV negative human plasma was tested and evaluated in accordance with methods defined in the CLSI EP6-A, using the 0.5 mL sample preparation protocol. The Abbott RealTi*me*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

Abbott Real*Time* HBV Linear Range Least Squares Linear Regression Analysis:

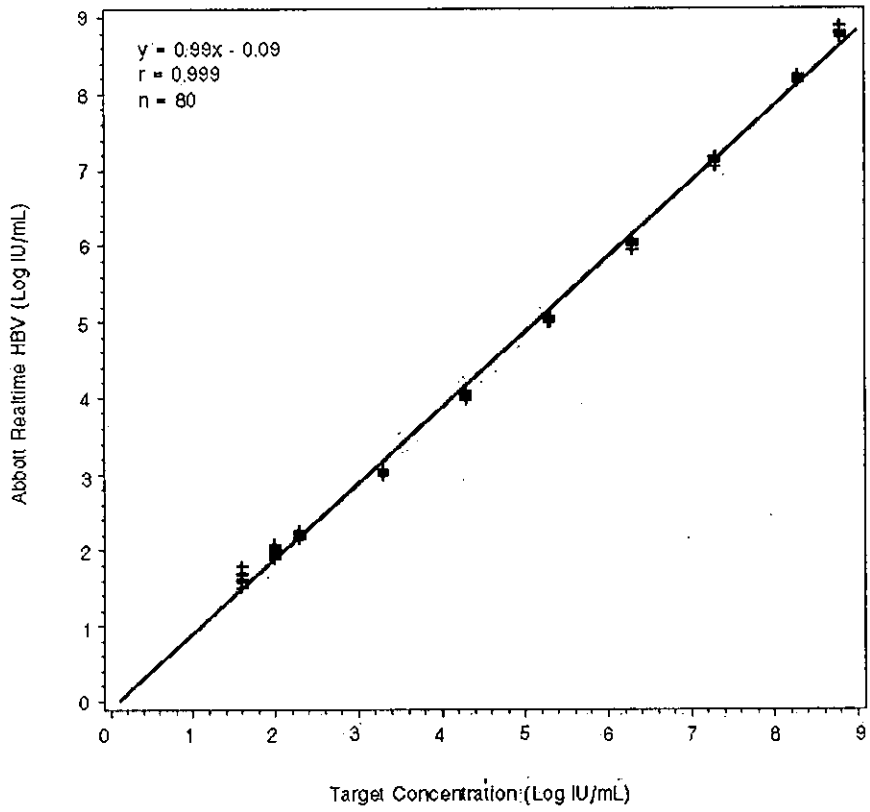


In a second study, one panel consisting of Genotype A and one panel consisting of Genotype C were tested. The two 10-member panels were prepared by diluting to concentrations targeted from 1.27 log IU/mL to 8.47 log IU/mL for Genotype A and 1.59 log IU/mL to 8.79 log IU/mL for Genotype C. The two panels 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 and Genotype C is shown in the two figures below. The Abbott RealTi*m*e 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.

Abbott Real*Time* HBV linear range least squares linear regression analysis Genotype A:



Abbott RealTi*me* HBV linear range least squares linear regression analysis Genotype C:



In addition, a 14-member serum panel and a 10-member plasma panel targeting the linear range of the assay was tested as part of the reproducibility studies and evaluated.
The Abbott RealTi*me* HBV assay was shown to give a linear response from 10 IU/mL (1.00 log IU/mL) HBV DNA to 10^9 IU/mL (9.00 log IU/mL) HBV DNA in both EDTA plasma and serum with deviation from linearity not more than 0.20 log IU/mL for the 0.5 mL sample preparation protocol.
The Abbott RealTi*me* HBV assay was shown to give a linear response from 15 IU/mL (1.18 log IU/mL) HBV DNA to 10^9 IU/mL (9.00 log IU/mL) HBV DNA in both EDTA plasma and serum with deviation from linearity not more than 0.20 log IU/mL for the 0.2 mL sample preparation protocol.

Limit of Detection (LoD) using the WHO international Standard

The LoD, defined as the HBV DNA concentration detected with a probability of 95%, 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 in plasma and serum at both sample volumes are summarized in the table below.

Sample Volume	Sample Matrix	Concentration Detected with 95% Probability	95% Confidence Interval	Concentration Detected with 95% Probability	95% Confidence Interval
		Log IU/mL		IU/mL	
0.5 mL	Plasma	0.81	(0.60, 1.12)	6.40	(3.97, 13.03)
0.2 mL	Plasma	1.03	(0.85, 1.29)	10.66	(7.11, 19.38)
0.5 mL	Serum	0.58	(0.19, 1.84)	3.82	(1.55, 69.76)
0.2 mL	Serum	0.75	(0.56, 1.04)	5.61	(3.62, 10.94)

Limit of detection (LoD) in plasma using 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 of the data determined that the concentration of HBV DNA detected with 95% probability was 6.40 IU/mL (95% CI 3.97-13.03 IU/mL).

Limit of detection (LoD) in serum using 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 of the data determined that the concentration of HBV DNA detected with 95% probability was 3.82 IU/mL (95% CI 1.55-69.76 IU/mL).

Limit of detection (LoD) in plasma using 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 of the data determined that the concentration of HBV DNA detected with 95% probability was 10.66 IU/mL (95% CI 7.11-19.38 IU/mL).

Limit of detection (LoD) in serum using 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[a]	25	86
2.50	30	27	90
1.00	30	17	57
0.50	30	17	57
0.20	30	4	13

[a] One replicate was deleted due to instrument error.
Probit analysis of the data determined that the concentration of HBV DNA detected with 95% probability was 5.61 IU/mL (95% CI 3.62-10.94 IU/mL).

The LoD of the Abbott RealTi*m*e HBV assay is determined as 10 IU/mL for the 0.5 mL sample preparation protocol and 15 IU/mL for the 0.2 mL sample preparation protocol.

Limit of Detection (LoD) by Genotype Using Clinical Specimens

The LoD was determined by analysis of a dilution series of patient samples representing HBV Genotypes A, B, C, D, E, F, G, H and of the WHO (World Health Organization) 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 preparation protocol; and target concentrations of 0.20 IU/mL, 0.50 IU/mL, 1.00 IU/mL, 2.50 IU/mL, 5.00 IU/mL, 10.00 IU/mL, 20.00 IU/mL, and 40.00 IU/mL for the 0.2 mL sample preparation protocol.

Probit analysis of the data was used to determine the concentration of each HBV genotype detected with 95% probability.
Summaries of the results of LoD by genotype at both volumes are shown in tables below.

Summary of LoD by genotype for 0.5 mL sample preparation protocol:

Genotype Tested	Plasma	Serum
	Concentration Detected (IU/mL) (95% Confidence Interval)	**Concentration Detected (IU/mL) (95% Confidence Intervals)**
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.

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

Summary of LoD by genotype for 0.2 mL sample preparation protocol:

Genotype Tested	Plasma	Serum
	Concentration Detected (IU/mL) (95% Confidence Interval)	**Concentration Detected (IU/mL) (95% Confidence Intervals)**
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.

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

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 the table below 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.

Plasma: Total Analytical Error Estimates (Log IU/mL)

Sample Protocol 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 Limit of Detection (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.
[d] Based on difference between two measurements approach.

Presented in the table below are the TAE estimates for the serum panel study. TAE was estimated by two different methods (see table footnotes).

Serum: Total Analytical Error Estimates (Log IU/mL)

Sample Protocol 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 Limit of Detection (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.
[d] Based on difference between two measurements approach.

These studies demonstrated that the Abbott RealTi*m*e 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 1.0 log IU/mL is statistically significant.

Linearity of assay by HBV genotype

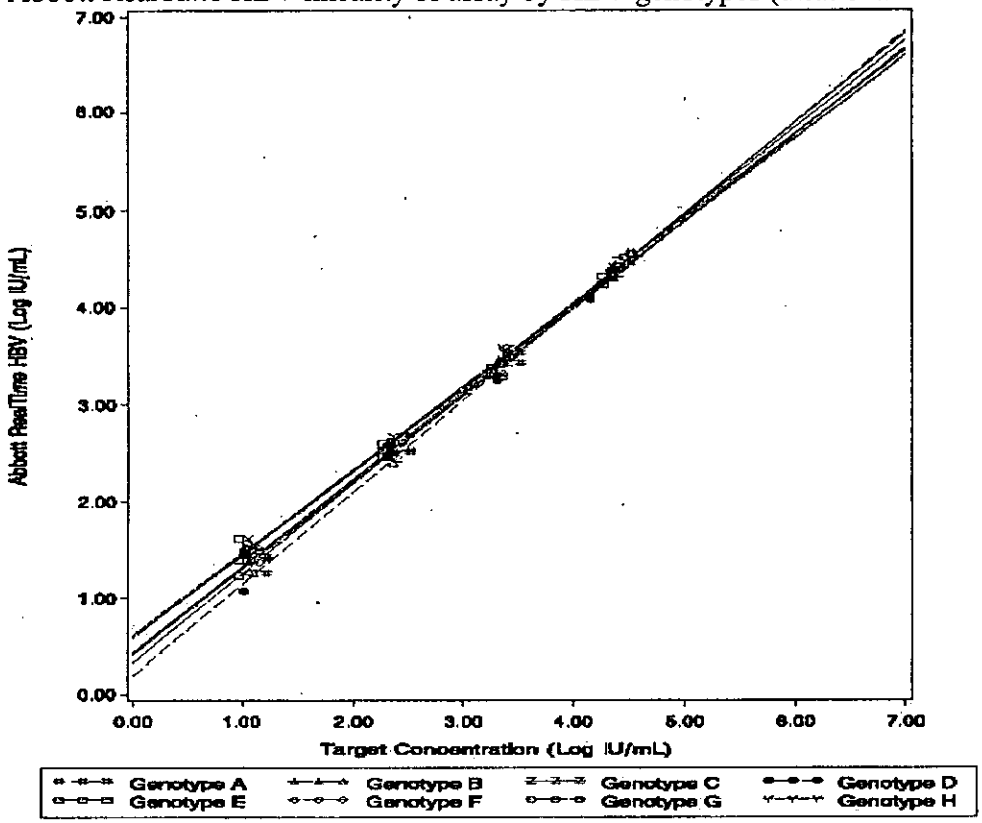
The ability of the RealTi*m*e HBV assay to detect and quantitate HBV genotypes was evaluated through linearity studies by diluting eight specimens, one of each genotype A through H, to target concentrations of 4.47 log IU/mL, 3.47 log IU/mL, 2.47 log IU/mL, and 1.17 log IU/mL in HBV serologically negative human plasma. Three replicates were tested at each concentration for each genotype, using the 0.5 mL sample preparation protocol. The results are summarized in the table and figure below.

Abbott RealTi*m*e HBV linearity of assay by HBV Genotype

HBV Genotype	Linear Equation in 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 ULoQ or LLoQ.

Abbott RealTi*me* HBV linearity of assay by HBV genotypes (dilutional linearity):



The data from the studies demonstrate that the Abbott RealTi*me* HBV assay is capable to quantitate different HBV genotypes across the linear range with deviation of not more 0.51 log IU/mL.

Precision

Within-Laboratory Precision (Lot-to-Lot)

The between instrument and lot precision of the assay was evaluated within a laboratory using an 8-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 *m*2000*sp* and *m*2000*rt* instrument pair. A total of 45 replicates were tested for each panel member across the three pairs of *m*2000*sp* and *m*2000*rt* 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 0.5 mL sample preparation protocol was used. Results are summarized in the table below.

Abbott RealTi*me* 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/Day 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 components of precision

The between-lot component of precision was less or equal to 0.14 log IU/mL.

Within-Laboratory Precision (Operator-to-Operator)

The within-run, between-run, and between-technician (operator) precision of the Abbott RealTi*me* HBV assay was evaluated by testing 84 replicates of each HBV panel member that span the dynamic range of the assay from approximately 1.0 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 the between-technician component and the total SD for the Abbott RealTi*me* HBV assay were 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 between-technician precision component was lower than the within-run component for all panel members. The results are summarized in the table below.

Abbott RealTi*me* 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 4457 "Internal Control Failed" for one sample
[d] Total precision includes within-run, between-run 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 US population. Each 5-member panel was prepared from a high copy source sample, which was comprised 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 than or equal to 0.10 log IU/mL. The results are summarized in tables below.

Abbott RealTi*me* HBV Reproducibility in Plasma - 0.5 mL Sample Preparation Protocol:

Panel	Genotype	n	Mean Concentration (Log	Mean Concentration (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]

			IU/mL)						
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[c]	27[c]	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.

Abbott RealTi*m*e HBV Reproducibility in Plasma (0.2 mL Sample Preparation Protocol):

Panel	Genotype	n	Mean Concentration (Log IU/mL)	Mean Concentration (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 tables below.

Abbott RealTime HBV Reproducibility in Serum (0.5 mL Sample Preparation Protocol):

Panel	Genotype	n	Mean Concentration (Log IU/mL)	Mean Concentration (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	9,725	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	9,068	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.

Abbott RealTime HBV Reproducibility in Serum (0.2 mL Sample Preparation Protocol):

Panel	Genotype	n	Mean Concentration (Log IU/mL)	Mean Concentration (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[c]	16[c]	0.21	0.12	0.07	0.18	0.31
6	A	86[a,b]	0.89[c]	10[c]	0.27	0.03	0.10	0.10	0.31
7	A	82[b]	0.62[c]	7[c]	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[c]	17[c]	0.22	0.10	0.11	0.12	0.29
13	C	87[a,b]	0.80[c]	8[c]	0.26	0.13	0.15	0.08	0.34
14	C	82[b]	0.73[c]	9[c]	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.

Analytical Specificity

Potentially Interfering Substances

The susceptibility of the Abbott RealTi*m*e 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 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 RealTi*m*e HBV assay was observed in the presence of high levels of hemoglobin (500 mg/dL), triglycerides (3,000 mg/dL), bilirubin (20 mg/dL), and protein (9 g/dL). For hemoglobin and protein, there was a slight trend towards 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 values 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 RealTi*m*e HBV assay was observed in the presence of the following drug pools for all HBV-positive and negative 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

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

Cross-reactivity studies with clinical specimens

The specificity of the assay was evaluated by testing 60 clinical specimens that were positive for at least one of the following DNA virus markers, RNA viruses, non-viral hepatitis, or autoimmune disease states (summarized in table below). Clinical specimens that were tested for DNA virus markers were serum. Clinical specimens that were tested for RNA virus markers were 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

Performance of the assay with HBV-Negative Specimens

Performance of the Abbott RealTi*m*e HBV assay was evaluated by testing 124 HBV seronegative serum and 125 HBV seronegative 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.

Cross-Reactivity studies using nucleic acid or viral lysate

The viruses and microorganisms in the table below 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 HBV DNA (3.47 log IU/mL). No interference in the Abbott RealTime HBV assay was observed in the presence of nucleic acids from potentially cross-reactant microorganisms or viruses for all the HBV-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

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 is defined as the number of HBV-negative samples that have a

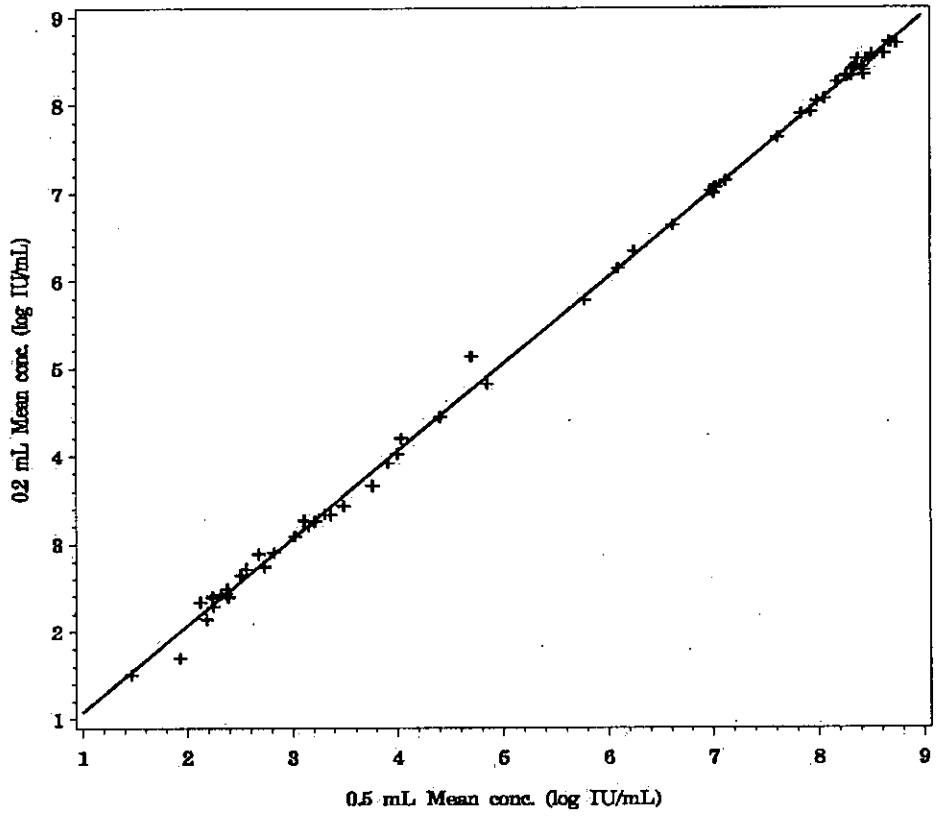
concentration reported greater than assay LoD over the total number of HBV-negative samples tested. The carryover rate in these representative studies ranged from 0% to 2% with an overall rate of 0.63% (95% CI 0.08%-2.24%). Results from the three studies are summarized in the table below.

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

Comparison of 0.2 mL vs. 0.5 mL Sample Preparation Protocols

This study used the Abbott RealTi*me* 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 study was designed to cover the dynamic range of the Abbott RealTi*me* HBV assay with actual patient samples representing genotypes (A, B, C, D) commonly encountered within the US. The data showed a slope of 0.99 and an intercept of 0.09. The results are summarized in the figure below.

Abbott RealTi*me* HBV
Correlation of 0.2 mL vs. 0.5 mL Sample Preparation Protocols
Least Squares Linear Regression



Sample Size (n)				60
Correlation Coefficient (r)				0.999
Slope				0.99
95% CI for Slope				(0.98, 1.00)
Intercept				0.09
95% CI for Intercept				(0.03, 0.14)
0.5 mL Mean conc. (log IU/mL)	Min	1.46	Max	8.75
0.2 mL Mean conc. (log IU/mL)	Min	1.51	Max	8.71

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 duplicate pair was 1.46 log IU/mL).

Sample Handling and Collection

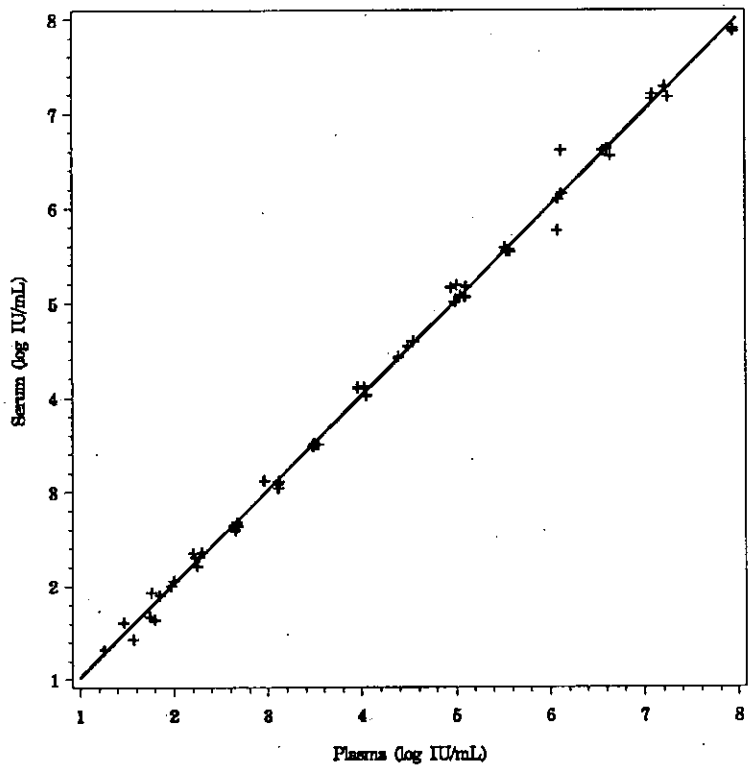
The assay is for use with serum or EDTA plasma specimens only.

Serum vs. Plasma Across the Linear Range

Specimens from 30 individual HBV serologically-negative donors were tested. 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 RealTi*m*e 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 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 results are summarized in the figure below.

Abbott RealTi*me* HBV
Specimen Type - Serum vs. Plasma
Least Squares Linear Regression



Sample Size (n)				58
Correlation Coefficient (r)				0.998
Slope				1.00
95% CI for Slope				(0.98, 1.01)
Intercept				0.03
95% CI for Intercept				(−0.04, 0.10)
Plasma (log IU/mL)	Min	1.26	Max	7.95
Serum (log IU/mL)	Min	1.32	Max	7.89

Recommended storage stability

The stability study data supports 18 month dating for the Abbott RealTi*me* HBV Amplification Reagent Kits.
The stability study data supports 18 month dating for the Abbott RealTi*me* HBV Control and Calibrator Kit components and the Internal Control from the Amplification Reagent Kit.

Recommended sample 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 the table below.

Specimen Stability (Log IU/mL)

Sample Type	Test Condition	Test Condition Mean	Baseline Condition Mean	Mean Difference
Plasma	24-26h at 28-32°C	3.781	3.722	0.059
	72-74h at 2-8°C	3.777	3.722	0.055
Serum	24-26h at 28-32°C	3.871	3.844	0.027
	72-74h at 2-8°C	3.870	3.844	0.026
Plasma (Whole Blood)	6-8h at 28-32°C	3.863	3.866	-0.003
	6-8h at 2-8°C	3.862	3.866	-0.004
Serum (Whole Blood)	6-8h at 28-32°C	3.823	3.628	0.195
	6-8h 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

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A summary of the clinical study is presented below.

B. Study Population and Baseline Parameters

The clinical performance of the Abbott RealTi*m*e 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[9] and one that evaluated patients with HBeAg-negative HBV infection with compensated liver function.[10] 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 subjects 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) and placebo patients (60) 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) and placebo patients (61) 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.

The table below summarizes the subject demographics.

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)
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] Clinical response data was 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.

The table below summarizes available subjects by treatment arm.

Summary of Available Subjects by Treatment Arm Population	Number of Subjects - Placebo	Number of Subjects -10 mg Adefovir	No. of Specimens per Subject[a]	Total No. of Specimens Tested
Chronic HBeAg+	60	169	2 to 7	1,036
Chronic HBeAg-	61	123	2 to 8	939
Total subjects tested	121	292	2 to 8	1,975

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

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 variability was similar to the within-subject variability since the assay 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 patient's and 81% of HBeAg-negative patient's change of viral load was less than 2.0 log IU/mL.

C. Safety and Effectiveness Results

Clinical Study Results and Statistical Analyses

Statistical analysis of clinical data was used to assess whether viral response to treatment measured with Abbott RealTi*m*e HBV Assay for use with the *m*2000 System is informative for assessing the response to treatment in HBeAg+ and HBeAg- 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.

HBeAg-Positive Patients

The table and figure below illustrate the efficacy of treating HBeAg-positive patients with 10 mg adefovir dipivoxil compared to placebo, based on HBV viral load testing results using Abbott RealTi*m*e HBV Assay for use with the *m*2000 System. At Week 48, 22.92% (33/144) of HBeAg-positive patients on treatment vs. 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 vs. 81.82% (45/55) on placebo had viral loads greater than or equal to 10^6 IU/mL.

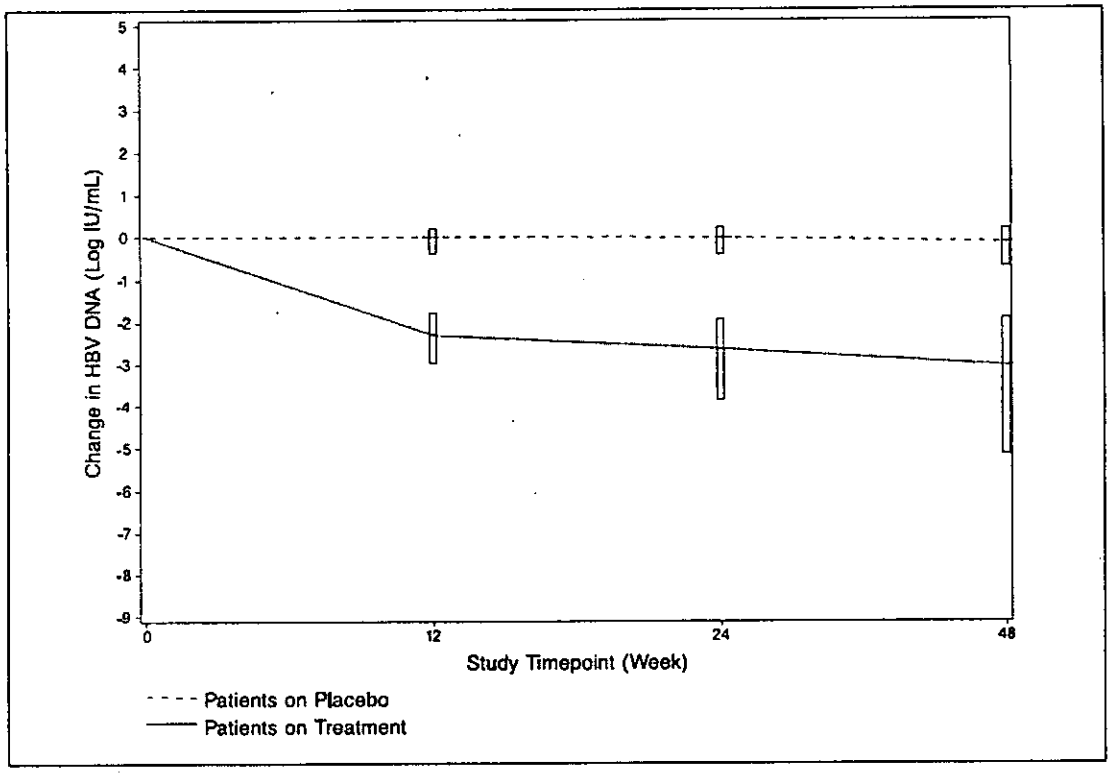
Distribution of HBV Viral Load at Week 48 for HBeAg-Positive Patients:

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 – < 10^9	32	22.22	98.61	43	78.18	96.36
≥ 10^9	2	1.39	100.00	2	3.64	100.00
Total	144	100.00		55	100.00	

[a] Target not detected.

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

Median and inter-quartile range of change in HBV DNA from baseline (HBeAg-positive subjects):



The goal of therapy for patients with chronic HBV infection is to reduce the HBV DNA to low or undetectable levels, and monitor for viral rebound that could be associated with resistance. Results in the table below 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) 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).

Distribution of the 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]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 the table below.

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*	TND*	TND*
Subject 2	8.59	6.57	6.85	6.72	5.68	1.45	**

* 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-negative population are provided in the three tables that follow.

Association between responses to treatment at week 48 and baseline covariates for HBeAg-positive patients association:

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	

Response to Treatment	Covariate	Category	n	No. of Patients with Response	Proportion (%) of Patients with Response	Unadjusted Odds Ratio (95% CI)
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 the two tables below. All lower limits of the 95% confidence intervals in these two tables are smaller than 1, except for Race and Genotype at Weeks 12 and 24 (when response is defined as < 2000 IU/mL). This is in agreement with logistic regression analyses resulting 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.

Odds ratios for the association between viral response (< 2000 IU/mL) and covariates, by week, for an HBeAg-positive population:

Covariate	Category	Week	n	No. Below 2000 IU/mL	Proportion (%) Below 2000 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	

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 an 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 was also 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 the following table:

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	[b]

[a] Target Not Detected
[b] The Abbott RealTime HBV result for the Week 240 time point was excluded due to technician error.

Viral load response was defined as either HBV DNA less than 2000 IU/mL or greater than or equal to 2 log IU/mL decrease from baseline. Statistical analysis (PPV) was performed to evaluate the association between the clinical responses at Weeks 48, 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 < 2000 IU/mL

As shown in the table below, early viral response (Weeks 12, 24, 48) is informative in predicting clinical responses at Weeks 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 exceeding 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.

An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48. PPV, NPV, and Odds Ratio for individual clinical responses during treatment predicted by early viral response (< 2000 IU/mL) in HBeAg-positive subjects (clinical response assessed at week 48):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV(95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio(95% CI)
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)	6.50 (1.95, 27.66)
		HBeAg Loss	64.0 (16/25)	(42.6, 81.3)	82.8 (82/99)	(73.6, 89.4)	8.58 (2.94, 25.54)
		Anti-HBe Gain	36.0 (9/25)	(18.7, 57.4)	91.9 (91/99)	(84.2, 96.2)	6.40 (1.85, 21.90)
		Seroconversion[a]	36.0 (9/25)	(18.7, 57.4)	91.9 (91/99)	(84.2, 96.2)	6.40 (1.85, 21.90)
24	48	Histologic	80.0 (28/35)	(62.5, 90.9)	49.3 (37/75)	(37.7, 61.0)	3.89 (1.42, 11.76)
		Biochemical	83.8 (31/37)	(67.3, 93.2)	59.7 (46/77)	(47.9, 70.6)	7.67 (2.68, 24.74)
		HBeAg Loss	62.2 (23/37)	(44.8, 77.1)	90.2 (74/82)	(81.2, 95.4)	15.20 (5.15, 46.52)
		Anti-HBe Gain	24.3 (9/37)	(12.4, 41.6)	92.7 (76/82)	(84.2, 97.0)	4.07 (1.16, 15.06)
		Seroconversion	24.3 (9/37)	(12.4, 41.6)	92.7 (76/82)	(84.2, 97.0)	4.07 (1.16, 15.06)
48	48	Histologic	74.0 (37/50)	(59.4, 84.9)	49.3 (35/71)	(37.3, 61.3)	2.77 (1.19, 6.62)
		Biochemical	78.0 (39/50)	(63.7, 88.0)	63.2 (48/76)	(51.3, 73.7)	6.08 (2.52, 15.15)
		HBeAg Loss	62.7 (32/51)	(48.1, 75.5)	97.5 (78/80)	(90.4, 99.6)	65.68 (14.10, 587.82)
		Anti-HBe Gain	33.3 (17/51)	(21.1, 48.0)	100.0 (80/80)	(94.3, 100.0)	>39.50[b]
		Seroconversion	33.3 (17/51)	(21.1, 48.0)	100.0 (80/80)	(94.3, 100.0)	>39.50[b]

[a] Seroconversion: HBeAg undetectable and antibody against HBeAg detected.

[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).

PPV, NPV, and Odds Ratio for individual clinical responses during treatment predicted by early viral response (< 2000 IU/mL) in HBeAg-positive subjects (clinical response assessed at week 144):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV(95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
Detection of HBeAg at 12 Weeks	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 [a]	33.3 (2/6)	(6.0, 75.9)	86.7 (39/45)	(72.5, 94.5)	3.25 (0.24, 28.53)
Detection of HBeAg at 24 Weeks	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)	7.00 (1.11, 42.84)
		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)
Undetectable	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)

[a] HBeAg undetectable and antibody against HBeAg detected.

PPV, NPV, and Odds Ratio for individual clinical responses during treatment predicted by early viral response (< 2000 IU/mL) in HBeAg-positive subjects (clinical response assessed at week 192):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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[a]	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)

[a] Seroconversion: HBeAg undetectable and antibody against HBeAg detected.

PPV, NPV, and Odds Ratio for individual clinical responses during treatment predicted by early viral response (< 2000 IU/mL) in HBeAg-positive subjects clinical response assessed at week 240):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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[a]	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)	24.00 (1.21, 1309.0)
		Seroconversion	50.0 (3/6)	(13.9, 86.1)	96.2 (25/26)	(78.4, 99.8)	25.00 (1.26, 1361.3)

* Undefined (division by zero).
[a] Seroconversion: HBeAg undetectable and antibody against HBeAg detected.

The following two tables demonstrate that the NPV is 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 < 2000 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.

PPV, NPV, and Odds Ratio (OR) for a combination of histologic, biochemical, and HBeAg loss responses during treatment predicted by an early viral response (<2000 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)
12	48	41.7 (10/24)	(22.8, 63.1)	89.9 (80/89)	(81.2, 95.0)	6.35 (1.90, 20.95)
24	48	42.9 (15/35)	(26.8, 60.5)	97.3 (72/74)	(89.7, 99.5)	27.00 (5.38, 252.97)
48	48	36.7 (18/49)	(23.8, 51.7)	98.6 (70/71)	(91.3, 99.9)	40.65 (5.75, 1716.7)
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).
An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

PPV, NPV, and Odds Ratio (OR) for a combination of histologic, biochemical, and seroconversion responses during treatment predicted by an early viral response (< 2000 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)
12	48	25.0 (6/24)	(10.6, 47.1)	97.8 (87/89)	(91.4, 99.6)	14.50 (2.28,

						152.63)
24	48	17.1 (6/35)	(7.2, 34.3)	98.6 (73/74)	(91.7, 99.9)	15.10 (1.68, 703.89)
48	48	16.3 (8/49)	(7.8, 30.2)	100.0 (71/71)	(93.6, 100.0)	>13.66[a]
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).

[a] 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).

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

Viral Response ≥ 2 Log IU/mL Decrease

As shown in the table below, early viral response (Weeks 12, 24, 48) is informative in predicting clinical responses at Weeks 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.

An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48. 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 (clinical response assessed at week 48):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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)	5.69 (1.76, 23.79)
		Anti-HBe Gain	18.8 (15/80)	(11.2, 29.4)	95.5 (42/44)	(83.3, 99.2)	4.85 (1.03, 45.38)
		Seroconversion[a]	18.8 (15/80)	(11.2, 29.4)	95.5 (42/44)	(83.3, 99.2)	4.85 (1.03, 45.38)
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)	14.00 (2.07,

							591.20)
		Anti-HBe Gain	16.7 (15/90)	(9.9, 26.3)	100.0 (29/29)	(85.4, 100.0)	>5.60[b]
		Seroconversion	16.7 (15/90)	(9.9, 26.3)	100.0 (29/29)	(85.4, 100.0)	>5.60[b]
48	48	Histologic	66.7 (60/90)	(55.9, 76.0)	58.1 (18/31)	(39.3, 74.9)	2.77 (1.11, 7.00)
		Biochemical	63.4 (59/93)	(52.8, 73.0)	75.8 (25/33)	(57.4, 88.3)	5.42 (2.06, 15.32)
		HBeAg Loss	35.8 (34/95)	(26.4, 46.3)	100.0 (36/36)	(88.0,100.0)	>19.51[b]
		Anti-HBe Gain	17.9 (17/95)	(11.1, 27.4)	100.0 (36/36)	(88.0,100.0)	>7.63[b]
		Seroconversion	17.9 (17/95)	(11.1, 27.4)	100.0 (36/36)	(88.0,100.0)	>7.63[b]

[a] Seroconversion: HBeAg undetectable and antibody against HBeAg detected.
[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).

PPV, NPV, and Odds Ratio for individual clinical responses during treatment predicted by early viral response ($\geq$ 2 Log IU/mL decrease) in HBeAg-positive subjects (clinical response assessed at week 144):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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[a]	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[b]
		Anti-HBe Gain	21.1 (8/38)	(10.1, 37.8)	100.0 (12/12)	(69.9, 100.0)	>2.93[b]
		Seroconversion	21.1 (8/38)	(10.1, 37.8)	100.0 (12/12)	(69.9, 100.0)	>2.93[b]
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)

[a] Seroconversion: HBeAg undetectable and antibody against HBeAg detected.

[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).

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 (clinical response assessed at week 192):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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 [a]	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)	>5.00[b]
		Seroconversion	33.3 (10/30)	(17.9, 52.9)	100.0 (11/11)	(67.9, 100.0)	>5.00[b]
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)

[a] Seroconversion: HBeAg undetectable and antibody against HBeAg detected.

[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).

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 (clinical response assessed at week 240):

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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[b]
		Seroconversion[a]	26.3 (5/19)	(10.1, 51.4)	100.0 (15/15)	(74.7, 100.0)	>5.00[b]
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[b]
		Seroconversion	21.7 (5/23)	(8.3, 44.2)	100.0 (9/9)	(62.9, 100.0)	>2.22[b]
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[b]
		Seroconversion	18.2 (4/22)	(6.0, 41.0)	100.0 (10/10)	(65.5, 100.0)	>2.00[b]

* Undefined (division by zero).

[a] Seroconversion: HBeAg undetectable and antibody against HBeAg detected.

[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).

The following two tables demonstrate that the NPV is very 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.

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

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)
12	48	23.7 (18/76)	(15.0, 35.1)	97.3 (36/37)	(84.2, 99.9)	11.17 (1.60, 478.17)
24	48	20.7 (17/82)	(12.9, 31.4)	100.0 (27/27)	(84.5, 100.0)	>6.80[a]
48	48	21.3 (19/89)	(13.7, 31.6)	100.0 (31/31)	(86.3, 100.0)	>8.14[a]
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] 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).

PPV, NPV, and Odds Ratio (OR) for a combination of histologic, biochemical, and seroconversion responses during treatment predicted by an early viral response ($\geq$ 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[a]
24	48	8.5 (7/82)	(3.8, 17.3)	100.0 (27/27)	(84.5, 100.0)	>2.43[a]
48	48	9.0 (8/89)	(4.2, 17.4)	100.0 (31/31)	(86.3, 100.0)	>2.96[a]
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] 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

The table 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 vs. 0% (0/55) on placebo

had achieved very low viral loads below 100 IU/mL. Furthermore, 3.42% (4/117) of patients on treatment vs. 30.91 % (17/55) on placebo had viral loads greater than or equal to 10^6 IU/mL.

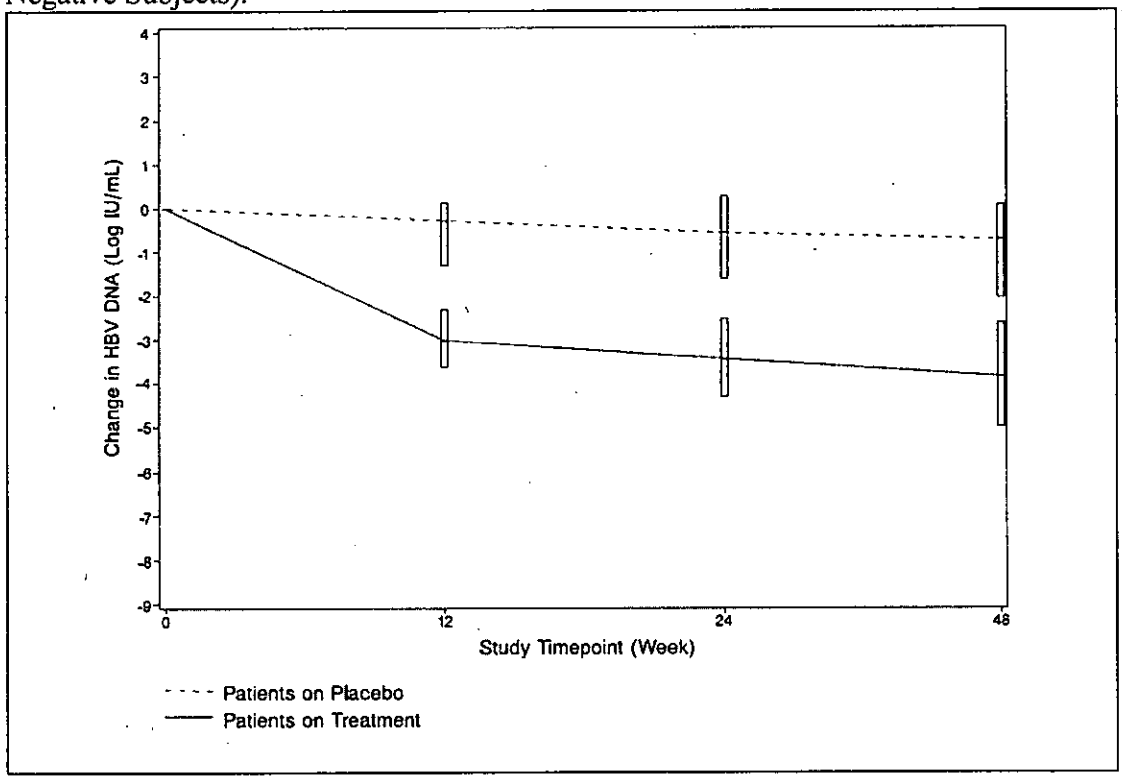
Distribution of HBV Viral Load at Week 48 for HBeAg-Negative Patients:

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] Target Not Detected.

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

Median and Inter-Quartile Range of Change in HBV DNA from Baseline (HeAg-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 the table below 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).

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[a]	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)		

[a] 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.

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*

[a] Target Not Detected
[b] Aliquot not available for testing

Summaries of the effect of baseline covariates for the HBeAg-negative population are provided in the two tables below.

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 the two tables below. All lower limits of the 95% confidence intervals in these two tables above 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.

Odds Ratios for the association between viral response (< 2000 IU/mL) and covariates, by week, for an HBeAg-negative population:

Covariate	**Category**	**Week**	**n**	**No. Below 2000 IU/mL**	**Proportion (%) Below 2000 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	26	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	

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 for HBeAg-Negative Patients

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 was also collected.

Viral load response was defined as either HBV DNA less than 2000 IU/mL or greater than or equal to 2 log IU/mL decrease from baseline. Statistical analysis (PPV) was performed to evaluate the association between the clinical responses at Weeks 48, 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 < 2000 IU/mL

As shown in the table below, viral response at Weeks 24, 48, (when defined as < 2000 IU/mL decrease in value from the baseline viral load result) 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. The PPV 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.

PPV, NPV, and Odds Ratio for individual clinical responses during treatment predicted by early viral response (< 2000 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)
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)	3.48 (1.15, 10.28)
48	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)	4.23 (1.39, 12.62)
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)	9.26 (1.97, 45.25)
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)	4.75 (1.06, 20.85)
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)

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV % (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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[a]
		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[a]
		Biochemical	80.6 (25/31)	(61.9, 91.9)	11.1 (1/9)	(0.6, 49.3)	0.52 (0.01, 5.51)

[a] 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).

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

The table below 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 < 2000 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).

PPV, NPV, and Odds Ratio (OR) for a combination of histologic and biochemical responses during treatment predicted by an early viral response (< 2000 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)
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)	3.45 (1.17, 10.66)
48	48	62.5 (50/80)	(50.9, 72.9)	70.0 (14/20)	(45.7, 87.2)	3.89 (1.22, 13.55)
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[a]
48	240	100.0 (12/12)	(69.9, 100.0)	42.9 (3/7)	(11.8, 79.8)	>8.25[a]

* Undefined (division by zero).

[a] 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).

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

Viral Response ≥ 2 Log IU/mL Decrease

As shown in the table below, viral response at Weeks 12, 24, 48, when defined as ≥ 2 log decrease in value from the screening 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 ≥ 78.9% for biochemical response at Week 48 and remained consistent through the end of the study.

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)
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)	4.51 (1.44, 13.91)
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)	4.48 (1.00, 20.41)
48	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)	15.00 (3.17, 92.12)
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)	5.29 (1.14, 24.06)
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)	16.00 (1.32, 805.02)
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)

Week of Viral Response	Week of Clinical Response	Clinical Response	PPV (%) (Proportion)	PPV (95% CI)	NPV (%) (Proportion)	NPV (95% CI)	Odds Ratio (95% CI)
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)

The table below 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).

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 decrease) 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)

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	48	60.4 (55/91)	(49.6, 70.4)	88.9 (8/9)	(50.7, 99.4)	12.22 (1.50, 551.92)
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)

* Undefined (Division by Zero)
An association with, rather than prediction of, clinical responses is demonstrated when measuring the viral response at Week 48.

X. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Guidelines for HBV DNA Testing in Clinical Practice

Published guidelines and the medical literature support the importance of measuring HBV levels at baseline prior to treatment, and at intervals during treatment.[1,2,3] The American Association for the Study of Liver Disease (AASLD) Practice Guidelines state that serum HBV DNA quantification using highly sensitive assays with a wide dynamic range, such as real-time PCR technology, is a crucial component in the evaluation of chronically HBV infected patients and the assessment of the efficacy of antiviral treatment.[1,2] The most recent guidelines highlight the importance of serial testing of HBV DNA levels, and describe issues with using the specific cutoff values to define treatment indications and response.[1]

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

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the

1 Lok ASF, McMahon BJ. Chronic hepatitis B: update 2009. AASLD Practice Guidelines. Hepatology 2009;50:661-662.

2 Lok ASF, McMahon BJ. Chronic hepatitis B. AASLD Practice Guidelines. Hepatology 2007;45:507-539.

3 Keeffe EB, Dieterich DT, Han SB, Jacobson IM, Martin P, Schiff ER, Tobias H, Wright TL. A treatment algorithm for the management of chronic hepatitis B virus infection in the U.S. Clin Gastroenterol Hepatol 2004;2:87-106.

information in the PMA substantially duplicates information previously reviewed by this panel.

XI. CONCLUSION.S DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

Based on the results of the preclinical and clinical laboratory studies, Abbott RealTi*me* HBV assay, when used according to the provided directions and in conjunction with other serological and clinical information, should be safe and pose minimal risk to the patient due to false test results.

B. Effectiveness Conclusions

The effectiveness of the Abbott RealTi*me* HBV assay has been demonstrated for use in quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma. A reasonable determination of effectiveness of the Abbott RealTi*me* HBV assay for aiding in the management of patients with chronic HBV infection undergoing anti-viral therapy, by measuring HBV DNA levels at baseline and during treatment, to aid in assessing response to treatment in conjunction with other laboratory results and clinical information has been demonstrated.

C. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the nonclinical studies demonstrated acceptable analytical sensitivity, traceability, linearity, precision, and analytical specificity of the Abbott RealTi*me* HBV assay when used according to the instructions for use as stated in the labeling, the warnings and precautions, and limitations sections of the labeling. The clinical studies and the statistical analysis of clinical data in this application has shown that viral response to treatment measured with Abbott RealTi*me* HBV assay is informative for assessing the effect of treatment in patients with chronic hepatitis B, and that the assay is safe and effective when used according to the directions for use in the labeling.

Risk and benefit analysis: As a diagnostic test, the Abbott RealTi*me* HBV assay involves removal of blood from an individual for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is having their blood drawn for any other diagnostic evaluation. The benefits to chronically HBV-infected individuals undergoing antiviral therapy tested by the assay outweigh any potential adverse event or risk to the patient or user due to assay malfunction or operator error. The potential risks encountered with this *in vitro* diagnostic test are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for the device.

Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

XII. CDRH DECISION

CDRH issued an approval order on August 13, 2010. The final conditions of approval cited in the approval order are described below.

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

XIII. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XIV. REFERENCES

1. Lok ASF, McMahon BJ. Chronic hepatitis B: update 2009. AASLD Practice Guidelines. Hepatology 2009;50:661-662.

2. Lok ASF, McMahon BJ. Chronic hepatitis B. AASLD Practice Guidelines. Hepatology 2007;45:507-539.

3. Keeffe EB, Dieterich DT, Han SB, Jacobson IM, Martin P, Schiff ER, Tobias H, Wright TL. A treatment algorithm for the management of chronic hepatitis B virus infection in the U.S. Clin Gastroenterol Hepatol 2004;2:87-106.4.