Summary of Safety and Effectiveness Data

I. GENERAL INFORMATION:

Device Generic Name: Nucleic acid assay for detection of Hepatitis B Virus (HBV) DNA

Device Trade Name: COBAS TaqMan HBV Test For Use With The High Pure System

Name and Address of Applicant: Roche Molecular Systems, Inc. (RMS)
4300 Hacienda Drive
Pleasanton, CA 94588

Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P050028

Date of Notice of Approval to the Applicant: September 04, 2008

II. INDICATIONS FOR USE:

The COBAS TaqMan HBV Test For Use With The High Pure System is an in vitro nucleic acid amplification test for the quantitation of Hepatitis B Virus (HBV) DNA in human serum or plasma (EDTA), using the High Pure Viral Nucleic Acid Kit for manual specimen preparation and the COBAS TaqMan 48 Analyzer for automated amplification and detection. The test 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 COBAS TaqMan HBV Test must be interpreted within the context of all relevant clinical and laboratory findings.

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.

The COBAS TaqMan HBV Test 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.

III.   CONTRAINDICATIONS: None known.

IV.   WARNINGS AND PRECAUTIONS: For *in vitro* diagnostic use only.

The warnings and precautions for the COBAS TaqMan HBV Test For Use With The High Pure System are stated in the respective product labeling.

V.   DEVICE DESCRIPTION:

Kit Configurations and Components

The COBAS TaqMan HBV Test For Use With The High Pure System consists of the following kits:

- High Pure System Viral Nucleic Acid Kit

- COBAS TaqMan HBV Test Kit Configuration

Each kit contains labeled reagents assembled according to storage temperature requirements and controlled room temperature.

A High Pure System Viral Nucleic Acid Kit (HPS) is a generic manual specimen preparation kit, for the isolation and purification of nucleic acids. Control, amplification, and detection reagents are provided separately in the COBAS TaqMan (CTM) HBV Test kit. The High Pure System Viral Nucleic Acid Kit contains the reagents and kit-specific disposables necessary for specimen and control processing. The following reagents and disposables are supplied in this kit:

LYS (Lysis/Binding Buffer): A Tris buffered solution containing guanidine hydrochloride, urea, and Triton X-100; 2 x 25 mL
CAR (RNA, lyophilized): Poly (A) carrier RNA is a lyophilized powder consisting of single stranded synthetic polymer, polyadenylic acid; 2 x 2 mg
IRB (Inhibitor Removal Buffer): A Tris buffered solution containing guanidine hydrochloride to which is added 80 mL of ethanol; 1 x 33 mL
WASH (Wash Buffer): A Tris buffered solution containing sodium chloride to which is added 20 mL of ethanol; 1 x 20 mL
ELB (Elution Buffer): PCR grade water; 1 x 30 mL
Disposables: RS (High Pure system Viral Nucleic Acid Rack Set) 4 x each; WR (High Pure System Viral Nucleic Acid Waste Rack) 8 x each

The CTM HBV Test Specimen Preparation and Control Reagents consist of an HBV Quantitation Standard (QS) DNA, two HBV DNA positive controls, and a negative control. The HBV QS and HBV positive controls have assigned HBV DNA expected ranges. The positive controls are prepared in a matrix of negative human plasma. The HBV QS is added, at a known quantity, to each specimen at

the beginning of the specimen preparation procedure. The addition of HBV QS allows for the calculated titer of HBV target DNA to be adjusted, accordingly, if the fluorescence of the HBV QS is delayed due to inhibition or poor sample recovery. The following Specimen Preparation and Control Reagents are provided in the CTM HBV Test kit:

HBV QS (HBV Quantitation Standard): Tris-HCl buffer, EDTA, <0.001% linearized, double stranded plasmid DNA containing an insert. The DNA insert contains HBV primer binding sequences and a unique probe binding region. Amaranth dye, < 0.005% Poly rA RNA (synthetic), 0.05% Sodium azide, urea, and Triton X-100; 2 x 1.0 mL

HBV H(+)C [HBV High (+) Control]: <0.001% linearized, double stranded plasmid DNA containing HBV sequences. Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods, 0.1% Proclin 300; 2 x 1.0 mL

HBV L(+)C [HBV Low (+) Control]: <0.001% linearized, double stranded plasmid DNA containing HBV sequences. Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody to HIV-1/2, and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods, 0.1% Proclin 300; 2 x 1.0 mL

CTM (-) C [COBAS TaqMan Negative Control (Human Plasma)]: Negative Human Plasma, non-reactive by US FDA licensed tests for antibody to HCV, antibody t0 HIV-1/2, and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods, 0.1% Proclin 300; 4 x 1.0 mL

The COBAS TaqMan HBV Test Amplification and Detection Reagents include reagents necessary to perform PCR amplification and detection of HBV and HBV QS target DNA. Additionally, these reagents include the manganese solution required as a co-factor for the DNA polymerase activity of the ZO5 DNA polymerase. The following Amplification and Detection Reagents are provided in the CTM HBV Test kit:

HBV MMX (COBAS TaqMan HBV Master Mix): Tricene buffer, Potassium hydroxide, Potassium acetate, Glycerol, < 0.001% dATP, dCTP, dGTP, dUTP, < 0.001% Upstream and downstream primers to the Pre-core/core region of HBV, < 0.001% Fluorescent-labeled oligonucleotide probes specific for HBV and the HBV Quantitation Standard, < 0.001% Oligonucleotide aptamer, <0.05% ZO5 DNA Polymerase (microbial), < 0.1% AmpErase (uracil-N-glycolase) enzyme (microbial), 0.09% Sodium azide; 2 x 1.4 mL

HBV Mn2+ (COBAS TaqMan Manganese Solution): < 0.2% Manganese acetate, Glacial acetic acid, 0.09% Sodium azide; 2 x 1.0 mL.

Assay Principle and Format

The COBAS TaqMan HBV Test is based on two major processes: (1) manual specimen preparation to obtain HBV DNA by lysis of viral particles followed by precipitation of the HBV DNA with alcohol and adsorption of the HBV DNA to

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glass fibers; (2) automated PCR amplification of target DNA using HBV specific complementary primers, and detection of cleaved dual fluorescent dye-labeled oligonucleotide detection probes that permit quantitation of HBV target amplified product (amplicon) and HBV Quantitation Standard (QS) DNA, which is processed, amplified, and detected simultaneously with the specimen. The HBV QS is incorporated into each individual specimen and control at a known copy number and is carried through the specimen preparation, PCR amplification and detection steps along with the HBV target. The COBAS TaqMan 48 (CTM 48) Analyzer calculates the HBV DNA titer in the test specimen by comparing the HBV signal to the HBV QS signal for each specimen and control.

The Master Mix reagent contains primer pairs and probes specific for both HBV DNA and HBV QS DNA. The Master Mix has been developed to ensure equivalent quantitation of genotypes A through G of HBV. The detection of amplified DNA is performed using target-specific and QS-specific dual labeled oligonucleotide probes that permit independent identification of HBV amplicon and HBV QS amplicon.

The quantitation of HBV viral DNA is performed using the HBV QS. The HBV QS is a non-infectious, linearized, double stranded plasmid that contains the identical primer binding sites as the HBV DNA target and a unique probe binding region that allows HBV QS amplicon to be distinguished from HBV target amplicon. The HBV QS is incorporated into each individual specimen and control at a known copy number and is carried through the specimen preparation, PCR amplification and detection steps along with the HBV target. The CTM 48 Analyzer calculates the HBV DNA titer in the test specimen by comparing the HBV signal to the HBV QS signal for each specimen and control. The HBV QS compensates for effects of inhibition and controls for the preparation and amplification processes to allow the accurate quantitation of HBV DNA in each specimen.

The CTM 48 Analyzer is a flexible bench top, batch analyzer that automates the incubation, timing, thermal cycling, and real time photometric measurement of the PCR process into a single analytical system. The primary operational components of the analyzer are the thermal cycler, incubator, and photometric detection systems.

The COBAS TaqMan HBV Test provides quantitative results over a very wide dynamic range since the monitoring of amplicon is performed during the exponential phase of amplification. The higher the HBV titer of a specimen, the earlier the fluorescence of the reporter dye of the HBV probe rises above the baseline fluorescence level. Since the amount of HBV QS DNA is constant between all specimens, the fluorescence of the reporter dye of the HBV QS probe should appear at the same cycle for all specimens. In cases where the QS amplification and detection is affected by inhibition or poor specimen recovery, the appearance of fluorescence will be delayed, thereby enabling the calculated titer of HBV target DNA to be adjusted accordingly. The appearance of the

specific fluorescent signal is reported as a critical threshold value (Ct). The Ct is defined as the fractional cycle number where reporter dye fluorescence exceeds a predetermined threshold (the Assigned Fluorescence Level), and starts the beginning of an exponential growth phase of this signal. A higher Ct value indicates a lower titer of initial HBV target DNA. A 2-fold increase in titer correlates with a decrease of 1 Ct for target HBV DNA, while a 10-fold increase in titer correlates with a decrease of 3.3 Ct. As the concentration of the virus increases, the growth curves shift to earlier cycles. Therefore the leftmost growth curve corresponds to the highest viral titer level whereas the rightmost growth curve corresponds to the lowest viral titer level.

Target Growth Curves for a Dilution Series of Virus Spanning over a 5-$\log_{10}$ Range:
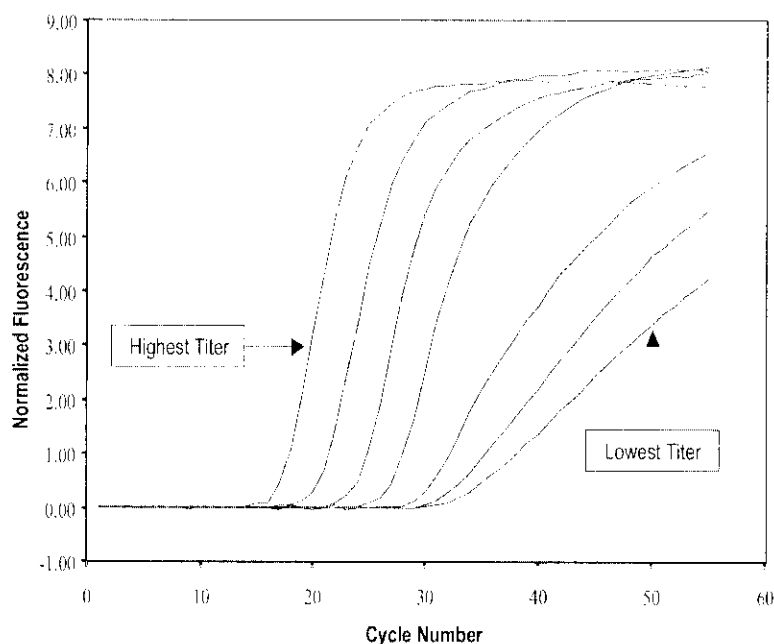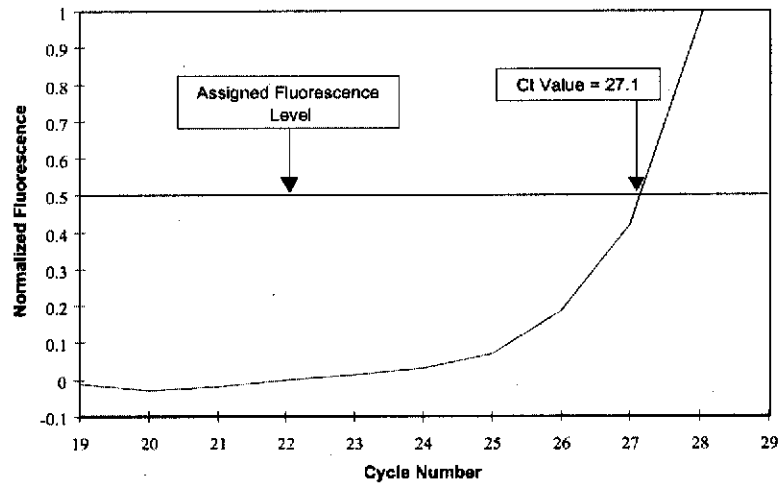

The amount of Quantitation Standard added to each specimen is constant for each reaction. The Ct value of the Quantitation Standard is similar regardless of the viral titer.

Quantitation Standard Growth Curves for a Dilution Series of Virus Spanning over a 5-$\log_{10}$ Range:
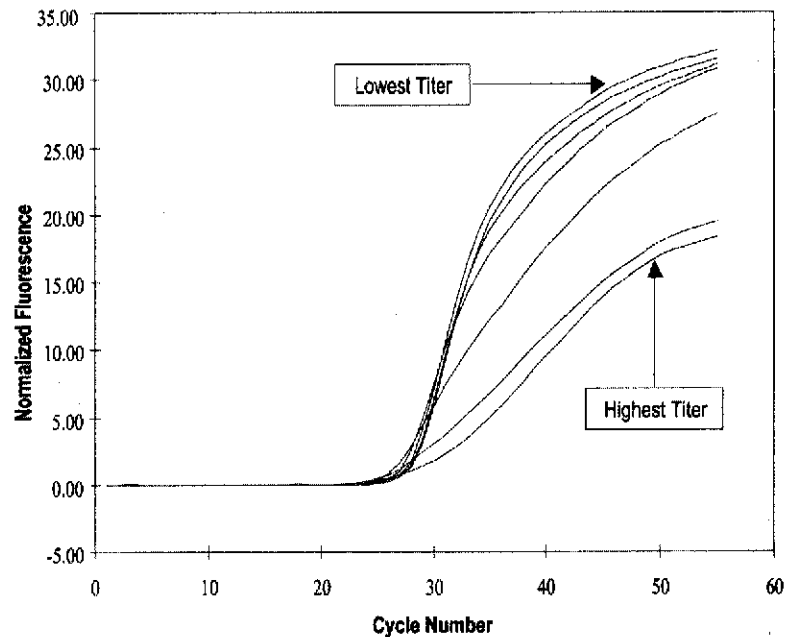
The fractional cycle number (Ct) is calculated where the fluorescence signal crosses the Assigned Fluorescence Level.

Fluorescence Values at Every Cycle are Normalized for Each Growth Curve:

HBV DNA Quantitation: The COBAS TaqMan HBV Test quantitates HBV viral DNA by utilizing a second target sequence (HBV Quantitation Standard) that is added to each test specimen at a known concentration. The HBV Quantitation Standard is a non-infectious, linearized, double stranded plasmid DNA construct, containing fragments of HBV sequences with primer binding regions identical to those of the HBV target sequence. The HBV Quantitation Standard also generates an amplification product of the same length and base composition as the HBV

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target DNA. The detection probe binding region of the HBV Quantitation Standard has been modified to differentiate HBV Quantitation Standard amplicon from HBV target amplicon.

During the annealing phase of the PCR on the COBAS TaqMan 48 Analyzer, the specimens are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the HBV DNA and HBV Quantitation Standard DNA data represent sets that are valid, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the HBV DNA and the HBV Quantitation Standard DNA. The lot-specific calibration constants provided with the COBAS TaqMan HBV Test are used to calculate the titer value for the specimens and controls based upon the HBV DNA and HBV Quantitation Standard DNA Ct values. The COBAS TaqMan HBV Test is standardized against the WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Technology (NAT) Assays Testing NIBSC 97/746 and titer results are reported in International Units (IU/mL).

Results

The COBAS TaqMan 48 Analyzer automatically determines the HBV DNA titer for the specimen or control. The HBV DNA titer is expressed in International Units (IU)/mL. The conversion factor between HBV copies/mL and HBV IU/mL is 5.82 copies/IU based on linkage to the WHO International Standard for HBV DNA Nucleic Acid Amplification Technology (NAT) Assays, NIBSC Code 97/746.

The COBAS TaqMan 48 Analyzer:
- Determines the Cycle Threshold value (Ct) for the HBV DNA and the HBV Quantitation Standard DNA.
- Determines the HBV DNA titer based upon the Ct values for the HBV DNA and HBV Quantitation Standard DNA and the lot-specific calibration coefficients.
- Determines that the calculated IU/mL titers for HBV L(+)C and HBV H(+)C fall within the assigned ranges stated on the Controls Value Card supplied with the kit.

Run Validation: The run is valid if no flags appear for the COBAS TaqMan HBV Controls.

Interpretation of Results

For a valid run, each individual specimen should be checked for flags or comments on the result printout. A valid run may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens. Specimen results are interpreted as follows:

Titer Result	Interpretation
Target Not Detected	No Ct value for HBV obtained. Report results as "HBV DNA not detected".
<2.9E+01 IU/mL	Below 2.9E+01 IU/mL (lower limit of quantitation, LLoQ). HBV DNA is not quantifiable.
≥2.9E+01 IU/mL and ≤1.1E+08 IU/mL	Calculated results greater than or equal to 29 IU/mL and less than or equal to 1.1E+08 IU/mL are within the Linear Range of the Assay.
> 1.10E+08 IU/mL	IU/mL are above the Linear Range of the assay. Report results as "greater than 1.10E+08 HBV DNA IU/mL". If quantitative results are desired, the original specimen should be diluted 1:100 with HBV-negative human plasma or serum depending upon the matrix of the original specimen (plasma samples must be diluted in plasma and serum samples must be diluted in serum), and the test repeated. Multiply the reported result by the dilution factor.

1.1E+08 IU/mL = 1.1×10^8 IU/mL

Note: In some rare instances, specimens with very high titers can produce an Invalid result with a flag "QS_INVALID." The result for these samples is not valid and must be repeated. If the sample continues to produce an invalid result the user can dilute the sample 1:100 in HBV negative plasma (for plasma samples) or HBV negative serum (for serum samples) in order to try to obtain a valid result. Multiply the reported result by the dilution factor.

VI.   ALTERNATIVE PRACTICES AND PROCEDURES

Currently, methods for following the progress of antiviral therapy include immunoassay (serological tests, EIA), biochemical (ALT), and histological (liver biopsy - fibrosis, inflammation). There has not been an adequate molecular method commercially available to follow HBV DNA response to antiviral therapy during the course of treatment.

VII.   MARKETING HISTORY

The COBAS TaqMan HBV Test For Use With The High Pure Viral Nucleic Acid System has not been withdrawn from the following markets for reasons related to safety or effectiveness. The test is currently available in the following countries:

Argentina	Iceland	Pakistan
Australia	India	Paraguay
Austria	Indonesia	Philippines
Belarus	Ireland	Poland
Belgium	Israel	Qatar
Canada	Japan	Russia
Chile	Korea	Saudi Arabia
Cyprus	Kuwait	Slovenia

Czech Republic	Lebanon	South Africa
Denmark	Luxembourg	Spain
Ecuador	Malaysia	Sweden
Egypt	Malta	Switzerland
Finland	Mexico	Syria
France	Netherlands	Turkey
Germany	New Zealand	United Arab Emirates
Greece	Norway	United Kingdom
Hong Kong	Oman	Venezuela
Hungary		

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 COBAS TaqMan HBV Test must be interpreted within the context of all relevant clinical and laboratory findings. Since the COBAS TaqMan HBV Test is for *in vitro* diagnostic use, there is no direct adverse effect on the health of the patient. However, failure of the product to perform as indicated or human error in use of the product may lead to a false result in the assessment of a response to HBV antiviral therapy, erroneously indicating the need for unnecessary change in treatment.

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.
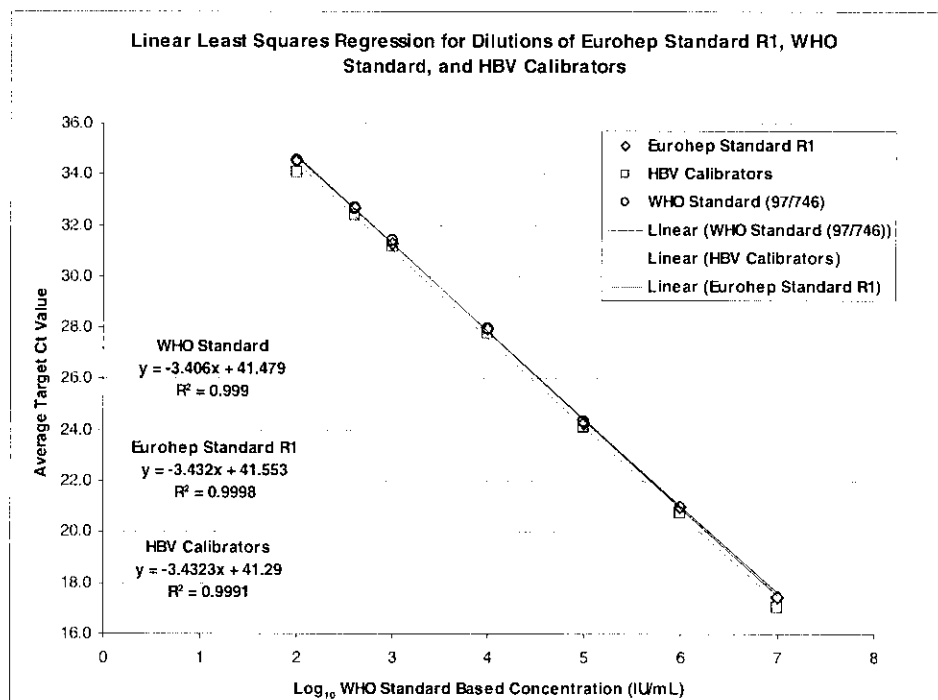
IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Traceability to the WHO Standard

Several standards and controls have been used to provide traceability to the WHO Standard. This includes the Eurohep R1 standard, the WHO Standard, and RMS HBV Calibrators. The Eurohep R1 standard, and the CTM HBV test calibrators behave similarly relative to the WHO standard with a deviation not more than 0.1 $\log_{10}$.
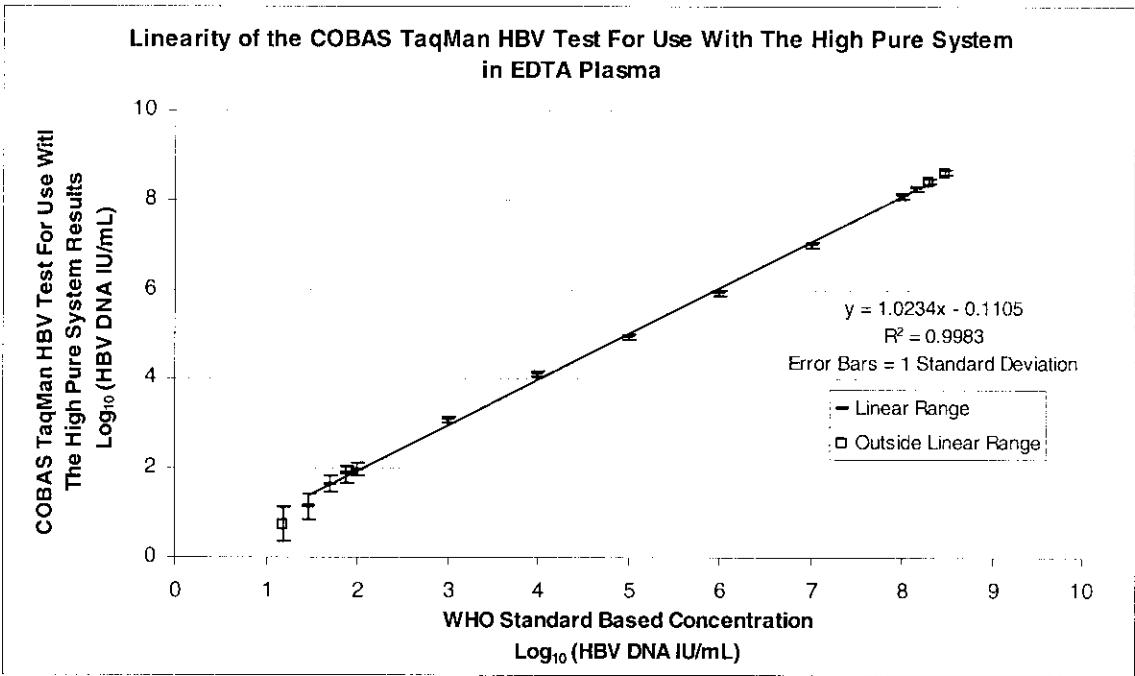
Traceability of the COBAS TaqMan HBV Test For Use With The High Pure System HBV Calibrators (2-7 on $\log_{10}$) to the WHO Standard (range 2-5 on $\log_{10}$) and Eurohep Standard R1 (range 2-7 on $\log_{10}$):

Linear Least Squares Regression for Dilutions of Eurohep Standard R1, WHO Standard, and HBV Calibrators

◇ Eurohep Standard R1
▫ HBV Calibrators
○ WHO Standard (97/746)
---- Linear (WHO Standard (97/746))
— Linear (HBV Calibrators)
······ Linear (Eurohep Standard R1)

WHO Standard
$y = -3.406x + 41.479$
$R^2 = 0.999$

Eurohep Standard R1
$y = -3.432x + 41.553$
$R^2 = 0.9998$

HBV Calibrators
$y = -3.4323x + 41.29$
$R^2 = 0.9991$

(Y-axis: Average Target Ct Value; X-axis: Log_{10} WHO Standard Based Concentration (IU/mL))
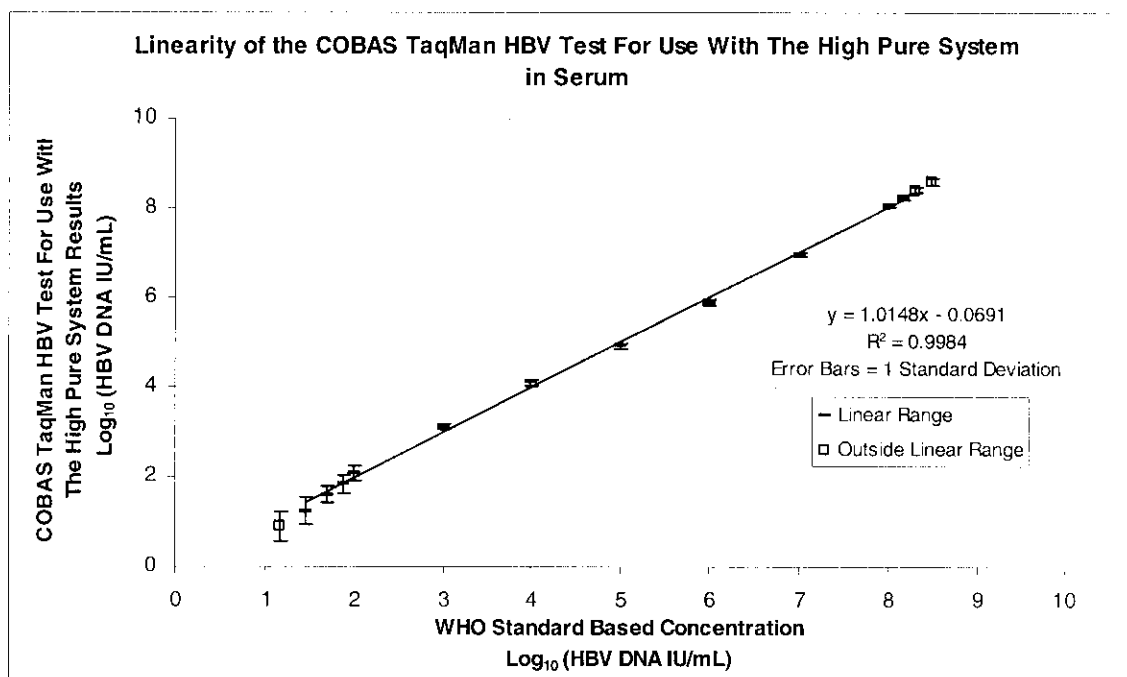
Linear Range

The linear range study was evaluated in accordance with the methods defined in the CLSI Guideline EP6-A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline." The COBAS TaqMan HBV Test For Use With the High Pure System was found to give a linear response from 2.9E1 ($\log_{10} = 1.46$) HBV DNA IU/mL to 1.10E8 ($\log_{10} = 8.02$) HBV DNA IU/mL in both EDTA plasma and serum with deviation from linearity not more than 0.20 $\log_{10}$, in both matrices. The study was performed using two lots of COBAS TaqMan HBV Test For Use With the High Pure System reagents and serial dilutions of an HBV positive genotype A specimen that was assigned relative to the WHO Standard. Twenty-seven replicates were tested per level in EDTA plasma and in serum.

Linearity of the COBAS TaqMan HBV Test For Use With The High Pure System in EDTA Plasma for Genotype A:

Linearity of the COBAS TaqMan HBV Test For Use With The High Pure System in EDTA Plasma

$$y = 1.0234x - 0.1105$$
$$R^2 = 0.9983$$

Error Bars = 1 Standard Deviation

— Linear Range
□ Outside Linear Range

X-axis: WHO Standard Based Concentration Log_{10} (HBV DNA IU/mL)

Y-axis: COBAS TaqMan HBV Test For Use With The High Pure System Results Log_{10} (HBV DNA IU/mL)

Linearity of the COBAS TaqMan HBV Test For Use With The High Pure System in Serum for Genotype A:

Linearity of the COBAS TaqMan HBV Test For Use With The High Pure System in Serum

$$y = 1.0148x - 0.0691$$
$$R^2 = 0.9984$$

Error Bars = 1 Standard Deviation

— Linear Range
□ Outside Linear Range

Y-axis: COBAS TaqMan HBV Test For Use With The High Pure System Results Log_{10} (HBV DNA IU/mL)

X-axis: WHO Standard Based Concentration Log_{10} (HBV DNA IU/mL)

Linearity for genotypes other than genotype A was not evaluated.

The analytical measurement range of analyte values that can be directly measured on a specimen without any dilution using the COBAS TaqMan HBV Test is 29 to 1.1E+08 IU/mL. The clinically reportable range of analyte values that can be measured on a sample with a maximum dilution of one to one-hundred using the COBAS TaqMan HBV Test is 29 to 1.1E+10 IU/mL.

Inclusivity (Genotype Titer Quantitation)

The performance of the COBAS TaqMan HBV Test on HBV genotypes was evaluated by analysis of 23 purified, linearized and quantitated plasmid DNAs containing representative sequence inserts from HBV genotypes A through G). In addition, a plasmid representing the common pre-Core mutation G:A at nucleotide position 1896 was tested. Each plasmid DNA was diluted to concentrations of 5.2E1, 5.2E2, 5.2E5 and 5.2E8 IU/mL. Each dilution was co-amplified with HBV QS DNA and analyzed in triplicate with the COBAS TaqMan HBV Test. The titers for all plasmids were compared with that of a control plasmid DNA.

COBAS TaqMan HBV Test Inclusivity Testing - Typed Plasmid DNA Tested:

Plasmid Designation	Genotype	Parent Specimen Origin
p8423-c1	A	India
p1115-c1	A	Burundi
p3952-c1	A	Cameroon
p4199-c2	A	Norway

p1764-c1	B	China
p1767-c1	B	China
p3958-c1	B	East Asia
p830-c1	B	Societe Island
p3982-c1	B	Vietnam
p1786-c1	C	China
p11549-1	C	Bangladesh
p3872-c1	D	Iran
p1103-c1	D	Tunisia
p3953-c2	D	North Africa
p18-c1	D	Sweden
p30893-5	D	Sweden
p4244-c1	D	Denmark
p3217-c1	E	Senegal
p3963-c2	E	Nigeria
p9203-c1	F	Colombia
p479-c1	F	Venezuela
p1009-c1	F	Spain
p00042975-4	G	United States
pIT1896	Pre-Core	Italy

The COBAS TaqMan HBV Test performance on all 23 plasmids and DNA copy numbers for all genotypes and the pre-Core mutation, and agreement with each other and the control plasmid DNA, can be seen from the of the COBAS TaqMan HBV Test results on HBV Genotypes A through G and a Pre-Core Mutant:
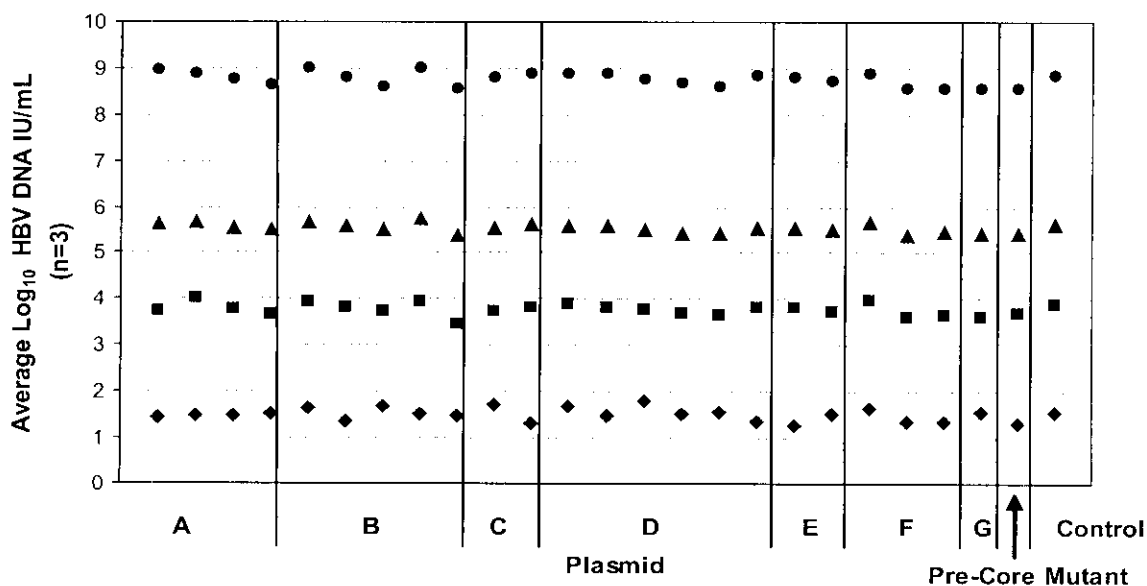
Limit of Detection Using Clinical Specimens Across All HBV Genotypes

The limit of detection was determined in accordance with the methods defined in the CLSI Guideline EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline." The LoD was determined using two lots of reagents for seven clinical specimens of HBV representing genotypes A through G diluted into both EDTA plasma and serum. One representative clinical sample of each genotype was tested. The HBV titer for each parent specimen was provided by the vendor or determined in-house. Dilution panels were generated from these final titer assignments. Each panel consisted of six members representing input levels at 15, 10, 8, 6, 4, and 1 IU/mL. Each level of each dilution was tested with 16 replicates split across two runs for each of two reagent lots for each genotype specimen in each matrix across eight days. A total of 32 replicates of each panel member were tested for each genotype in each matrix. The hit rate was determined at each input level and the Limit of Detection is defined as the lowest level demonstrating a ≥95% hit rate and where all higher input levels have ≥ 95% hit rate.

The LoD for the various HBV genotypes for the EDTA plasma and serum is summarized in the table below. The data shown represents the combined results from testing with two lots of reagents:

Genotype	EDTA Plasma		Serum	
	LOD, IU/mL	Hit Rate (95% Confidence Limits)	LOD, IU/mL	Hit Rate (95% Confidence Limits)
A	10	100% (89.1 – 100%)	4	100% (89.1 – 100%)
B	4	97% (83.8 – 99.9%)	4	100% (89.1 – 100%)
C	4	97% (83.8 – 99.9%)	4	100% (89.1 – 100%)
D	4	100% (89.1 – 100%)	4	97% (83.8 – 99.9%)
E	4	97% (83.8 – 99.9%)	4	100% (89.1 – 100%)
F	4	100% (89.1 – 100%)	4	100% (89.1 – 100%)
G	6	97% (83.8 – 99.9%)	4	100% (89.1 – 100%)

The LoD for the COBAS TaqMan HBV Test, detecting any of the seven genotypes tested, was determined to be **10 IU/mL**. No significant difference between the seven genotypes was observed. In addition, there was no significant difference between plasma and serum or between the two lots of reagents tested. Considering that COBAS TaqMan HBV Test does not differentiate between HBV genotypes, the overall LoD of the assay to detect HBV in clinical specimens is determined to be 10 IU/mL.

Limit of Detection Using the WHO International Standard

The WHO Standard was freshly diluted into each of five unique EDTA plasma specimens and each of five unique serum specimens. Each level of each dilution

was tested with six replicates split across two runs for each of two reagent lots for each matrix. A total of 10 runs were conducted over five days for each reagent lot for each matrix to give a total of 60 replicates for each level for each matrix. These studies demonstrate that the COBAS TaqMan HBV Test can detect HBV DNA in EDTA plasma and serum at concentrations as low as 10 IU/mL with a positivity rate greater than 95%. The concentration of HBV DNA using the WHO international standard in EDTA plasma and serum that can be detected with a positivity rate of greater than 95% as determined by Probit Analysis, is 3.5 IU/mL and 3.4 IU/mL, respectively.

Limit of Detection in EDTA Plasma of the COBAS TaqMan HBV Test For Use With the High Pure System using the WHO International Standard (Genotype A):

WHO Standard Based Concentration (HBV DNA IU/mL)	No. Valid Replicates	No. Positives	Positivity Rate
29.0	59	59	100%
22.0	59	59	100%
15.0	60	60	100%
12.0	59	59	100%
10.0	59	59	100%
8.0	60	59	98%
6.0	60	59	98%
4.0	60	59	98%
2.0	60	51	85%
1.0	60	37	62%
0.5	60	23	38%
0.0	60	0	0%
Probit 95% Hit Rate	3.5 IU/mL [95% confidence limits of 2.8 – 4.7 IU/mL]		

Limit of Detection in Serum of the COBAS TaqMan HBV Test For Use With the High Pure System using the WHO International Standard (Genotype A):

WHO Standard Based Concentration (HBV DNA IU/mL)	No. Valid Replicates	No. Positives	Positivity Rate
29.0	59	59	100%
22.0	59	59	100%
15.0	60	60	100%
12.0	60	60	100%
10.0	59	59	100%
8.0	59	58	98%

6.0	60	59	98%
4.0	60	58	97%
2.0	59	52	88%
1.0	59	39	66^
0.5	60	19	32%
0.0	60	0	0%
Probit 95% Hit Rate	3.4 IU/mL [95% confidence limits of 2.7 – 4.6 IU/mL]		

Limit of Quantitation

The limit of quantitation was determined using the WHO International Standard in accordance with the methods defined in the CLSI Guideline EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline." The WHO Standard was freshly diluted into each of five unique EDTA plasma specimens and each of five unique serum specimens. Each level of each dilution was tested with six replicates split across two runs for each of two reagent lots for each matrix. A total of 10 runs were conducted across five days for each reagent lot for each matrix to give a total of 60 replicates for each level for each matrix.

The studies demonstrated that the COBAS TaqMan HBV Test can determine the concentration of HBV DNA in EDTA plasma and serum at concentrations as low as 29 IU/mL with an acceptable level of accuracy:

Matrix	Expected Concentration	Expected $\log_{10}$ Concentration	Observed Avg. $\log_{10}$ Concentration	Absolute Bias	SD $\log_{10}$ Concentration	Total Analytical Error[1]
EDTA Plasma	29 IU/mL	1.462	1.25	0.21	0.24	0.69
Serum	29 IU/mL	1.462	1.247	0.22	0.17	0.56

[1] *The Total Analytical Error, or TAE, is defined as Bias + 2SD; the TAE was 0.69 for plasma, and 0.56 for serum. At this concentration, the difference between two measurements of more than 1.0 $\log_{10}$ IU/mL is statistically significant.*

### *Sample Handling and Collection*

Sample Types (Serum and Plasma)

The COBAS TaqMan HBV Test is for use with serum or EDTA plasma specimens only. To demonstrate the equivalency of the use of EDTA plasma or serum, 60 matched clinical specimen sets (each set is EDTA plasma and serum drawn from a single HBV-infected or HBsAg-positive individual) were tested to demonstrate plasma and serum equivalency. Each sample was tested in duplicate

and the mean titer for each sample was calculated. A Deming linear regression analysis was also performed using the calculated mean titers. Fifty matched sets were used for data analysis which included 41 matched sets testing within the linear range of the test and nine matched sets with titers above the linear range of the assay upon initial testing and retested following 1:100 dilution to obtain the titer of the original sample. The results from ten sets (five negative for HBV DNA and five sets positive for HBV DNA but with titers too low to quantify) were not included in the final analysis.

The individual log titer difference (log titer EDTA plasma – log titer) for 49 of the 50 matched sets was ≤ 0.30 with the one set having a difference of -0.37. The mean difference was -0.05 log (95% CI: -0.036, 0.025), indicating that the results between serum and EDTA plasma were not significantly different.

The result from the linear regression analysis which demonstrates the effect of matrix type on HBV DNA results from patient specimens is shown in the figure below, with slope=1.0131 (95% confidence interval is [1.0045 – 1.0216]) with an intercept of -0.0605 (95% confidence interval is [-0.1065 to -0.0144]).

Regression Analysis of Matched Serum – EDTA Plasma Samples (n=50):

$y = 1.0131x - 0.0605$
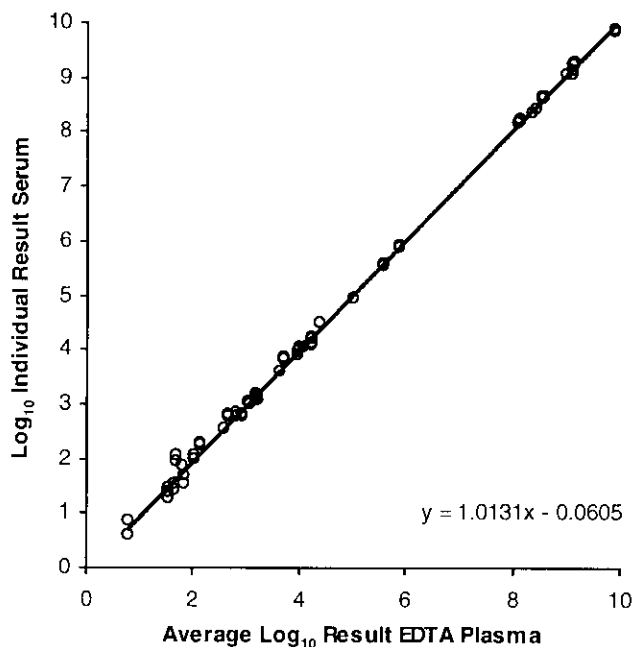
The pooled standard deviation estimates for the EDTA plasma and serum samples in the matrix equivalency study are shown in the table below.

Matrix	Mean log Titer	Pooled SD
EDTA Plasma	4.621	0.060
Serum	4.616	0.093

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Specimen Collection

Blood should be collected in BD SST Serum Separator Tubes or in tubes using EDTA (lavender top) as the anticoagulant. Whole blood can be stored at 2-25°C for no longer than 1 day. Serum or plasma should be separated from whole blood within 1 day of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature, and transferred to a sterile polypropylene tube.

The figure below illustrates the data from specimen collection studies. The largest observed difference between the EDTA plasma conditions was not more than $\pm$ 0.12 $\log_{10}$ and the largest observed difference between the serum conditions was not more than $\pm$ 0.16 $\log_{10}$.

HBV Stability in Whole Blood With EDTA Anticoagulant or in Serum Separator Tubes Before Separation into Plasma or Serum:
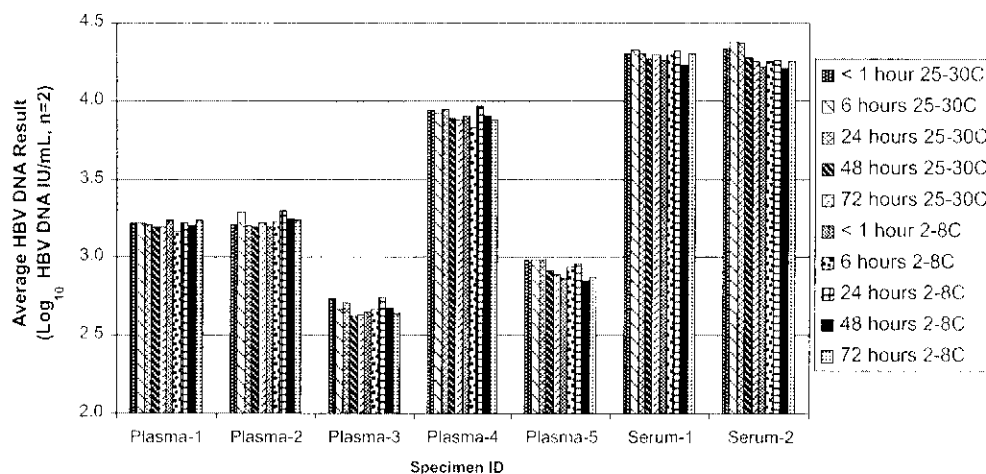

Specimen Transport

Transportation of whole blood, serum or plasma must comply with country, federal, state and local regulations for the transport of etiologic agents. Whole blood must be transported at 2-25°C and processed within 6 hours of collection.

Specimen Storage

Serum or plasma specimens may be stored at room temperature for up to 3 days, at 2-8°C for up to 7 days or frozen at -20°C to -80°C for up to six weeks. The largest observed difference between the EDTA plasma conditions was not more than ± 0.11 $\log_{10}$ and the largest observed difference between the serum conditions was not more than ± 0.05 $\log_{10}$ across the tested conditions. It is recommended that specimens be stored in 800-900 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes. Figure below shows the HBV stability data in EDTA-plasma or serum from these specimen storage studies:
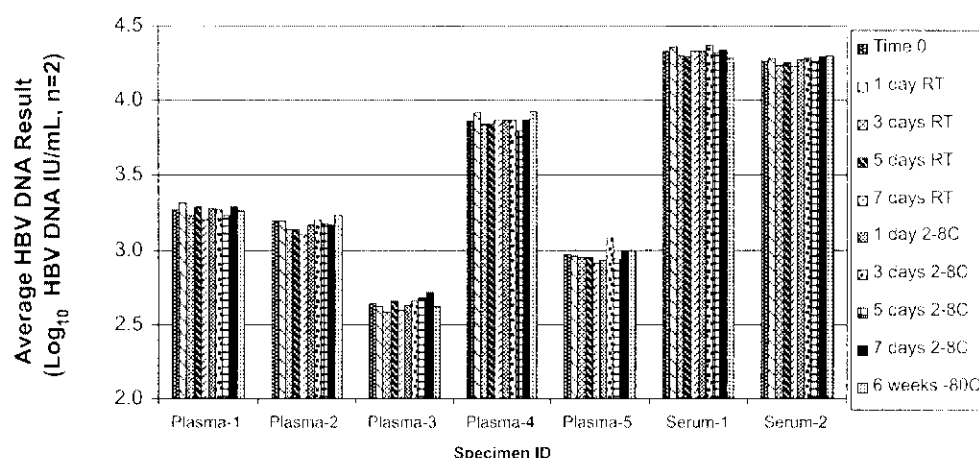
Serum and plasma specimens may be frozen and thawed up to five times without a loss of HBV DNA. The largest observed difference between the EDTA plasma conditions was not more than ± 0.19 $\log_{10}$ and the largest observed difference between the serum conditions was not more than ± 0.10 $\log_{10}$. Figure below illustrates the HBV results after up to five freeze-thaw (F-T) cycles data from these freeze-thaw studies:
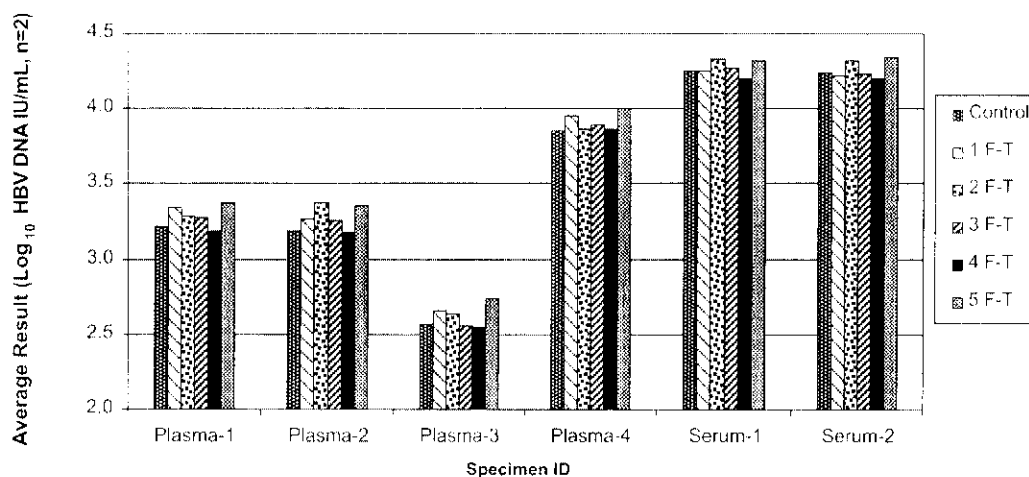
Analytical Specificity

Cross-reactivity

The analytical specificity of the COBAS TaqMan HBV Test was evaluated by adding cultured virus into HBV-negative human EDTA plasma or analyzing specimens from subjects positive for other viral agents (listed in table below). Except for one CMV infected specimen and HPV strain 18, none of the non-HBV DNA or RNA viruses tested were positive for HBV DNA. Subsequent testing of the CMV infected specimen did not consistently confirm the initial result. Subsequent testing of HPV strain 18 indicated that no positive results for HBV were detected at HPV concentrations less than 2.0E+09 cp/mL.

Analytical specificity specimens tested:

Virus Added Into Plasma	Specimens from Infected Patients (n)
Adenovirus type 7	Cytomegalovirus infected patients (2)[2]
Cytomegalovirus AD-169	Epstein-Barr Virus infected patients (2)
Epstein-Barr Virus (RAJI Burkitt's Lymphoma cells)	Hepatitis A Virus infected patients (2)
Hepatitis A Virus PA21	Hepatitis C Virus infected patient genotype 4 (1)
Herpes Simplex type 1, MacIntyre	Hepatitis C Virus infected patient genotype 6a (1)
Herpes Simples type 2, MS	HIV-1 infected patients (2)
Human Papilloma Virus Strain 18[1]	
Influenza A virus A/Hong Kong/8/68	
Influenza B virus B/R75	
Varicella-Zoster Ellen	
West Nile Virus	

[1]*HPV strain 18 returned a positive HBV result at an HPV concentration of 2.0E+09 cp/mL. Subsequent testing indicated no HBV positive results for HPV concentrations less than 2.0E+09 cp/mL.*

[2]*One of the two CMV specimens from infected patients returned a positive result which was not consistently confirmed in subsequent testing.*

Interfering Substances

Clinical specimens with elevated levels of triglycerides, bilirubin, albumin and hemoglobin were tested in the absence and presence (approximately 150 IU/mL) of HBV and did not interfere with the quantitation of HBV DNA in the ranges tested:

	Range of Specimens Tested	Normal Range
Triglycerides	655 – 1,378 mg/dL	45 – 190 mg/dL
Bilirubin	3.7 – 8.2 mg/dL	0.25 – 1.2 mg/dL
Albumin	5,100 – 6,600 mg/dL	2,800 – 5,000 mg/dL

Hemoglobin	27.5 – 243.2 mg/dL	0 – 2.5 mg/dL

The following drug compounds were tested at $1xC_{max}$ and $3xC_{max}$ for each drug in the absence and presence (approximately 150 IU/mL) of HBV and did not interfere with the quantitation of HBV DNA by this test:

Nucleotide DNA Polymerase Inhibitors	**Nucleoside Reverse Transcriptase and DNA Polymerase Inhibitors**
Adefovir dipivoxil	Lamivudine
Tenofovir disoproxil fumarate	Zidovudine
	Zalcitabine
	Stavudine
	Abacavir
HIV Protease Inhibitors	**Non-nucleoside HIV Reverse Transcriptase Inhibitors**
Indinavir	
Ritonavir	Nevirapine
Nelfinavir	Efavirenz
Saquinavir	
Amprenavir	
Lopinavir/Ritonavir	
Immune Modulators	**HIV Fusion Inhibitor**
Interferon alpha-2a	Enfuvirtide
Interferon alpha-2b	
CMV Treatment Compounds	
Ganciclovir	
Valganciclovir hydrochloride	
Acyclovir	
Valacyclovir hydrochloride	

Within-Laboratory Precision

Within-Run, Run-to-Run and Total Precision were evaluated in accordance with the methods defined in the CLSI (formerly NCCLS) Guideline EP5-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition." This procedure permits the determination of both Within-Run and Total Precision through the performance of a single multiple-day and multiple operator study. A run, consisting of three replicates of each of ten panel members diluted from an HBV genotype A clinical specimen, was performed daily for 15 days. Each panel member was taken through the entire COBAS TaqMan HBV Test procedure, including specimen preparation, amplification and detection, representing all aspects of the test procedure. The study was performed for three lots of COBAS TaqMan HBV Test reagents, and

the combined results are presented in HBV DNA IU/mL and HBV DNA $\log_{10}$ IU/mL.

Precision of the COBAS TaqMan HBV Test (in IU/mL):

Specimen	1	2	3	4	5	6	7	8	9	10
Average Observed HBV DNA Titer (IU/mL)	1.07E8	5.33E7	1.04E7	8.52E5	9.28E4	1.21E4	1200	111	49	14
Within-Run CV	19%	10%	12%	7%	14%	16%	14%	22%	27%	50%
Run-to-Run CV	11%	14%	12%	14%	16%	18%	18%	26%	17%	22%
Total CV	23%	17%	17%	16%	22%	24%	22%	34%	32%	54%
Total No. Replicates	132	134	134	135	135	134	135	135	135	135

Precision of the COBAS TaqMan HBV Test (in $\log_{10}$ IU/mL):

Specimen	1	2	3	4	5	6	7	8	9	10
Average Observed HBV DNA Titer ($\log_{10}$ IU/mL)	8.02	7.72	7.01	5.92	4.94	4.06	3.07	2.03	1.67	1.05
Within-Run Standard Deviation	0.07	0.04	0.05	0.03	0.21	0.18	0.06	0.09	0.11	0.59
Run-to-Run Standard Deviation	0.05	0.06	0.05	0.06	0.07	0.07	0.08	0.10	0.07	0.00
Total Standard Deviation	0.08	0.07	0.07	0.07	0.22	0.19	0.10	0.13	0.13	0.59

Reproducibility

The reproducibility of the COBAS TaqMan HBV Test For Use With the High Pure System was evaluated by two operators at each of three external clinical sites. Each operator performed three days of testing on each of three lots of reagents with each panel. Each run comprised a single panel with each panel member tested in triplicate. The results of the reproducibility study are summarized in tables below for EDTA plasma and serum.

Components of Variance (Percentage of Total Variance) of HBV DNA Concentration ($\log_{10}$ IU/mL) — EDTA Plasma:

Geno-type	Mean of HBV DNA Concentration (log$_{10}$ IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/Instru-ment	Operator	Day/Run	Within-Run	Total Precision Variance of log$_{10}$ HBV DNA Concentration
A	1.32	21	162[a]	0.0015 (3%)	0.0000 (0%)	0.0008 (1%)	0.0044 (7%)	0.0521 (89%)	0.0588 (100%)
	2.34	220	162	0.0004 (5%)	0.0000 (0%)	0.0008 (11%)	0.0015 (20%)	0.0048 (64%)	0.0074 (100%)
	3.36	2,314	162	0.0003 (4%)	0.0000 (0%)	0.0019 (30%)	0.0014 (22%)	0.0027 (44%)	0.0062 (100%)
	4.35	22,369	162	0.0010 (12%)	0.0002 (2%)	0.0019 (24%)	0.0019 (23%)	0.0032 (39%)	0.0081 (100%)
	5.19	154,752	162	0.0014 (24%)	0.0003 (5%)	0.0011 (20%)	0.0011 (20%)	0.0017 (31%)	0.0056 (100%)
	7.29	19,444,058	162	0.0000 (1%)	0.0000 (0%)	0.0005 (14%)	0.0007 (21%)	0.0022 (64%)	0.0035 (100%)
C	1.38	24	162	0.0020 (9%)	0.0006 (3%)	0.0006 (3%)	0.0059 (25%)	0.0143 (61%)	0.0235 (100%)
	2.34	219	160[b]	0.0022 (34%)	0.0004 (6%)	0.0005 (7%)	0.0000 (0%)	0.0035 (54%)	0.0064 (100%)
	3.43	2,686	162	0.0004 (7%)	0.0016 (29%)	0.0009 (17%)	0.0008 (15%)	0.0019 (33%)	0.0057 (100%)
	4.39	24,479	161[c]	0.0004 (8%)	0.0010 (21%)	0.0008 (16%)	0.0011 (22%)	0.0015 (32%)	0.0047 (100%)
	5.24	172,515	162	0.0007 (17%)	0.0009 (20%)	0.0008 (19%)	0.0006 (13%)	0.0014 (31%)	0.0044 (100%)
	7.38	23,791,720	162	0.0013 (21%)	0.0001 (1%)	0.0004 (8%)	0.0008 (14%)	0.0033 (56%)	0.0059 (100%)

[a] 14 out of 162 (8.6%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."
[b] 2 results were invalid.
[c] 1 result was invalid.

Standard Deviation Components of HBV DNA Concentration (log$_{10}$ IU/mL) — EDTA Plasma:

Geno-type	Mean of HBV DNA Concen-tration (log$_{10}$ IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/Instru-ment	Operator	Day/Run	Within-Run	Total Standard Deviation of log$_{10}$ HBV DNA Concentration
A	1.32	21	162[a]	0.0391	0.0000	0.0277	0.0662	0.2283	0.2425
	2.34	220	162	0.0188	0.0000	0.0291	0.0381	0.0691	0.0860
	3.36	2,314	162	0.0161	0.0000	0.0431	0.0368	0.0522	0.0787
	4.35	22,369	162	0.0311	0.0128	0.0441	0.0431	0.0564	0.0900

Geno-type	Mean of HBV DNA Concen-tration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/Instru-ment	Operator	Day/Run	Within-Run	Total Standard Deviation of log_{10} HBV DNA Concentration
	5.19	154,752	162	0.0368	0.0161	0.0329	0.0337	0.0416	0.0748
	7.29	19,444,058	162	0.0051	0.0000	0.0222	0.0268	0.0474	0.0592
C	1.38	24	162	0.0449	0.0251	0.0246	0.0770	0.1196	0.1533
	2.34	219	160[b]	0.0466	0.0190	0.0212	0.0000	0.0589	0.0800
	3.43	2,686	162	0.0205	0.0402	0.0307	0.0286	0.0431	0.0755
	4.39	24,479	161[c]	0.0196	0.0319	0.0274	0.0327	0.0391	0.0686
	5.24	172,515	162	0.0270	0.0292	0.0290	0.0242	0.0368	0.0663
	7.38	23,791,720	162	0.0354	0.0079	0.0212	0.0285	0.0574	0.0768

[a] 14 out of 162 (8.6%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."
[b] 2 results were invalid.
[c] 1 result was invalid.

Reproducibility results summary: Total %CV for HBV panel members — EDTA Plasma:

Genotype	Mean of HBV DNA Concentration (log_{10} IU/mL)	Geometric Mean of HBV DNA Concen-tration (IU/mL)	No. of Tests[1]	Total Precision Variance of log_{10} HBV DNA Concentration	Total Precision Standard Deviation of log_{10} HBV DNA Concentration	lognormal CV (%) [2]
A	1.32	21	162[a]	.0588	0.24	60
	2.34	220	162	.0074	0.09	20
	3.36	2,314	162	.0062	0.08	18
	4.35	22,369	162	.0081	0.09	21
	5.19	154,752	162	.0056	0.07	17
	7.29	19,444,058	162	.0035	0.06	14
C	1.38	24	162	.0235	0.15	36
	2.34	219	160[b]	.0064	0.08	19
	3.43	2,686	162	.0057	0.08	17
	4.39	24,479	161[c]	.0047	0.07	16
	5.24	172,515	162	.0044	0.07	15
	7.38	23,791,720	162	.0059	0.08	18

[a] 14 out of 162 (8.6%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."
[b] 2 results were invalid.
[c] 1 result was invalid

Components of Variance (Percentage of Total Variance) of HBV DNA
Concentration ($\log_{10}$ IU/mL) — Serum:

Geno-type	Mean of HBV DNA Concentration ($\log_{10}$ IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/ Instru-ment	Operator	Day/ Run	Within-Run	Total Precision Variance of $\log_{10}$ HBV DNA Concentration
A	1.04	11	162[a]	0.0054 (5%)	0.0127 (13%)	0.0010 (1%)	0.0044 (4%)	0.0767 (77%)	0.1001 (100%)
	2.10	127	160[b]	0.0000 (0%)	0.0022 (16%)	0.0007 (5%)	0.0043 (30%)	0.0072 (50%)	0.0144 (100%)
	3.34	2,194	162	0.0000 (1%)	0.0022 (28%)	0.0009 (11%)	0.0016 (21%)	0.0031 (39%)	0.0078 (100%)
	4.34	21,749	161[c]	0.0020 (20%)	0.0029 (29%)	0.0008 (8%)	0.0023 (23%)	0.0019 (19%)	0.0099 (100%)
	5.17	147,146	162	0.0024 (35%)	0.0011 (16%)	0.0007 (10%)	0.0016 (24%)	0.0011 (16%)	0.0069 (100%)
	7.27	18,732,744	162	0.0000 (0%)	0.0003 (6%)	0.0001 (4%)	0.0003 (7%)	0.0033 (83%)	0.0039 (100%)
C	1.38	24	162	0.0005 (2%)	0.0000 (0%)	0.0032 (9%)	0.0068 (19%)	0.0244 (70%)	0.0349 (100%)
	2.34	218	161[c]	0.0010 (11%)	0.0000 (0%)	0.0005 (5%)	0.0040 (48%)	0.0030 (35%)	0.0084 (100%)
	3.43	2,664	162	0.0003 (5%)	0.0003 (5%)	0.0009 (15%)	0.0029 (46%)	0.0018 (29%)	0.0062 (100%)
	4.39	24,555	162	0.0009 (12%)	0.0004 (6%)	0.0005 (6%)	0.0040 (53%)	0.0018 (24%)	0.0076 (100%)
	5.22	167,232	162	0.0009 (13%)	0.0006 (9%)	0.0005 (7%)	0.0032 (46%)	0.0017 (24%)	0.0070 (100%)
	7.37	23,676,552	161[c]	0.0006 (8%)	0.0000 (0%)	0.0004 (5%)	0.0041 (51%)	0.0030 (37%)	0.0082 (100%)

[a] *65 out of 162 (40.1%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid*

Standard Deviation Components of HBV DNA Concentration ($\log_{10}$ IU/mL) —
Serum:

Geno-type	Mean of HBV DNA Concentration ($\log_{10}$ IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/ Instru-ment	Operator	Day/ Run	Within-Run	Total Standard Deviation of $\log_{10}$ HBV DNA Concentration

Geno-type	Mean of HBV DNA Concentration (log$_{10}$ IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests	Lot	Site/ Instru-ment	Operator	Day/ Run	Within-Run	Total Standard Deviation of log$_{10}$ HBV DNA Concentration
A	1.04	11	162[a]	0.0737	0.1127	0.0312	0.0660	0.2769	0.3164
	2.10	127	160[b]	0.0000	0.0473	0.0260	0.0654	0.0850	0.1200
	3.34	2,194	162	0.0067	0.0470	0.0292	0.0406	0.0553	0.0883
	4.34	21,749	161[c]	0.0451	0.0539	0.0281	0.0482	0.0435	0.0995
	5.17	147,146	162	0.0489	0.0332	0.0262	0.0403	0.0328	0.0831
	7.27	18,732,744	162	0.0000	0.0160	0.0121	0.0164	0.0573	0.0624
C	1.38	24	162	0.0232	0.0000	0.0566	0.0823	0.1562	0.1868
	2.34	218	161[c]	0.0309	0.0000	0.0215	0.0636	0.0547	0.0917
	3.43	2,664	162	0.0181	0.0179	0.0306	0.0536	0.0421	0.0787
	4.39	24,555	162	0.0297	0.0210	0.0214	0.0634	0.0424	0.0872
	5.22	167,232	162	0.0302	0.0251	0.0221	0.0569	0.0413	0.0837
	7.37	23,676,552	161[c]	0.0249	0.0000	0.0204	0.0644	0.0549	0.0906

[a] *65 out of 162 (40.1%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*
[c] *1 result was invalid*

Reproducibility Results Summary: Total %CV for HBV Panel Members — Serum:

Genotype	Mean of HBV DNA Concentration (log$_{10}$ IU/mL)	Geometric Mean of HBV DNA Concentration (IU/mL)	No. of Tests[1]	Total Precision Variance of log$_{10}$ HBV DNA Concentration	Total Precision Standard Deviation of log$_{10}$ HBV DNA Concentration	lognormal CV (%) [2]
A	1.04	11	162[a]	.1001	0.32	84
	2.10	127	160[b]	.0144	0.12	28
	3.34	2,194	162	.0078	0.09	21
	4.34	21,749	161[c]	.0099	0.10	23
	5.17	147,146	162	.0069	0.08	19
	7.27	18,732,744	162	.0039	0.06	15
C	1.39	24	162	.0349	0.19	45
	2.34	218	161[c]	.0084	0.09	21
	3.43	2,664	162	.0062	0.08	18
	4.39	24,555	162	.0076	0.09	20
	5.22	167,232	162	.0070	0.08	19
	7.37	23,676,552	161[c]	.0082	0.09	21

[a] *65 out of 162 (40.1%) results were "HBV DNA detected, less than 10.00 HBV DNA IU/mL."*
[b] *2 results were invalid.*

ᶜ 1 result was invalid

The table below summarizes the results for HBV Negative Panel Members from the reproducibility study for each matrix. Eleven false positives were observed in the study and all were below the LOD. Specificity was 100% in EDTA plasma [95% CI = (0.98, 1.00)] and 97% in serum [95% CI = (0.94, 0.99)]:

Matrix	Total Valid Results	Target Not Detected	Target Detected but Below LOD[1]	≥10 and <29 IU/mL[2]	Within Linear Range[3]
EDTA Plasma	324	323	1	0	0
Serum	322	312	10	0	0

[1] *The limit of detection (LOD) for the assay is 10 IU/mL. Results < 10 IU/mL are below the LOD.*
[2] *Results 10 IU/mL to < 29 IU/mL are above the LOD, but below the linear range.*
[3] *Linear range (>29 IU/mL to 1.10E+8 IU/mL).*

Performance of COBAS TaqMan HBV Test with HBV-Negative Samples

The performance of the COBAS TaqMan HBV Test with HBV-negative samples was determined by analysis of HBV-negative serum and EDTA plasma from blood donors. A total of 220 specimens (110 individual EDTA plasma and 110 serum specimens) that were non-reactive for HBsAg and anti-HBc were tested. All specimens were noted as Target Not Detected for HBV DNA for both EDTA plasma and serum (100%, or 110/110, with a 95% confidence interval of 96.6% to 100%).

Carryover / Cross-Contamination Studies

The objective of this study was to verify that any cross contamination potential occurring when the entire process (sample processing and amplification / detection) is performed as prescribed by the manufacturer. Testing consisted of at least five runs consisting of alternating high titer positive and negative samples. Each run consisted of twelve replicates of high positive sample at ~ 2E7 IU/mL and twelve replicates of HBV-negative human EDTA plasma in an alternating pattern. Each run also included four replicates of COBAS TaqMan Negative Control (NC), two replicates of COBAS TaqMan HBV Low Positive Control (LPC) and two replicates of COBAS TaqMan HBV High Positive Control (HPC). All 60 replicates of HBV-negative EDTA plasma produced a result of "Target Not Detected" and all 60 replicates of high titer sample were valid. All of the individual controls were valid and within the specified range.

Therefore, the carryover contamination rate was 0% across 5 runs consisting alternating high positive HBV specimen (~2E7 IU/mL) and HBV-negative plasma.

Recommended Storage Stability

Results of real-time stability studies indicate that the COBAS TaqMan HBV Test is stable for 12 months when stored at its labeled storage conditions of 2 to 8°C.

Open Bottle Reagent Stability

The study was designed to confirm the stability of the opened, reconstituted and unused portion of reagents between the first and second use.

Three levels of clinical HBV Proficiency Sample Panel in EDTA Plasma and three levels of clinical HBV Proficiency Sample Panel in Serum were used to assess the stability of opened vials of reagents. The panel descriptions:

HBV Proficiency Panel (Serum)	HBV Proficiency Sample Panel (Plasma)	Titer
Proficiency Sample 1 (PS1)	Proficiency Sample 1 (PS1)	~150
Proficiency Sample 2 (PS2)	Proficiency Sample 2 (PS2)	1 x 10E5
Proficiency Sample 3 (PS3)	Proficiency Sample 3 (PS3)	5 x 10E8

The stability of the following twelve reagents were assessed after the storage under the conditions indicated below:

Reagent		Storage Temp	Storage Time (weeks)
COBAS TaqMan HBV MMX	HBV MMX	2 - 8°C	2, 3, 4 ,5, 6, 7
COBAS TaqMan Mn2+	CTM Mn2+	2 - 8°C	2, 3, 4 ,5, 6, 7
COBAS TaqMan HBV QS	HBV QS	2 - 8°C	2, 3, 4 ,5, 6, 7
COBAS TaqMan Negative Control1	CTM (–) C	-20°C	2, 3, 4 ,5, 6, 7
COBAS TaqMan HBV Low Positive Control	HBV L(+)C	-20°C	2, 3, 4 ,5, 6, 7
COBAS TaqMan HBV High Positive Control	HBV H(+)C	2 - 8°C	2, 3, 4 ,5, 6, 7
Reconstituted PolyA carrier RNA	CAR	2 - 8°C	2, 3, 4 ,5, 6, 7
Reconstituted Proteinase K	PK	2 - 8°C	2, 3, 4 ,5, 6, 7
Lysis Buffer	LYS	15 - 25°C	2, 3, 4 ,5, 6, 7
Working Wash Buffer (following Ethanol addition)	WASH	15 - 25°C	2, 3, 4 ,52
Working Inhibitor Removal Buffer (following Ethanol addition)	IRB	15 - 25°C	2, 3, 4 ,52
Elution Buffer	ELB	15 - 25°C	2, 3, 4 ,5, 6, 7

(1) Although the stability of CTM (-) C was evaluated, the CTM (-) is a single use reagent. Once opened, any unused portion of CTM (–) C must be discarded.
(2) Wash Buffer and Inhibitor Removal Buffer was prepared freshly on weeks 2 and 3.

At each time point, testing was performed using 3 replicates of each panel member and 3 and 1 replicate of each kit control (NC, LPC and HPC).

Once opened, ELB and LYS are stable for 30 days when stored at 15-25°C in original vial. After addition of ELB to reconstitute the CAR and PK, unused reconstituted CAR and PK are stable for 30 days when stored at -15 to -25°C in original vial. After addition of ethanol, IRB and WASH Working Solutions are stable for 30 days when stored at 15-25°C. Once opened, unused portions of HBV MMX, HBV L(+)C, HBV H(+)C, HBV QS and CTM Mn^{2+} are stable for 30 days when stored at 2-8°C in the original vial.

X.   SUMMARY OF CLINICAL STUDIES

Study Population and Baseline Parameters

The clinical performance of the COBAS TaqMan HBV Test For Use With The High Pure 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+ HBV infection and compensated liver function and one that evaluated patients with presumed precore mutant (HBeAg-/ HBV DNA+) chronic HBV infection with compensated liver function. Marcellin et el.[4] and Hadziyannis et al.[6] previously described subject selection for both studies.

The study population consisted of 407 chronic HBV infected patients enrolled in double-blind, randomized, placebo-controlled studies of Adefovir Dipivoxil. Demographic data, drug dosing data, HBV genotype, HBeAg and anti-HBe results, ALT results, and Baseline (pre-treatment) and end-point liver biopsy results were available for each patient. Viral load testing was performed at Screening and at Weeks 4, 8, 16, 28, 44, and 48 (when available).

Table below summarizes the study population at Screening:

Characteristic	Category	Summary Statistics	HBeAg+	HBeAg-	Total
Total Number of Subjects		N	264	143	407
Placebo		n (%)	129 (49)	50 (35)	179 (44)
10 mg Adefovir Dipivoxil		n (%)	135 (51)	93 (65)	228 (56)
Age (yr)		Median (Min, Max)	34 (16, 65)	46 (18, 65)	39 (16, 65)
Weight (kg)		Median (Min, Max)	70 (41, 118)	74 (46, 111)	72 (41, 118)
Sex	Male	N (%)	191 (72)	119 (83)	310 (76)
	Female	N (%)	73 (28)	24 (17)	97 (24)
Race	White	N (%)	86 (33)	90 (63)	176 (43)
	Asian	N (%)	167 (63)	48 (34)	215 (53)
	Other	N (%)	11 (4)	5 (3)	16 (4)
Genotype	A	N (%)	73 (28)	9 (6)	82 (20)

Characteristic	Category	Summary Statistics	HBeAg+	HBeAg-	Total
	B	N (%)	49 (19)	28 (20)	77 (19)
	C	N (%)	111 (42)	19 (13)	130 (32)
	D	N (%)	25 (9)	84 (59)	109 (27)
	Other	N (%)	6 (2)	3 (2)	9 (2)
HBV DNA < 1.72E+04 IU/mL		N (%)	3 (1)	5 (3)	8 (2)
ALT <= ULN[1]		N (%)	5 (2)	7 (5)	12 (3)
Knodell Score		N	259	139	398
Total		Mean (SD)	9.5 (3.3)	9.4 (3.4)	9.5 (3.3)
Necroinflammatory		Mean (SD)	7.7 (2.8)	7.5 (2.8)	7.6 (2.7)
Fibrosis		Mean (SD)	1.7 (1.1)	1.9 (1.2)	1.8 (1.1)

[1]*ULN = Upper Limit of Normal Range*

Screening samples were obtained six to 125 days before study start. On average, HBeAg+ patients were younger than the HBeAg- patients (median age = 34 years vs 46 years), predominantly Asian (63% vs 34%), were female (28% vs 17%) and were infected with primarily HBV genotypes A and C (70% vs 19%). HBeAg- patients were predominantly White (63%) and infected with HBV genotype D (59%). The Knodell necropsy scores for necroinflammation and fibrosis at Baseline (pre-treatment) were comparable for both populations.

Patients included in the clinical performance analysis received either the standard 10 mg Adefovir dipivoxil dosing or placebo, as indicated in the following table summarizing available samples by treatment arm:

Population	No. Subjects — Placebo	No. Subjects — 10 mg Adefovir	No. Samples per Subject	Total No. Samples
Chronic HBeAg+	129	135	7	1848
Chronic HBeAg-	50	93	6	858
Grand Total				2706

Within-Subject Variability in Absence of Treatment

One hundred and seventy nine subjects were enrolled into the placebo arms of the HBeAg+ and HBeAg- studies of which 137 of 179 had results within the linear range of the assay for testing at Weeks 0, 4, and 8. These results were used to estimate within subject variability, which includes biological variability as well as total assay variability. The within subject variability from these results was estimated to be 0.58 $\log_{10}$ IU/mL for HBeAg- patients and 0.78 $\log_{10}$ IU/mL for HBeAg+ patients. Biological variability was similar to the within-subject variability since the assay variability was negligible. The median change of viral load within a subject was estimated to be 0.30 $\log_{10}$ IU/mL for HBeAg+ and 0.58

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$\log_{10}$ IU/mL for HBeAg- patients. Approximately 90% of the HBeAg+ patients' and 80% of the HBeAg- patients' change of viral load was less than 2.0 $\log_{10}$ IU/mL.

## Safety and Effectiveness Results

Clinical Performance of the COBAS TaqMan HPS HBV Test in Patients on Therapy

Assessment was performed at Screening and at Weeks 4, 8, 16, 28, 44, and 48 (when available). The primary objective was to determine the relationship between viral levels at various treatment time points compared with histological, serological, and biochemical responses to treatment.

The results from testing with the COBAS TaqMan HBV Test For Use With The High Pure System were used to determine whether change (or absence of change) in HBV viral load at various time points may predict improvement (or lack of improvement) in a patient's immune response marker or liver histology at different treatment time points. Statistical analysis of clinical data was used to assess whether viral response to treatment measured with COBAS TaqMan HBV Test For Use With the High Pure 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+ Patients

A graph in the figure below illustrates the change of the median and inter-quartile range of change in HBV DNA from Screening in patients on adefovir dipivoxil and on placebo. It demonstrates efficacy of treatment of the HBeAg+ patients with chronic hepatitis B with adefovir dipivoxil compared to placebo.

YEAR 1 TREATMENT (AS TREATED)   - - - PLACEBO
——— 10 MG

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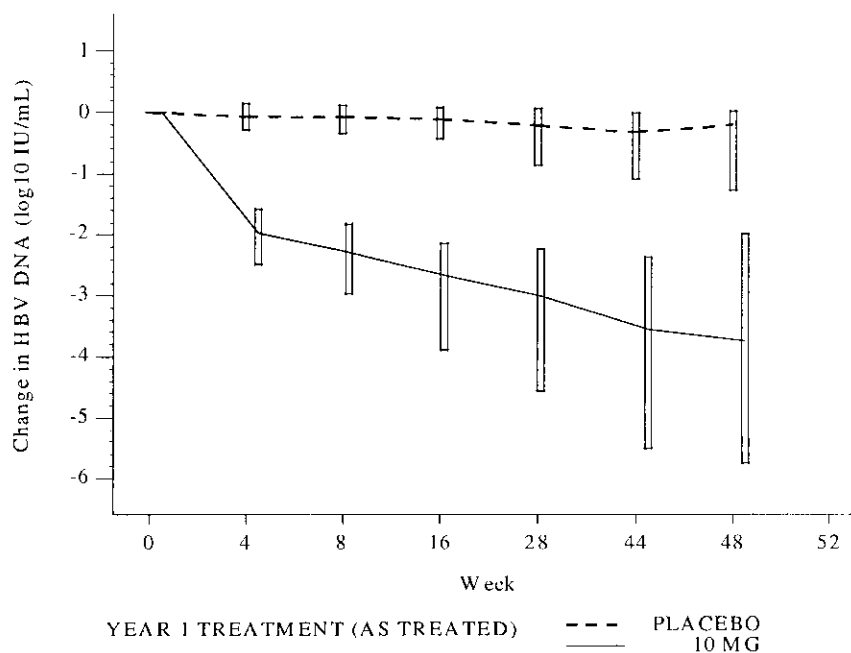
A useful monitoring tool is to observe whether there is an HBV viral load increase of more than 1 $\log_{10}$ after reaching a nadir. Results presented in the table below show that 60.7% (82/135) of the patients reached a nadir in viral load by week 44 on treatment. Seventeen patients had more than 1 $\log_{10}$ increase in viral load by week 48 after achieving the nadir, a 12.6% (17/135) of the total number of patients on treatment and 20.7% (17/82) of the patients who achieved a nadir. Table below summarizes the data for the distribution of the 135 HBeAg+ patients on adefovir dipivoxil 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 |
	4	8	16	28	44	48		
HBV DNA not detected	0	0	0	0	1 (0.7)	0	1 (0.7)	1 (0.7)
< 10	0	0	2 (1.5)	3 (2.2)	7 (5.2)	6 (4.4)	18 (13.3)	19 (14.1)
10 – < 100	0	0	0	0	9 (6.7)	12 (8.9)	21 (15.6)	40 (29.6)
100 – < 10^3	0	0	2 (1.5)	1 (0.7)	0	10 (7.4)	13 (9.6)	53 (39.3)
10^3 – < 10^4	1 (0.7)	1 (0.7)	2 (1.5)	2 (1.5)	8 (5.9)	6 (4.4)	20 (14.8)	73 (54.1)
10^4 – < 10^5	0	0	1 (0.7)	5 (3.7)	3 (2.2)	10 (7.4)	19 (14.1)	92 (68.1)
10^5 – < 10^6	0	2 (1.5)	1 (0.7)	3 (2.2)	10 (7.4)	5 (3.7)	21 (15.6)	113 (83.7)
$\geq 10^6$	3 (2.2)	5 (3.7)	4 (3.0)	4 (3.0)	2 (1.5)	4 (3.0)	22 (16.3)	135 (100)
Total By Week	4 (3.0)	8 (5.9)	12 (8.9)	18 (13.3)	40 (29.6)	53 (39.3)		
Cumulative By Week	4 (3.0)	12 (8.9)	24 (17.8)	42 (31.1)	82 (60.7)	135 (100)		

The results of the analysis of the associations between the responses to treatment at week 48 and baseline covariates for HBeAg+ patients are summarized in table below. Since the lower limits of the 95% CIs of the odds ratios are smaller than 1 (0.26 - 0.96), there are no statistically significant associations:

Response to Treatment	Covariate	Category	N	Number of Patients With Response	Proportion (%) of Patients With Response	Unadjusted Odds Ratio (95% CI)
Antigen Loss	Race	Asian	84	23	27.4	0.75
		Other	45	15	33.3	(0.32, 1.80)
	Sex	Male	99	28	28.3	0.79
		Female	30	10	33.3	(0.31, 2.14)
	Age	≤30	60	15	25.0	0.67
		>30	69	23	33.3	(0.28, 1.54)
	Genotype	B,C	82	21	25.6	0.61
		Non-B,C	47	17	36.2	(0.26, 1.43)

Histological	Race	Asian	79	52	65.8	1.20
		Other	47	29	61.7	(0.52, 2.69)
	Sex	Male	95	65	68.4	2.03
		Female	31	16	51.6	(0.81, 5.02)
	Age	$\leq$30	57	39	68.4	1.39
		>30	69	42	60.9	(0.63, 3.13)
	Genotype	B,C	77	52	67.5	1.43
		Non-B,C	49	29	59.2	(0.64, 3.21)
Biochemical	Race	Asian	84	52	61.9	2.09
		Other	48	21	43.8	(0.96, 4.58)
	Sex	Male	100	58	58.0	1.57
		Female	32	15	46.9	(0.65, 3.78)
	Age	$\leq$30	61	39	63.9	1.93
		>30	71	34	47.9	(0.91, 4.13)
	Genotype	B,C	83	51	61.4	1.96
		Non-B,C	49	22	44.9	(0.90, 4.26)

Further data analysis of the HPS/CTM HBV Test results in HBeAg+ population was performed using two different definitions of the early virological response to treatment: (1) HBV viral load < 2000 IU/mL (or approximately 104 cp/mL), (2) a decrease in serum HBV DNA from an initial Screening viral load value by $\geq$ 2 $\log_{10}$. The statistical significance of the associations of the Race, Sex, Age and Genotype covariates with the viral response was studied by calculating odds ratios plus their exact 95% confidence intervals for both definitions of the viral response and summarized in two tables below. The logistic regression analysis of viral response as a function of the covariates showed no statistical significance of such associations for either definition of the viral response. Table below lists odds ratios for the association between viral response (< 2000 IU/mL) and covariates, by week, for an HBeAg+ population:

Covariate	Category	Week	N	Number With $\geq$2-$\log_{10}$ Decrease	Proportion (%) With $\geq$2-$\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	85	46	54.1	2.43 (1.10, 5.45)
	Other		49	16	32.7	
	Asian	8	84	55	65.5	1.10 (0.49, 2.44)
	Other		49	31	63.3	
	Asian	16	83	66	79.5	1.02 (0.38, 2.65)
	Other		48	38	79.2	
	Asian	28	84	68	81.0	1.09 (0.40, 2.85)
	Other		49	39	79.6	

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Covariate	Category	Week	N	Number With ≥2-$\log_{10}$ Decrease	Proportion (%) With ≥2-$\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
	Asian	44	82	66	80.5	1.11 (0.41, 2.93)
	Other		47	37	78.7	
	Asian	48	84	65	77.4	1.51 (0.63, 3.58)
	Other		49	34	69.4	
Sex	Male	4	101	44	43.6	0.64 (0.27, 1.53)
	Female		33	18	54.5	
	Male	8	100	62	62.0	0.61 (0.23, 1.55)
	Female		33	24	72.7	
	Male	16	99	77	77.8	0.65 (0.17, 2.00)
	Female		32	27	84.4	
	Male	28	100	80	80.0	0.89 (0.26, 2.62)
	Female		33	27	81.8	
	Male	44	97	77	79.4	0.89 (0.26, 2.63)
	Female		32	26	81.3	
	Male	48	100	74	74.0	0.91 (0.32, 2.42)
	Female		33	25	75.8	

Covariate	Category	Week	N	Number With ≥2-$\log_{10}$ Decrease	Proportion (%) With ≥2-$\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
Age	≤30	4	63	34	54.0	1.80 (0.86, 3.79)
	>30		71	28	39.4	
	≤30	8	62	42	67.7	1.29 (0.59, 2.82)
	>30		71	44	62.0	
	≤30	16	61	48	78.7	0.92 (0.36, 2.37)
	>30		70	56	80.0	
	≤30	28	62	50	80.6	1.02 (0.40, 2.67)
	>30		71	57	80.3	
	≤30	44	60	48	80.0	1.02 (0.39, 2.67)
	>30		69	55	79.7	
	≤30	48	62	48	77.4	1.34 (0.57, 3.22)
	>30		71	51	71.8	
Genotype	B,C	4	83	44	53.0	2.07 (0.95, 4.54)
	Non-B,C		51	18	35.3	
	B,C	8	82	53	64.6	1.00 (0.45, 2.20)
	Non-B,C		51	33	64.7	
	B,C	16	81	65	80.2	1.15 (0.43, 2.94)
	Non-B,C		50	39	78.0	
	B,C	28	82	67	81.7	1.23 (0.46, 3.18)
	Non-B,C		51	40	78.4	
	B,C	44	80	65	81.3	1.25 (0.47, 3.27)
	Non-B,C		49	38	77.6	
	B,C	48	82	64	78.0	1.63 (0.68, 3.85)
	Non-B,C		51	35	68.6	

Odds ratios for association between viral response (≥ 2 $\log_{10}$ decrease from initial screening result) and covariates, by week, for HBeAg+ population:

Covariate	Category	Week	N	Number With ≥2-$\log_{10}$ Decrease	Proportion (%) With ≥2-$\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	85	46	54.1	2.43 (1.10, 5.45)
	Other		49	16	32.7	
	Asian	8	84	55	65.5	1.10 (0.49, 2.44)
	Other		49	31	63.3	
	Asian	16	83	66	79.5	1.02 (0.38, 2.65)
	Other		48	38	79.2	

Covariate	Category	Week	N	Number With ≥2-$\log_{10}$ Decrease	Proportion (%) With ≥2-$\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
	Asian	28	84	68	81.0	1.09 (0.40, 2.85)
	Other		49	39	79.6	
	Asian	44	82	66	80.5	1.11 (0.41, 2.93)
	Other		47	37	78.7	
	Asian	48	84	65	77.4	1.51 (0.63, 3.58)
	Other		49	34	69.4	
Sex	Male	4	101	44	43.6	0.64 (0.27, 1.53)
	Female		33	18	54.5	
	Male	8	100	62	62.0	0.61 (0.23, 1.55)
	Female		33	24	72.7	
	Male	16	99	77	77.8	0.65 (0.17, 2.00)
	Female		32	27	84.4	
	Male	28	100	80	80.0	0.89 (0.26, 2.62)
	Female		33	27	81.8	
	Male	44	97	77	79.4	0.89 (0.26, 2.63)
	Female		32	26	81.3	
	Male	48	100	74	74.0	0.91 (0.32, 2.42)
	Female		33	25	75.8	

Covariate	Category	Week	N	Number With $\geq 2\text{-}\log_{10}$ Decrease	Proportion (%) With $\geq 2\text{-}\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
Age	≤30	4	63	34	54.0	1.80 (0.86, 3.79)
	>30		71	28	39.4	
	≤30	8	62	42	67.7	1.29 (0.59, 2.82)
	>30		71	44	62.0	
	≤30	16	61	48	78.7	0.92 (0.36, 2.37)
	>30		70	56	80.0	
	≤30	28	62	50	80.6	1.02 (0.40, 2.67)
	>30		71	57	80.3	
	≤30	44	60	48	80.0	1.02 (0.39, 2.67)
	>30		69	55	79.7	
	≤30	48	62	48	77.4	1.34 (0.57, 3.22)
	>30		71	51	71.8	
Genotype	B,C	4	83	44	53.0	2.07 (0.95, 4.54)
	Non-B,C		51	18	35.3	
	B,C	8	82	53	64.6	1.00 (0.45, 2.20)
	Non-B,C		51	33	64.7	
	B,C	16	81	65	80.2	1.15 (0.43, 2.94)
	Non-B,C		50	39	78.0	
	B,C	28	82	67	81.7	1.23 (0.46, 3.18)
	Non-B,C		51	40	78.4	
	B,C	44	80	65	81.3	1.25 (0.47, 3.27)
	Non-B,C		49	38	77.6	
	B,C	48	82	64	78.0	1.63 (0.68, 3.85)
	Non-B,C		51	35	68.6	

All lower limits of the 95% confidence intervals in two tables above are smaller than 1, except for Race at Week 28 (when response defined as < 2000 IU/mL) and Week 4 (when response defined as $\geq 2 \log_{10}$ decrease from initial screening result), which were both 1.1. This is in concordance with logistic regression analysis resulting in no statistically significant associations between the four covariates and viral load. Therefore, the virological responses at Weeks 4, 8, 16, 28 and 44 do not appear to be correlated with Race, Sex, Age, and HBV Genotype.

Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Odds Ratio (OR) Calculations for Week 48 Responses to Therapy with Respect to Viral Response at Various Times on Treatment in an HBeAg+ Population

For each patient, three responses - Histological, Biochemical and HBeAg Loss - were measured at various times on treatment. These three responses were defined as positive at week 48 as follows:

- Positive Histological response — improvement of histological status at Week 48 by at least 2 units of the Knodell necroinflammatory score without deterioration of the fibrosis score compared to the histological status at baseline

- Positive Biochemical response — normalization of ALT test result at Week 48 compared to the biochemical status at the baseline

- Positive HBeAg Loss response — HBeAg undetectable at week 48.

Statistical analysis was performed to evaluate whether there is an association between each of the above Week 48 positive responses and a positive viral load response (defined as HBV DNA < 2000 IU/mL or $\geq$ 2 log_{10} decrease from screening) at Weeks 4, 8, 16, 28 or 44 on treatment. Conversely, statistical analysis was performed to evaluate whether there is an association between each of the above Week 48 negative responses and a negative viral load response (defined as HBV DNA $\geq$ 2000 IU/mL or < 2 log_{10} decrease from screening) at Weeks 4, 8, 16, 28 or 44 of treatment.

Based on the information in two tables below, the viral response at Weeks 4, 8, 16, 28 and 44 is informative for predicting various responses at Week 48 (i.e., lower bound of the 95% Confidence Interval (CI) of OR exceeding 1). For the HBeAg+ population, the highest PPVs of the HPS/CTM HBV test take place with individual responses between 53.8% and 85.7%, while the highest NPVs of the HPS/CTM HBV test take place with the combination of all three responses being between 81.9% and 96.2%. This data indicates that between 81.9% and 96.2% of the HBeAg+ patients with negative early viral response ($\geq$2000 IU/mL) are not expected to achieve all three responses to treatment at week 48 of treatment.

Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Odds Ratio (OR) for Three Individual Responses at Week 48 of Treatment Predicted by an Early Viral Response (< 2000 IU/mL) in HBeAg+ Patients:

Week of Viral Response	Week 48 Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
4	Histology	36.4 (44/121)	66.7 (4/6)	1.14 (0.16, 13.10)
	Biochemical	45.6 (57/125)	71.4 (5/7)	2.10 (0.33, 22.67)
	HBeAg Loss	73.0 (89/122)	71.4 (5/7)	6.74 (1.02, 72.78)

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Week of Viral Response	Week 48 Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
8	Histology	36.6 (41/112)	71.4 (10/14)	1.44 (0.38, 6.69)
	Biochemical	47.0 (55/117)	73.3 (11/15)	2.44 (0.67, 11.05)
	HBeAg Loss	75.4 (86/114)	66.7 (10/15)	6.14 (1.71, 24.52)
16	Histology	39.4 (39/99)	76.0 (19/25)	2.06 (0.71, 6.83)
	Biochemical	51.0 (53/104)	80.8 (21/26)	4.36 (1.44, 15.79)
	HBeAg Loss	76.2 (77/101)	53.8 (14/26)	3.74 (1.38, 10.11)
28	Histology	41.6 (37/89)	78.4 (29/37)	2.58 (1.00, 7.24)
	Biochemical	54.7 (52/95)	81.1 (30/37)	5.18 (1.96, 15.21)
	HBeAg Loss	85.7 (78/91)	65.8 (25/38)	11.54 (4.35, 31.03)
44	Histology	43.8 (32/73)	75.5 (37/49)	2.41 (1.02, 5.88)
	Biochemical	62.8 (49/78)	85.7 (42/49)	10.14 (3.79, 29.75)
	HBeAg Loss	93.2 (69/74)	66.0 (33/50)	26.79 (8.41, 97.69)

[1]*The denominator is the number of patients predicted not to have an individual response to treatment at week 48 based on the lack of an early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of an early viral response of < 2000 IU/mL.*

[2] *The denominator is the number of patients predicted to have an individual week 48 response to treatment based on an early viral response of < 2000 IU/mL; the numerator is the number of patients who had a respective week 48 response to treatment among the patients with early viral response of <2000 IU/mL.*

PPV, NPV and OR for a Combination of All Three Responses at Week 48 on of Treatment Predicted by an Early Viral Response (< 2000 IU/mL) for an HBeAg+ Population:

Week of Viral Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
4	81.9 (104/127)	16.7 (1/6)	0.90 (0.02, 8.66)
8	83.9 (99/118)	35.7 (5/14)	2.89 (0.68, 10.85)
16	85.7 (90/105)	36.0 (9/25)	3.38 (1.09, 9.91)
28	91.6 (87/95)	43.2 (16/37)	8.29 (2.84, 25.10)

Week of Viral Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
44	96.2 (76/79)	42.9 (21/49)	19.00 (4.98, 104.34)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response of < 2000 IU/mL.*

[2] *The denominator is the number of patients predicted to have week 48 response to treatment based on early viral response of < 2000 IU/mL; the numerator is the number of patients who had all three responses to treatment at week 48 among the patients with early viral response of < 2000 IU/mL.*

With the viral response defined as $\geq 2 \log_{10}$ decrease in value from the Screening viral load result, for the HBeAg+ population, the PPVs of HPS/CTM HBV Test are larger for the individual responses, 36.3% to 70.5%, while the NPVs are the largest for the combination of all three responses, ranging from 87.5% to 96.3%, as shown in two tables below.

PPV, NPV and OR for Three Individual Responses at Week 48 on Treatment Predicted by Early Viral Response ($\geq 2 \log_{10}$ Decrease from Screening) in HBeAg+ Patients:

Week of Viral Response	Week 48 Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
4	Histology	43.1 (28/65)	70.5 (43/61)	1.81 (0.81, 4.05)
	Biochemical	58.6 (41/70)	70.5 (43/61)	3.38 (1.54, 7.48)
	HBeAg Loss	82.4 (56/68)	43.3 (26/60)	3.57 (1.49, 8.77)
8	Histology	47.6 (20/42)	69.9 (58/83)	2.11 (0.91, 4.85)
	Biochemical	60.9 (28/46)	63.5 (54/85)	2.71 (1.22, 6.08)
	HBeAg Loss	89.1 (41/46)	40.2 (33/82)	5.52 (1.88, 19.55)
16	Histology	43.5 (10/23)	65.0 (65/100)	1.43 (0.50, 3.94)
	Biochemical	63.0 (17/27)	59.8 (61/102)	2.53 (0.97, 6.80)
	HBeAg Loss	96.3 (26/27)	37.4 (37/99)	15.52 (2.31, 652.99)
28	Histology	47.6 (10/21)	66.3 (69/104)	1.79 (0.61, 5.14)

Week of Viral Response	Week 48 Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
	Biochemical	76.0 (19/25)	62.3 (66/106)	5.23 (1.79, 17.15)
	HBeAg Loss	96.2 (25/26)	36.3 (37/102)	14.23 (2.12, 599.78)
44	Histology	45.5 (10/22)	65.7 (65/99)	1.59 (0.55, 4.48)
	Biochemical	76.9 (20/26)	64.0 (64/100)	5.93 (2.03, 19.45)
	HBeAg Loss	96.0 (24/25)	37.8 (37/98)	14.56 (2.15, 614.51)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease); the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 48 response to treatment based on early viral response ($\geq 2 \log_{10}$ IU/mL decreas); the numerator is the number   of patients who had respective week 48 response to treatment among the patients with early viral response ($\geq 2 \log_{10}$ IU/mL decrease).*

PPV, NPV and OR for a Combination of All Three Responses at Week 48 on Treatment Predicted by an Early Viral Response ($\geq 2 \log_{10}$ Decrease from Screening Viral Load) for HBeAg+ population:

Week of Viral Response	NPV % (Proportion[1])	PPV % (Proportion [2])	OR (95% CI)
4	87.5 (63/72)	25.0 (15/60)	2.33 (0.86, 6.58)
8	93.6 (44/47)	25.0 (21/84)	4.89 (1.32, 26.88)
16	96.3 (26/27)	22.5 (23/102)	7.57 (1.10, 323.62)
28	96.2 (25/26)	21.9 (23/105)	7.01 (1.02, 300.23)
44	96.2 (25/26)	22.8 (23/101)	7.37 (1.07, 315.62)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease); the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 48 response to treatment based on early viral response ($\geq 2 \log_{10}$ IU/mL decrease); the numerator is the number of patients who had all three week 48 responses to treatment among the patients with early viral response ($\geq 2 \log_{10}$ IU/mL decrease).*

HBeAg loss is the most favorable endpoint in HBeAg+ patients receiving anti-viral therapy, since this allows possible safe discontinuation of therapy with a low likelihood of off-treatment relapse. The results demonstrate that HBV DNA measurements of less than 2000 IU/mL or more than a 2 $\log_{10}$ decrease from a Screening HBV viral load at Weeks 4, 8, 16, 28 and 44 are associated with HBeAg loss at Week 48. A NPV of not achieving a 2 $\log_{10}$ decrease from a

Screening HBV viral load result at an early stage of treatment appears medically associated with not achieving HBeAg loss at Week 48.
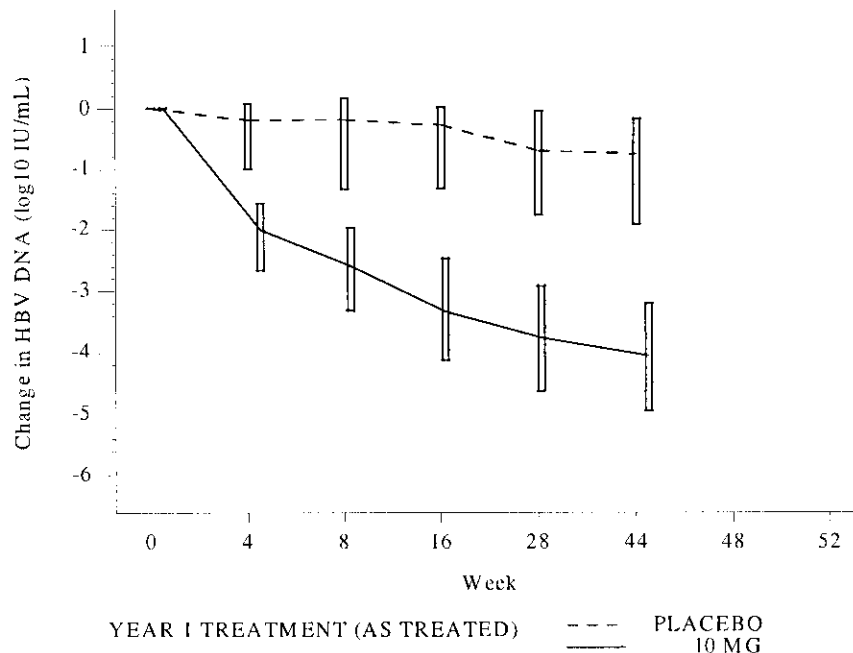
HBeAg- Patients

Table below demonstrates the efficacy, based on HBV viral load testing, of treating HBeAg- patients with 10 mg Adefovir Dipivoxil compared to placebo. At week 44 on treatment, 46.2% of HBeAg- patients on medication vs. 0% on placebo had achieved very low viral loads below 100 IU/mL. Furthermore, 74% of patients on medication vs. 2 % on placebo had achieved viral loads measuring below 400 IU/mL. Only 1.1% of patients on medication vs. 34% on placebo had a viral load exceeding 10^6 IU/mL.

Distribution of the HBV Viral Load at Week 44 on Treatment for HBeAg- Patients:

Viral Load (IU/mL)	Adefovir Dipivoxil			Placebo		
	N	%	Cumul %	N	%	Cumul %
TND*	5	5.4	5.4	0	0.0	0.0
< 10	22	23.7	29.0	0	0.0	0.0
10 - < 100	16	17.2	46.2	0	0.0	0.0
100 - <400	26	28.0	74.2	1	2.0	2.0
400 - <10^3	6	6.5	80.6	2	4.0	6.0
10^3 - <10^4	6	6.5	87.1	8	16.0	22.0
10^4 - <10^5	5	5.4	92.5	12	24.0	46.0
10^5 - <10^6	6	6.5	98.9	10	20.0	66.0
10^6 - <10^8	1	1.1	100.0	17	34.0	100.0
Total	93			50		

TND=HBV DNA not detected.

The graph in the figure below shows the curves drawn through the median viral loads at various times on treatment, along with the inter-quartile ranges, for the change in HBV DNA from Screening, in HBeAg- patients both on medication (lower curve) and on placebo (upper curve).

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YEAR 1 TREATMENT (AS TREATED)   – – –   PLACEBO
——   10 MG

Another way of assessing the effect of antiviral treatment for HBeAg- patients is looking for the HBV viral load increase of more than 1 $\log_{10}$ after reaching a nadir. The following table summarizes distribution by week on treatment and the viral load at which nadir was reached for the 90 HBeAg- patients on adefovir dipivoxil treatment:

Nadir Viral Load (IU/mL)	Number (%) of Patients With the Nadir Viral Load Achieved by Week					Total By Viral Load	Cumulative By Viral Load
	4	8	16	28	44		
HBV DNA not detected	1 (1.1)	2 (2.2)	4 (4.3)	1 (1.1)	3 (3.2)	11 (11.8)	11 (11.8)
< 10	0	0	3 (3.2)	3 (3.2)	12 (12.9)	18 (19.4)	29 (31.2)
10 – < 100	0	1 (1.1)	0	11 (11.8)	12 (12.9)	24 (25.8)	53 (57.00)
$100 - < 10^3$	0	0	0	8 (8.6)	15 (16.1)	23 (24.7)	76 (81.7)
$10^3 - < 10^4$	1 (1.1)	0	0	0	6 (6.5)	7 (7.5)	83 (89.2)
$10^4 - < 10^5$	0	2 (2.2)	1 (1.1)	0	2 (2.2)	5 (5.4)	88 (94.6)
$10^5 - < 10^6$	0	0	1 (1.1)	0	3 (3.2)	4 (4.3)	92 (98.9)
$\geq 10^6$	0	0	0	1 (1.1)	0	1 (1.1)	93 (100)
Total By Week	2 (2.2)	5 (5.4)	9 (9.7)	24 (25.8)	53 (57.00)		
Cumulative By Week	2 (2.2)	7 (7.6)	16 (17.3)	40 (43.1)	93 (100)		

In this study, 43% (40/90) of the patients reached a nadir in viral load by week 28 on treatment. Out of those, seven patients had more than 1 $\log_{10}$ increase in viral load by week 44 after achieving the nadir, or 7.5% (7/93) of the total number of patients on treatment and 17.5% (7/40) of the patients who achieved a nadir.

Table below summarizes the results of analysis of association of the responses to treatment at week 44 and baseline covariates for HBeAg- patients. With all lower

limits of the 95% CIs being smaller than 1 (i.e., between 0.00 and 0.52), there are no statistically significant associations:

Response to Treatment	Covariate	Category	N	Number of Patients With Response	Proportion (%) of Patients With Response	Unadjusted Odds Ratio (95% CI)
Histological	Race	Asian	28	16	57.1	0.47 (0.16, 1.35)
		Other	58	43	74.1	
	Sex	Male	73	52	71.2	2.12 (0.52, 8.31)
		Female	13	7	53.8	
	Age	≤30	8	6	75.0	1.42 (0.23, 15.24)
		>30	78	53	67.9	
	Genotype	B,C	28	16	57.1	0.47 (0.16, 1.35)
		Non-B,C	58	43	74.1	
Biochemical	Race	Asian	30	18	60.0	0.75 (0.28, 2.05)
		Other	63	42	66.7	
	Sex	Male	79	47	59.5	0.11 (0.00, 0.84)
		Female	14	13	92.9	
	Age	≤30	8	5	62.5	0.91 (0.16, 6.26)
		>30	85	55	64.7	
	Genotype	B,C	30	18	60.0	0.75 (0.28, 2.05)
		Non-B,C	63	42	66.7	

Additional data analysis of the HBeAg- population to demonstrate clinical performance of the HPS/CTM HBV Test was done using two different definitions of the early virological response to treatment: (1) HBV viral load < 2000 IU/mL (or approximately 10^4 cp/mL), (2) a decrease in serum HBV DNA from initial Screening viral load value by ≥ 2 $\log_{10}$.

The statistical significance of the associations of the Race, Sex, Age and Genotype covariates with the viral response was studied. The logistic regression analysis of viral response as a function of the covariates showed no statistical significance of such associations for either definition of the viral response. In addition, odds ratios plus their exact 95% confidence intervals were calculated for both definitions of the viral response.

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

Covariate	Category	Week	N	Number Below 2000 IU/mL	Proportion (%) Below 2000 IU/mL	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	30	11	36.7	1.70 (0.59, 4.75)
	Other		63	16	25.4	
	Asian	8	28	16	57.1	1.78 (0.66, 4.84)
	Other		63	27	42.9	
	Asian	16	30	22	73.3	1.69 (0.60, 5.10)
	Other		63	39	61.9	
	Asian	28	29	22	75.9	1.26 (0.42, 4.10)
	Other		63	45	71.4	
	Asian	44	30	26	86.7	1.69 (0.46, 7.79)
	Other		63	50	79.4	
Sex	Male	4	79	23	29.1	1.03 (0.26, 4.95)
	Female		14	4	28.6	
	Male	8	78	38	48.7	1.52 (0.40, 6.43)
	Female		13	5	38.5	
	Male	16	79	51	64.6	0.73 (0.15, 2.84)
	Female		14	10	71.4	
	Male	28	78	57	73.1	1.09 (0.22, 4.30)
	Female		14	10	71.4	
	Male	44	79	64	81.0	0.71 (0.07, 3.76)
	Female		14	12	85.7	
Age	≤30	4	8	2	25.0	0.80 (0.07, 4.89)
	>30		85	25	29.4	
	≤30	8	8	3	37.5	0.65 (0.09, 3.58)
	>30		83	40	48.2	
	≤30	16	8	4	50.0	0.49 (0.09, 2.87)
	>30		85	57	67.1	
	≤30	28	8	5	62.5	0.59 (0.11, 4.15)
	>30		84	62	73.8	
	≤30	44	8	6	75.0	0.64 (0.10, 7.15)
	>30		85	70	82.4	
Genotype	B,C	4	30	11	36.7	1.70 (0.59, 4.75)
	Non-B,C		63	16	25.4	
	B,C	8	28	16	57.1	1.78 (0.66, 4.84)
	Non-B,C		63	27	42.9	
	B,C	16	30	22	73.3	1.69 (0.60, 5.10)
	Non-B,C		63	39	61.9	
	B,C	28	29	22	75.9	1.26 (0.42, 4.10)

Covariate	Category	Week	N	Number Below 2000 IU/mL	Proportion (%) Below 2000 IU/mL	Unadjusted Odds Ratio (95% CI)
	Non-B,C		63	45	71.4	
	B,C	44	30	26	86.7	1.69 (0.46, 7.79)
	Non-B,C		63	50	79.4	

Odds ratios for the association between viral response ($\geq 2\log_{10}$ decrease from initial screening result) and covariates, by week, for an HBeAg- population:

Covariate	Category	Week	N	Number With ≥ 2 $\log_{10}$ Decrease	Proportion (%) With ≥ 2 $\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
Race	Asian	4	30	15	50.0	1.07 (0.41, 2.79)
	Other		62	30	48.4	
	Asian	8	28	20	71.4	0.80 (0.27, 2.54)
	Other		62	47	75.8	
	Asian	16	30	26	86.7	1.40 (0.37, 6.61)
	Other		62	51	82.3	
	Asian	28	29	27	93.1	2.60 (0.50, 25.81)
	Other		62	52	83.9	
	Asian	44	30	28	93.3	2.38 (0.44, 23.90)
	Other		62	53	85.5	
Sex	Male	4	78	37	47.4	0.68 (0.18, 2.47)
	Female		14	8	57.1	
	Male	8	77	56	72.7	0.48 (0.05, 2.53)
	Female		13	11	84.6	
	Male	16	78	64	82.1	0.35 (0.01, 2.76)
	Female		14	13	92.9	
	Male	28	77	65	84.4	0.00 (0.00, 1.49)
	Female		14	14	100.0	
	Male	44	78	67	85.9	0.00 (0.00, 1.69)
	Female		14	14	100.0	
Age	≤ 30	4	8	2	25.0	0.32 (0.03, 1.93)
	>30		84	43	51.2	
	≤ 30	8	8	6	75.0	1.03 (0.17, 11.22)
	>30		82	61	74.4	
	≤ 30	16	8	6	75.0	0.55 (0.09, 6.19)
	>30		84	71	84.5	

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Covariate	Category	Week	N	Number With ≥2 $\log_{10}$ Decrease	Proportion (%) With ≥2 $\log_{10}$ Decrease	Unadjusted Odds Ratio (95% CI)
	≤30	28	8	6	75.0	0.41 (0.06, 4.76)
	>30		83	73	88.0	
	≤30	44	8	6	75.0	0.36 (0.05, 4.23)
	>30		84	75	89.3	

Covariate	Category	Week	N	Number With ≥2 log_{10} Decrease	Proportion (%) With ≥2 log_{10} Decrease	Unadjusted Odds Ratio (95% CI)
Genotype	B,C	4	30	15	50.0	1.07 (0.41, 2.79)
	Non-B,C		62	30	48.4	
	B,C	8	28	20	71.4	0.80 (0.27, 2.54)
	Non-B,C		62	47	75.8	
	B,C	16	30	26	86.7	1.40 (0.37, 6.61)
	Non-B,C		62	51	82.3	
	B,C	28	29	27	93.1	2.60 (0.50, 25.81)
	Non-B,C		62	52	83.9	
	B,C	44	30	28	93.3	2.38 (0.44, 23.90)
	Non-B,C		62	53	85.5	

All lower limits of the 95% confidence intervals in the above two tables are smaller than 1, indicating no statistically significant associations between the four covariates and the viral load, and, therefore, the virological responses at Weeks 4, 8, 16, 28 and 44 do not appear to be correlated with race, sex, age, and HBV genotype.

Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Odds Ratio (OR) Calculations for Week 44 Responses to Therapy with Respect to Viral Response (HBV viral load using the HPS/CTM HBV Test) at Various Times on Treatment for HBeAg- Patients

For each patient, two responses - Histological and Biochemical - were measured at various times on treatment. The two responses were defined as positive at week 44:

- Positive Histological response — improvement of histological status at Week 44 by at least 2 units of the Knodell necroinflammatory score without deterioration of the fibrosis score compare to the histological status at baseline

- Positive Biochemical response — normalization of ALT test result at Week 44 compared to the biochemical status at the baseline.

Statistical analysis was performed to evaluate whether there is an association between each of the above Week 44 positive responses and a positive viral load response (defined as HBV DNA < 2000 IU/mL or ≥ 2 log_{10} decrease from screening) at Weeks 4, 8, 16 or 28 on treatment. Conversely, statistical analysis was performed to evaluate whether there is an association between each of the above Week 44 negative responses and a negative viral load response (defined as HBV DNA ≥ 2000 IU/mL or < 2 log_{10} decrease from screening) at weeks 4, 8, 16 or 28 of treatment.

The viral response (HBV DNA < 2000 IU/mL) at Weeks 4, 8, 16 and 28 is not informative for predicting various Week 44 responses for HBeAg- patients with the data available (the lower limits of the 95% CIs are between 0.11 and 0.89, below 1, for various responses at various times on treatment). PPV, NPV and OR for two individual responses at week 44 of treatment predicted by early viral response (< 2000 IU/mL) in HBeAg- patients are presented here:

Week of Viral Response	Week 44 on Treatment Response	NPV (Proportion[1])	PPV (Proportion[2])	OR (95% CI)
4	Histology	25.0 (16/64)	50.0 (11/22)	0.33 (0.11, 1.04)
	Biochemical	31.8 (21/66)	55.6 (15/27)	0.58 (0.21, 1.63)
8	Histology	23.4 (11/47)	57.9 (22/38)	0.42 (0.15, 1.18)
	Biochemical	35.4 (17/48)	65.1 (28/43)	1.02 (0.40, 2.66)
16	Histology	30.0 (9/30)	67.9 (38/56)	0.90 (0.30, 2.59)
	Biochemical	43.8 (14/32)	68.9 (42/61)	1.72 (0.64, 4.54)
28	Histology	33.3 (8/24)	68.9 (42/61)	1.11 (0.35, 3.33)
	Biochemical	52.0 (13/25)	70.1 (47/67)	2.55 (0.89, 7.26)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 44 based on the lack of early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 48 response to treatment among the patients with the lack of early viral response of < 2000 IU/mL.*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response of < 2000 IU/mL; the numerator is the number of patients who had respective week 48 response to treatment among the patients with early viral response of <2000 IU/mL.*

PPV, NPV and OR for both biochemical and histological responses at week 44 of treatment predicted by early viral response (< 2000 IU/mL) in HBeAg- patients:

Week of Viral Response	NPV (Proportion[1])	PPV (Proportion[2])	OR (95% CI)
4	49.2 (32/65)	29.2 (7/24)	0.40 (0.12, 1.19)
8	50.0 (24/48)	40.0 (16/40)	0.67 (0.26, 1.69)
16	62.5 (20/32)	49.1 (28/57)	1.61 (0.61, 4.32)
28	72.0 (18/25)	50.8 (32/63)	2.65 (0.89, 8.53)

[1]*The denominator is the number of patients predicted not to have the response to treatment at week 48 based on the lack of early viral response of < 2000 IU/mL; the numerator is the number of patients who had no respective week 44 response (no histological or biochemical response) to treatment at week 44 among the patients with the lack of early viral response of < 2000 IU/mL;*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response of < 2000 IU/mL; the numerator is the number of patients who had both (histological and biochemical) responses to treatment at week 44 among the patients with early viral response of <2000 IU/mL.*

Viral response at weeks 8, 16 and 28, when defined as $\geq 2 \log_{10}$ decrease in value from the screening viral load result, is informative (i.e., lower limits of the 95% CIs exceed 1) for prediction of biochemical response at week 44 on treatment; it is also informative at weeks 16 and 28 for prediction of having both responses (histological and biochemical) at week 44 on treatment for HBeAg- patients. The NPV for the biochemical response is from 73.3% at week 16 to 83.3% at week 28. The PPV is approximately 71% at both weeks 16 and 28.

PPV, NPV and OR for two individual responses at week 44 of treatment predicted by early viral response ($\geq 2 \log_{10}$ decrease from screening viral load) in HBeAg- patients:

Week of Viral Response	Week 44 Response	NPV (Proportion[1])	PPV (Proportion[2])	OR (95% CI)
4	Histology	28.3 (13/46)	64.1 (25/39)	0.70 (0.25, 1.94)
	Biochemical	44.7 (21/47)	73.3 (33/45)	2.22 (0.85, 5.90)
8	Histology	31.8 (7/22)	67.7 (42/62)	0.98 (0.29, 3.07)
	Biochemical	56.5 (13/23)	71.6 (48/67)	3.28 (1.10, 9.86)
16	Histology	30.8 (4/13)	68.1 (49/72)	0.95 (0.19, 3.85)
	Biochemical	73.3 (11/15)	71.4 (55/77)	6.88 (1.76, 32.07)
28	Histology	18.2 (2/11)	65.8 (48/73)	0.43 (0.04, 2.31)
	Biochemical	83.3 (10/12)	70.9 (56/79)	12.17 (2.27, 119.14)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 44 based on the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease); the numerator is the number of patients who had no respective week 44 response to treatment among the patients with the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response ($\geq 2 \log_{10}$ IU/mL decrease); the numerator is the number of patients who had respective week 44 response to treatment among the patients with early viral response ($\geq 2 \log_{10}$ IU/mL decrease).*

PPV, NPV and OR for combination of both biochemical and histological responses at week 44 of treatment predicted by early viral response ($\geq 2 \log_{10}$ decrease from screening viral load) in HBeAg- patients:

Week of Viral Response	NPV (Proportion[1])	PPV (Proportion[2])	Odds Ratio (95% CI)
4	59.6 (28/47)	48.8 (20/41)	1.40 (0.55, 3.56)
8	73.9 (17/23)	51.6 (33/64)	3.02 (0.96, 10.47)
16	86.7 (13/15)	50.7 (37/73)	6.68 (1.34, 63.95)
28	91.7 (11/12)	49.3 (37/75)	10.71 (1.40, 473.19)

[1] *The denominator is the number of patients predicted not to have the response to treatment at week 44 based on the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease); the numerator is the number of patients who had no respective week 44 response (no histological or biochemical response) to treatment among the patients with the lack of early viral response ($\geq 2 \log_{10}$ IU/mL decrease).*

[2] *The denominator is the number of patients predicted to have week 44 response to treatment based on the early viral response($\geq$ 2 log$_{10}$ IU/mL decrease); the numerator is the number of patients who had both two responses to treatment at week 44 among the patients with early viral response ($\geq$ 2 log$_{10}$ IU/mL decrease).*

XI.   SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Literature Review of HBV DNA in Clinical Practice

Guidelines published in the medical literature support the importance of measuring HBV levels at baseline prior to treatment, at intervals during treatment to monitor antiviral response as well as at intervals on therapy to survey for the development of drug resistance[1,2]. The American Association for the Study of Liver Disease (AASLD) Practice Guidelines from 2007 make specific recommendations for initiating HBV therapy based on the level of pre-treatment serum ALT, HBeAg status and level of HBV DNA. In these guidelines, HBV DNA values of >20,000 IU/mL and >2,000 IU/mL for chronic hepatitis B (CHB) and cirrhosis respectively were selected as the level at which to initiate treatment[1]. The threshold level of HBV DNA for determination of candidacy differs in other published guidelines ($\geq$20,000 IU/mL or ~10^5 copies/mL for patients with HBeAg-positive CHB; $\geq$2,000 IU/mL or ~10^4 copies/mL for patients with HBeAg-negative CHB; and $\geq$200 IU/mL or ~10^3 copies/mL for patients with decompensated cirrhosis)[2].

Treatment goals have evolved over time although goals are consistent across recently published guidelines[1,2,3]. Hoofnagle et al state that the major goals of therapy are not immediate amelioration of symptoms (since CHB is typically silent), but rather long-term prevention of progression, development of cirrhosis and hepatocellular carcinoma (HCC)[3]. Improvement in histology, a surrogate for improvement in natural history, has been a primary efficacy end-point in the design of clinical trials that led to FDA-approval of lamivudine and adefovir[4,5,6]. Other end-points include HBeAg seroconversion in HBeAg positive patients,

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

[2] 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.

[3] Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok ASF. Management of hepatitis B: summary of a clinical research workshop. Hepatology 2007;45:1056-1075.

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normalization of ALT and undetectable or reduced HBV DNA. Loss of HBsAg is the most desirable end-point of treatment, but this is rarely achieved with nucleoside analogs[3]. The AASLD[1] guidelines state that the aims of treatment of chronic hepatitis B are to achieve sustained suppression of HBV replication and remission of liver disease. Similarly, the guideline of Keeffe et al[2] states that the goal of therapy for CHB should be to significantly suppress HBV replication and prevent clinical progression of liver disease. Hoofnagle et al state that virological responses based upon testing for levels of HBV DNA in serum are probably the most appropriate criteria for assessing outcome of antiviral therapy[3]. Hence, a goal of treatment is to reduce and maintain serum HBV DNA at the lowest possible levels (durable HBV DNA suppression). Viral suppression was also the primary goal of therapy in the Asian Pacific Association for the Study of the Liver APASL guidelines[7]. Virological response can be defined as the lack of detectable HBV DNA in serum using an assay that is sensitive to 20-100 IU/mL (~100-500 copies/mL)[3]. Viral suppression should lead to other goals of therapy, including histological improvement, HBeAg loss and/or HBeAg seroconversion, and ALT normalization. In patients who are HBeAg-positive before therapy, an additional goal of treatment is loss of HBeAg with seroconversion to anti-HBe. The latter is preferable because attainment of complete HBeAg seroconversion indicates that antiviral therapy may be discontinued, and the likelihood is high that the benefit will persist off-therapy. Loss of HBsAg is rarely achieved with short-term antiviral therapy and therefore not a common goal for antiviral trials.

To measure durable viral suppression, Keeffe et al, recommend that patients be monitored at least every six months while on therapy with either entecavir or adefovir, and more frequently with lamivudine to identify resistance[2]. Patients should be treated after HBeAg seroconversion as long as HBV DNA levels are decreasing until there are undetectable HBV DNA levels by PCR[2]. Treatment should then be continued for an additional six to twelve months. In patients who have demonstrated HBeAg seroconversion but in whom HBV DNA levels are detectable and stable, treatment should be continued for six months; seroconversion should be documented again, then consideration given to stopping treatment (in noncirrhotic patients). It should be noted that these recommendations reflect the opinions of the expert panel that convened this treatment algorithm, and although not always based on randomized controlled trials, are aimed at ultimately reducing the severity of liver disease.

Overall, the goal of therapy for patients with chronic HBV infection is to prevent progression of liver disease to cirrhosis and HCC. Because HBV replication is implicated in these outcomes, the primary aim of therapy is durable suppression of serum HBV DNA[1]. This goal requires sensitive HBV DNA tests with a broad dynamic range. Quantitative "real-time" PCR enables establishing of a patient's baseline HBV DNA prior to treatment, and monitoring response to antiviral

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[7] Liaw YF, Leung N, Guan R, Lau GKK, Merican I, McCaughan G, Gane E, Kao JH and Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2005 update. Liver Int 2005;25:472-489.

therapy. The threshold level of HBV DNA to initiate treatment differs in different populations, ranging from 2,000 IU/mL for patients with cirrhosis to 20,000 IU/mL for patients with chronic HBV disease. Therefore, HBV DNA testing is integral to many aspects of the management of patients with chronic HBV infection, and is critical for assessing the response to antiviral therapy, along with other laboratory and clinical considerations.

XII. PANEL RECOMMENDATIONS

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

Based on the results of the preclinical and clinical laboratory studies, the COBAS TaqMan HBV Test For Use With the High Pure System, 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 COBAS TaqMan HBV Test For Use With the High Pure System 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 COBAS TaqMan HBV Test For Use With the High Pure System 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 COBAS TaqMan HBV Test For Use With the High Pure System 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 COBAS TaqMan HBV Test For Use With the High Pure System 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 COBAS TaqMan HBV Test For Use With the High Pure System 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.

XIV. CDRH DECISION

CDRH issued an approval order on September 4, 2008. The final conditions of approval cited in the approval order are described below.

CONDITIONS OF APPROVAL:

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT.
Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e) or (f). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations that require a PMA supplement cannot be briefly summarized; therefore, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing

designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report (see below). FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

Alternate submissions permitted under 21 CFR 814.39(f) for manufacturing process changes include the use of a 30-day Notice. The manufacturer may distribute the device 30 days after the date on which the FDA receives the 30-day Notice, unless the FDA notifies the applicant within 30 days from receipt of the notice that the notice is not adequate.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

1.   Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

2.   Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

a.   unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

b.   reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

1.   A mix-up of the device or its labeling with another article.

2.   Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and:

a.   has not been addressed by the device's labeling; or

b.   has been addressed by the device's labeling but is occurring with unexpected severity or frequency.

3.   Any significant chemical, physical or other change or deterioration in the device, or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued

reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984. This regulation was replaced by the reporting requirements of the Safe Medical Devices Act of 1990 which became effective July 31, 1996 and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to the FDA whenever they receive or otherwise become aware of information, from any source, that reasonably suggests that a device marketed by the manufacturer or importer:

1.   May have caused or contributed to a death or serious injury; or

2.   Has malfunctioned and such device or similar device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for a PMA, the manufacturer shall submit the appropriate reports required by the MDR Regulation within the time frames as identified in 21 CFR 803.10(c) using FDA Form 3500A, i.e., 30 days after becoming aware of a reportable death, serious injury, or malfunction as described in 21 CFR 803.50 and 21 CFR 803.52 and 5 days after becoming aware that a reportable MDR event requires remedial action to prevent an unreasonable risk of substantial harm to the public health. The manufacturer is responsible for submitting a baseline report on FDA Form 3417 for a device when the device model is first reported under 21 CFR 803.50. This baseline report is to include the PMA reference number. Any written report and its envelope is to be specifically identified, e.g., "Manufacturer Report," "5-Day Report," "Baseline Report," etc.

Any written report is to be submitted to:

> Food and Drug Administration
> Center for Devices and Radiological Health
> Medical Device Reporting
> PO Box 3002
> Rockville, Maryland 20847-3002

Additional information on MDR is available at
http://www.fda.gov/cdrh/devadvice/351.html.

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The applicant's manufacturing facility was inspected on July 7-22, 2008, and found to be in compliance with the Quality Systems (QS) regulation (21 CFR 820).

XV.   APPROVAL SPECIFICATIONS

Directions for use: See the labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI.   REFERENCES

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

2. 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.

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