SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time PCR test

Device Trade Name: **cobas**® HBV

Device Procode: MKT

Applicant's Name and Address: Roche Molecular Systems, Inc.
4300 Hacienda Drive
Pleasanton, CA 94588-2722

Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P150014

Date of FDA Notice of Approval: October 14, 2015

II. INDICATIONS FOR USE

cobas® HBV is an in vitro nucleic acid amplification test for the quantitation of hepatitis B virus (HBV) DNA in human EDTA plasma or serum of HBV-infected individuals. This test is intended for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy. The test can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment. The results from **cobas**® HBV must be interpreted within the context of all relevant clinical and laboratory findings.

The **cobas**® HBV is not intended for use as a screening test for the presence of HBV in blood or blood products or as a diagnostic test to confirm the presence of HBV infection.

III. CONTRAINDICATIONS

There are no known contraindications for use for this test.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the **cobas**® HBV labeling.

V. DEVICE DESCRIPTION

A. cobas® HBV test Description

The **cobas**® HBV is a quantitative test performed on the **cobas**® 6800 System and cob **cobas**® as 8800 System. The **cobas**® HBV allows for the detection and quantitation of HBV DNA in EDTA plasma or serum of infected patients for use in clinical laboratories for routine clinical practice in the management of patients with HBV. A single probe is used to detect and quantify, but not distinguish genotypes A-H. The viral load is quantified against a non-HBV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample preparation. The DNA-QS also functions to monitor the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

The **cobas**® HBV test system consists of:

- the **cobas**® 6800/8800 Systems
- the **cobas**® HBV Assay Specific Analysis Package (ASAP) software
- **cobas**® HBV in cassettes
- **cobas**® HBV/HCV/HIV-1 Control Kit (HPC and LPC) in cassettes
- **cobas**® NHP Negative Control Kit in cassettes
- Specimen preparation reagents (**cobas**® omni Reagents)

The **cobas**® HBV is based on automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 Systems Software which assigns test results for all tests. Results can be reviewed directly on the system screen, exported, or printed as a report.

B. Principle of Procedure

1. Sample Preparation (Nucleic Acid Extraction and Purification)

   Nucleic acid from patient samples, external controls and added DNA-QS molecules are simultaneously extracted. Viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

2. Nucleic Acid Amplification

   Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from

highly conserved regions of the HBV genome. Selective amplification of DNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the HBV genome. A thermostable DNA polymerase enzyme is used for amplification.

The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55 °C.

3. Nucleic Acid Detection

   The **cobas**® HBV master mix contains detection probes which are specific for the HBV target sequences and the DNA-QS nucleic acid, respectively. The specific HBV and DNA-QS detection probes are each labeled with one of two unique fluorescent dyes which act as reporters. Each probe also has a second dye which acts as a quencher. The two reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified HBV target and the DNA-QS.

   When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probe is generated and the cumulative signal of the reporter dye increases concomitantly. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified HBV target and the DNA-QS are possible.

4. Controls

   - Normal Human Plasma Negative Control - Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods.
   - HBV/HCV/HIV-1 Low Positive Control - < 0.001% HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein armored, < 0.001% synthetic plasmid) HBV DNA encapsulated in Lambda bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma.
   - HBV/HCV/HIV-1 High Positive Control - < 0.001% synthetic (armored) HIV-1 Group M RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) HBV DNA encapsulated in Lambda

bacteriophage coat protein, < 0.001% synthetic (armored) HCV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma.

- Interpretation of Control Results: The **cobas**® 6800/8800 software determines whether control results are valid or invalid and therefore whether the run is valid or invalid.

C. Instrumentation and Software

The **cobas**® 6800/8800 platform is configured as two instrument versions: the **cobas**® 6800 System, and the **cobas**® 8800 System.

Each system is comprised of a **cobas**® 6800 or **cobas**® 8800 instrument, system software, Assay Specific Analysis Packages (ASAP), and a sample source unit, which can be connected to a conveyor system for automated transport of samples to and from the system. The test kits consist of assay-specific reagents and omni reagents (common reagents) which can be used with any of the **cobas**® 6800/8800 assays, and on either the **cobas**® 6800 or the **cobas**® 8800 instrument.

In addition, the omni reagents and consumables, such as such as the P-plates, racks, AD-plates, waste bags, pipette tips, and secondary tubes, can be used by any of the **cobas**® 6800/8800 System assays, and on either the **cobas**® 6800 or the **cobas**® 8800 instrument.

Either system can be interfaced to an uninterruptible power supply (UPS), a Laboratory Information System (LIS), or middleware, and/or office PCs for remote viewing and messaging functionalities.

D. Interpretation of Results

Table 1: Target Result Interpretation

Results	Interpretation
Target Not Detected	HBV DNA not detected. Report results as "HBV not detected."
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "HBV detected, less than (Titer Min)." Titer min = 10 IU/mL
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as "(Titer) of HBV detected".
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "HBV detected, greater than (Titer Max)." Titer max = 1.00E+09 IU/mL

[a] Sample result > Titer Max refers to HBV positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with HBV-negative EDTA plasma or serum, depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently three (3) FDA approved alternative in vitro nucleic acid amplification tests for the quantitation of HBV:

- COBAS TaqMan HBV Test for Use with the High Pure System (Roche)
- COBAS AmpliPrep/COBAS TaqMan HBV Test, v2 (Roche)
- Abbott RealTime HBV Test (Abbott)

Each alternative has its own advantages and disadvantages. The patient and physician should fully discuss alternatives to select the best method.

VII. MARKETING HISTORY

The test is currently available in the following countries listed in Table 2. To date, there have been no adverse incidents or potentially-critical complaints reported.

Table 2: Marketed Countries

Austria	Greece	Norway
Belgium	Hungary	Poland
Bulgaria	Iceland	Portugal
Croatia	Ireland	Romania
Cyprus	Italy	Slovakia
Czech Republic	Latvia	Slovenia
Denmark	Liechtenstein	Spain
Estonia	Lithuania	Sweden
Finland	Luxembourg	Switzerland
France	Malta	Turkey
Germany	Netherlands	United Kingdom

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects (e.g., complications) associated with the use of the device.

The primary adverse events from use of the device are caused by an inaccurate measurement result of HBV DNA viral load. Failure of the **cobas**® HBV to perform as indicated, or human error in the use of the test or the interpretation of the test result, may result in an incorrect test result that is too low or too high.

An erroneous low test result or a false negative result may lead to inappropriate patient management decisions, a premature discontinuation of antiviral therapy, or may instill a false sense of security in a patient or clinician.

An erroneous high test result, or a false positive result, may contribute to a change in therapy, unnecessary treatment, prolonged duration of therapy, or create anxiety in the patient.

Inaccurate results would be most significant between 2,000 – 20,000 IU/mL, where treatment may be considered under different scenarios; however, serial measurements are common, and clinicians are likely to repeat measurements when there is consideration of treatment but a deciding factor is a low HBV value. The risks from 'overtreatment' (i.e., treatment initiated due to an unexpectedly high HBV viral load) are relatively small absent the cost of medication.

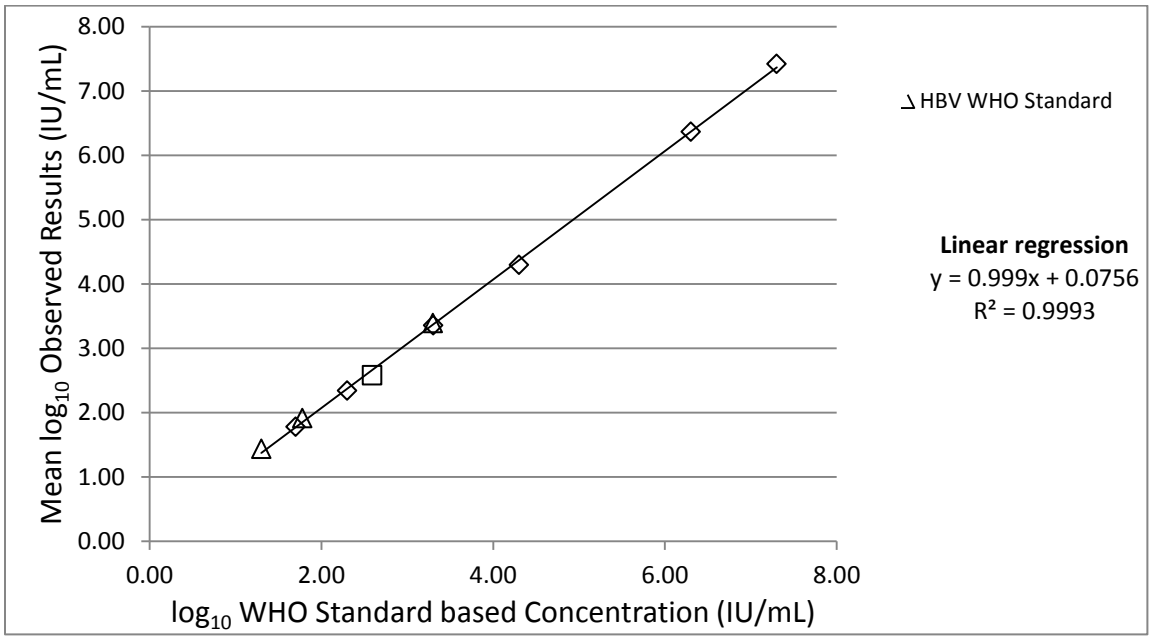
IX. SUMMARY OF PRE-CLINICAL STUDIES

A. Traceability to the WHO International Standard

Several standards and controls have been used during development of this test to provide traceability to the WHO standard (2nd WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Amplification Techniques (NIBSC 97/750). The standards used during development of the test include the HBV WHO Standard, the RMS HBV Secondary Standard, and the RMS HBV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the HBV WHO Standard was from 2.00E+01 IU/mL to 2.00E+03 IU/mL (1.30 - 3.30 $\log_{10}$ IU/mL), the RMS HBV Secondary Standard was tested at 3.89E+02 IU/mL (2.59 $\log_{10}$ IU/mL), and the RMS HBV Calibration Panel was tested from 5.00E+01 to 2.00E+07 IU/mL (1.70 - 7.30 $\log_{10}$ IU/mL).

All materials performed similarly and demonstrated co-linear dilution performance across the linear range of **cobas**® HBV (Figure 1). Based on these results, the calibration and standardization process of **cobas**® HBV provides quantitation values for the calibration panel, the RMS HBV Secondary Standard, and the HBV WHO Standard that are similar to the expected values with deviation of not more than 0.14 $\log_{10}$ IU/mL. The maximum deviation was obtained around the test LLoQ using combined regression analyses for the RMS HBV Calibration Panel, the RMS HBV Secondary Standard and the HBV WHO Standard.

Figure 1: Traceability to WHO International Standard (mean observed $\log_{10}$ titer versus $\log_{10}$ WHO standard based titer) Using **cobas**® HBV



B. Analytical Sensitivity - Limit of Detection (LOD)

The limit of detection (LoD) of **cobas**® HBV was determined with the WHO International Standard (genotype A), genotypes B through H, and the predominant precore mutant. The LoD was determined to be 6.6 IU/mL for EDTA plasma and 3.5 IU/mL for serum by PROBIT analysis. The same LoD was verified at the same level for genotypes B through H and for the predominant precore mutant.

WHO International Standard

The limit of detection of **cobas**® HBV was determined by analysis of serial dilutions of the WHO International Standard for Hepatitis B Virus DNA for Nucleic Acid Amplification Technology Assays (2nd WHO International Standard) genotype A obtained from NIBSC, in HBV-negative human EDTA plasma and serum. Panels of eight concentration levels plus a negative were tested over three lots of **cobas**® HBV test reagents, multiple runs, days, operators, and instruments.

The study demonstrates that **cobas**® HBV detected HBV DNA for the WHO International Standard at a concentration of 2.7 IU/mL for LoD by PROBIT with a hit rate of 95% in EDTA plasma. For serum, the study demonstrates that **cobas**® HBV detected HBV DNA at a concentration of 2.4 IU/mL for LoD by PROBIT with a hit rate of 95%.

C. Analytical Sensitivity – Limit of Detection (LOD) for Genotypes

The limit of detection of **cobas**® HBV was determined by analysis of serial dilutions for seven different genotypes (B, C, D, E, F, G, H) and the predominant precore mutant (G1896A; C1858T). Dilution of clinical specimens or contrived plasmids were made in HBV-negative human EDTA plasma and serum. Panels of eight

concentration levels plus a negative were tested using three lots of **cobas**® HBV test reagents, over multiple runs, days, operators, and instruments.

The results for EDTA plasma and serum are shown in Table 3 and Table 4, respectively. The study demonstrates that **cobas**® HBV detected all HBV genotypes tested with a similar LoD as that for HBV genotype A.

Table 3: LoD for HBV DNA genotypes B through H and the Predominant Precore Mutant in EDTA plasma (500 µL)

Genotype	95% LoD by PROBIT	95% Confidence Interval
GT B	3.5 IU/mL	2.95 IU/mL - 4.32 IU/mL
GT C	4.1 IU/mL	3.32 IU/mL - 5.82 IU/mL
GT D	4.5 IU/mL	3.59 IU/mL - 6.49 IU/mL
GT E	3.2 IU/mL	2.76 IU/mL - 3.98 IU/mL
GT F	1.9 IU/mL	1.66 IU/mL - 2.24 IU/mL
GT G	2.5 IU/mL	2.17 IU/mL - 3.02 IU/mL
GT H	6.6 IU/mL	5.33 IU/mL - 8.77 IU/mL
precore mutant	2.4 IU/mL	2.08 IU/mL - 2.90 IU/mL

Table 4: LoD for HBV DNA Genotypes B through H and the Predominant Precore in Serum (500 µL)

Genotype	95% LoD by PROBIT	95% Confidence Interval
GT B	3.3 IU/mL	2.76 IU/mL - 4.30 IU/mL
GT C	3.3 IU/mL	2.83 IU/mL - 4.23 IU/mL
GT D	2.6 IU/mL	2.17 IU/mL - 3.42 IU/mL
GT E	2.7 IU/mL	2.25 IU/mL- 3.49 IU/mL
GT F	2.0 IU/mL	1.72 IU/mL - 2.45 IU/mL
GT G	2.1 IU/mL	1.75 IU/mL - 2.66 IU/mL
GT H	3.5 IU/mL	2.89 IU/mL - 4.60 IU/mL
precore mutant	1.6 IU/mL	1.43 IU/mL - 2.03 IU/mL

D. Linear Range & Lower Limit of Quantitation

The linearity study for **cobas**® HBV was performed with a dilution series consisting of 15 panel members spanning the intended linear range for the predominant genotype (GT A). High titer panel members were prepared from a high titer HBV plasmid DNA stock whereas the lower titer panel members were prepared from a clinical sample. The linearity panel was designed to have an approximate 2 $\log_{10}$ titer overlap between the two material sources. The linearity panel was designed to range from one concentration below LLoQ (e.g., 7.5 IU/mL) to one concentration level

above ULoQ (e.g., 2.0E+09 IU/mL) and to include medical decision points. Moreover, the linearity panel was designed to partly support steps of 1.0 $\log_{10}$ throughout the linear range. For each panel member the nominal concentration in IU/mL and the source of the HBV DNA were given. The data were analyzed and the best fitting polynomial regression fit and the LLoQ were calculated.

The **cobas**® HBV is linear for EDTA plasma and serum from 10 IU/mL to 1.00E+09 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.2 $\log_{10}$. Across the linear range, the accuracy of the test was within ± 0.24 $\log_{10}$.

See Figure 2 and Figure 3 for representative results.

Figure 2: Linear Range Determination in EDTA Plasma

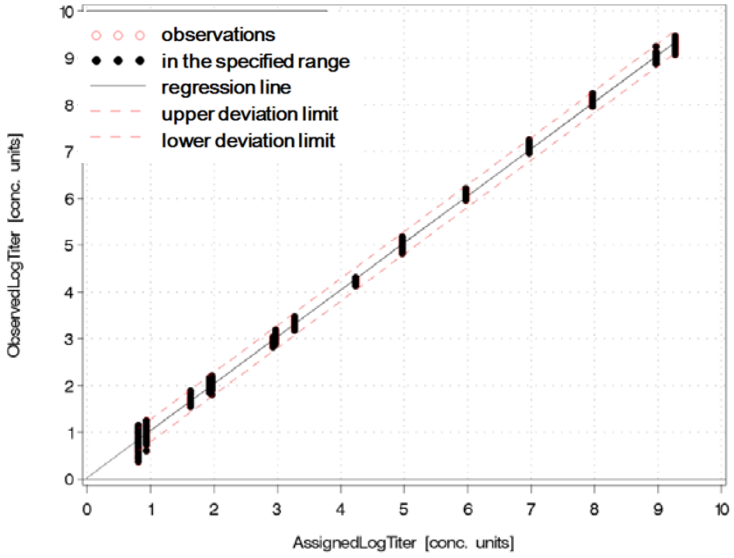
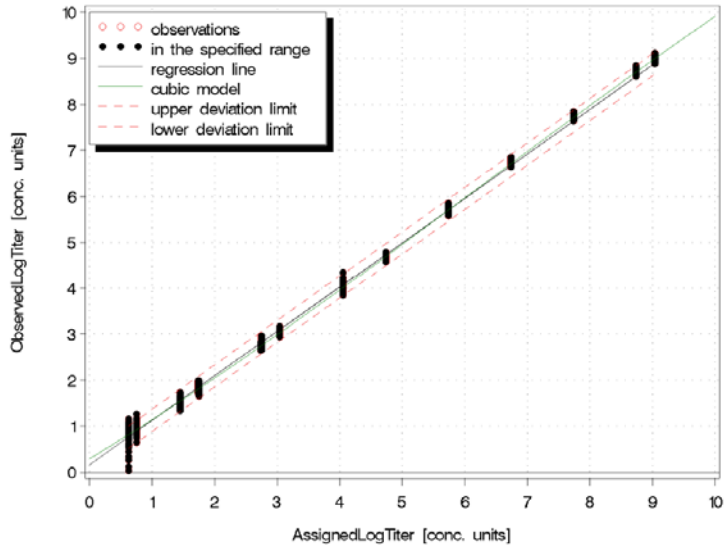


Figure 3: Linear Range Determination in Serum



E. Linear Range – Genotypes

The dilution series used to verify linearity of **cobas**® HBV for genotypes consisted of 10 panel members spanning the intended linear range. High titer panel members were prepared from a high titer plasmid DNA stock whereas the lower titer panel members were made from a high titer clinical sample. The linearity panel was designed to have an approximate 2 $\log_{10}$ titer overlap between the two material sources. The linear range of **cobas**® HBV spanned from below the LLoQ (10 IU/mL) to the ULoQ (1.00E+09 IU/mL) and included at least one medical decision point. Twenty-one replicates were tested across three lots of **cobas**® HBV reagent for each level in EDTA plasma and serum.

The linearity within the linear range of **cobas**® HBV was verified for all seven genotypes (B, C, D, E, F, G, H) and predominant precore mutant (G1896A; C1858T). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.2 $\log_{10}$.

F. Precision – within Laboratory

Precision of **cobas**® HBV was determined by analysis of serial dilutions of clinical HBV (Genotype A) samples (CS) or of HBV plasmid DNA in HBV negative EDTA plasma or in serum. Ten to 12 dilution levels were tested in 48 replicates for each level across three lots of **cobas**® HBV test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas**® HBV test procedure on the **cobas**® 6800/8800 Systems. The results are shown in Table 5 and Table 6.

Table 5: Within-Laboratory Precision of cobas® HBV (EDTA Plasma Samples)*

Nominal Concentration (IU/mL)	Assigned Concentration (IU/mL)	Source Material	EDTA Plasma			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+09	9.32E+08	plasmid DNA	0.04	0.07	0.09	0.07
1.00E+08	9.32E+07	plasmid DNA	0.04	0.08	0.05	0.06
1.00E+07	9.32E+06	plasmid DNA	0.06	0.05	0.04	0.05
1.00E+06	9.32E+05	plasmid DNA	0.06	0.07	0.04	0.06
1.00E+05	9.32E+04	plasmid DNA	0.06	0.06	0.07	0.06
2.00E+04	1.71E+04	clinical specimen	0.05	0.03	0.03	0.04
2.00E+03	1.86E+03	plasmid DNA	0.05	0.04	0.07	0.05
1.00E+03	8.54E+02	clinical specimen	0.04	0.05	0.04	0.04
1.00E+03	9.32E+02	plasmid DNA	0.06	0.06	0.05	0.06
1.00E+02	8.54E+01	clinical specimen	0.07	0.08	0.07	0.07
1.00E+02	9.32E+01	plasmid DNA	0.10	0.08	0.09	0.09
5.00E+01	4.27E+01	clinical specimen	0.09	0.04	0.08	0.08

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 6: Within-Laboratory Precision of cobas® HBV (Serum Samples)*

Nominal Concentration (IU/mL)	Assigned Concentration (IU/mL)	Source Material	Serum			
			Lot 1	Lot 2	Lot 3	All Lots
			SD	SD	SD	Pooled SD
1.00E+09	5.47E+08	plasmid DNA	0.05	0.06	0.03	0.05
1.00E+08	5.47E+07	plasmid DNA	0.03	0.04	0.03	0.04
1.00E+07	5.47E+06	plasmid DNA	0.05	0.05	0.03	0.05
1.00E+06	5.47E+05	plasmid DNA	0.04	0.06	0.06	0.05
1.00E+05	5.47E+04	plasmid DNA	0.04	0.03	0.03	0.04
2.00E+04	1.12E+04	clinical specimen	0.10	0.07	0.08	0.08
2.00E+03	1.09E+03	plasmid DNA	0.05	0.05	0.03	0.05
1.00E+03	5.62E+02	clinical specimen	0.03	0.14	0.03	0.09
1.00E+03	5.47E+02	plasmid DNA	0.04	0.05	0.04	0.04
1.00E+02	5.62E+01	clinical specimen	0.09	0.06	0.07	0.07
1.00E+02	5.47E+01	plasmid DNA	0.05	0.07	0.04	0.06
5.00E+01	2.81E+01	clinical specimen	0.07	0.06	0.10	0.08

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

G. Multi-Site Reproducibility Studies

The reproducibility and lot-to-lot variability of the **cobas®** HBV were evaluated in separate studies with EDTA plasma on the **cobas®** 6800 System using a mixed model to estimate the total variance.

Lot-to-Lot Variability

Lot-to-lot variability testing was performed for genotypes A and C at 1 test site, using three reagent lots. Two operators at the site tested each lot for 6 days. Two runs were performed each day.

Table 9 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected $\log_{10}$ HBV DNA concentration for the **cobas®** 6800 System.

Table 9: Summary of Lot-to-Lot Variability Study on the cobas® 6800 System

	HBV DNA Concentration ($\log_{10}$ IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
A	1.48	1.50	107	13%	0%	0%	0%	87%	0.157	37.27

	HBV DNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Lot	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
				(12.90)	(0.00)	(0.00)	(0.00)	(34.68)		
	2.70	2.72	108	52% (11.96)	0% (0.00)	0% (0.00)	0% (0.00)	48% (11.56)	0.072	16.69
	3.70	3.64	108	60% (14.29)	0% (0.00)	4% (3.55)	1% (1.57)	36% (11.01)	0.080	18.53
	4.70	4.65	107	47% (13.05)	0% (0.00)	3% (3.22)	1% (2.32)	49% (13.29)	0.082	19.14
	5.70	5.67	107	53% (13.66)	2% (2.59)	0% (0.00)	0% (0.00)	45% (12.54)	0.081	18.80
	6.70	6.71	105	50% (11.66)	0% (0.00)	0% (0.00)	5% (3.82)	44% (10.92)	0.071	16.48
	7.70	7.41	108	55% (13.08)	0% (0.00)	0% (0.00)	4% (3.59)	40% (11.18)	0.076	17.65
	8.70	8.41	107	51% (12.52)	0% (0.00)	0% (0.00)	10% (5.61)	38% (10.75)	0.075	17.51
C	1.48	1.49	107	23% (13.62)	1% (2.83)	0% (0.00)	0% (0.00)	76% (25.26)	0.124	29.05
	2.70	2.71	105	53% (13.92)	2% (2.63)	3% (3.48)	0% (0.00)	41% (12.27)	0.082	19.16
	3.70	3.64	107	61% (11.67)	0% (0.00)	0% (0.80)	0% (0.00)	39% (9.37)	0.065	15.02
	4.70	4.65	106	47% (11.44)	0% (0.00)	0% (0.00)	0% (0.00)	53% (12.25)	0.073	16.82
	5.70	5.69	107	60% (14.76)	0% (0.00)	1% (1.51)	0% (0.00)	39% (11.86)	0.082	19.08
	6.70	6.69	107	48% (11.79)	0% (0.00)	2% (2.31)	0% (0.00)	50% (12.13)	0.074	17.14
	7.70	7.38	107	51% (11.22)	0% (0.00)	0% (0.00)	1% (1.57)	48% (10.94)	0.068	15.80
	8.70	8.42	106	56% (13.92)	0% (0.00)	0% (0.00)	4% (3.54)	40% (11.72)	0.080	18.62

Note: Results with detectable viral load are included in this table; Within the range of assay are results from 1.0E+01 IU/mL to 1.0E+09 IU/mL.

[a] Calculated using the SAS MIXED procedure.

[b] Number of valid tests with detectable viral load.

[c] Calculated using the total variability from the SAS MIXED procedure.

[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100

CV(%) = percent coefficient of variation; DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; SD = standard deviation; sqrt = square root.

In Table 10 below, the negative percent agreement (NPA) for the **cobas**® 6800 System using negative panel member results was 100%.

Table 10: Negative Percent Agreement Using the Negative Panel Member (Lot-to-Lot)

Expected HBV DNA Concentration	No. of Valid Tests	Positive Results	Negative Results	Negative Percent Agreement[a]	95% CI[b]
Negative	106	0	106	100.00	(96.58, 100.00)

[a] NPA = (number of negative results / total number of valid tests in negative panel member) * 100.
[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.
CI = confidence interval; DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; NPA = negative percent agreement.

Reproducibility

Reproducibility testing was performed at three sites for genotypes A and C, using one reagent lot. Two operators at each site tested for 6 days. Two runs were performed each day.

Table 11 below shows attributable percentages of total variance, total precision SDs, and lognormal CVs by genotype and expected $\log_{10}$ HBV DNA concentration on the **cobas**® 6800 System.

Table 11: Summary of Reproducibility Study on the cobas® 6800

	HBV DNA Concentration ($\log_{10}$ IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Site	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
A	1.48	1.48	107	1% (4.21)	0% (0.00)	5% (7.75)	1% (3.56)	93% (34.98)	0.153	36.41
	2.70	2.66	108	34% (9.53)	0% (0.00)	0% (0.00)	16% (6.40)	50% (11.52)	0.070	16.33
	3.70	3.60	108	34% (7.49)	2% (1.90)	7% (3.42)	0% (0.00)	56% (9.58)	0.055	12.80
	4.70	4.62	107	13% (5.40)	0% (0.00)	0% (0.00)	12% (5.28)	75% (13.05)	0.065	15.12
	5.70	5.63	107	37% (7.82)	1% (1.26)	0% (0.00)	0% (0.00)	62% (10.04)	0.055	12.81
	6.70	6.67	106	20% (5.99)	3% (2.16)	4% (2.57)	15% (5.16)	60% (10.48)	0.059	13.59
	7.70	7.37	108	3% (2.70)	2% (2.06)	0% (0.00)	0% (0.00)	95% (15.12)	0.067	15.50
	8.70	8.36	107	12% (4.32)	0% (0.00)	0% (0.00)	2% (1.53)	86% (11.46)	0.053	12.36

	HBV DNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%))					Total Precision	
Geno-type	Expected	Observed Mean[a]	No. of Tests[b]	Site	Oper-ator	Day	Run	Within-Run	SD[c]	Log-normal CV(%)[d]
C	1.48	1.48	107	2% (11.79)	1% (7.06)	0% (0.00)	0% (0.00)	97% (84.30)	0.324	86.20
	2.70	2.67	105	19% (5.94)	3% (2.22)	0% (0.00)	0% (0.00)	79% (12.27)	0.060	13.84
	3.70	3.61	107	14% (4.49)	0% (0.00)	7% (3.15)	0% (0.00)	78% (10.48)	0.051	11.84
	4.70	4.62	106	24% (6.45)	0% (0.00)	0% (0.00)	0% (0.00)	76% (11.59)	0.057	13.29
	5.70	5.65	107	18% (5.96)	0% (0.00)	3% (2.29)	0% (0.00)	80% (12.68)	0.061	14.22
	6.70	6.65	107	23% (6.35)	6% (3.26)	0% (0.00)	1% (1.33)	70% (11.10)	0.057	13.29
	7.70	7.34	106	0% (0.00)	3% (2.38)	0% (0.00)	13% (5.12)	84% (13.11)	0.062	14.30
	8.70	8.36	107	4% (2.24)	0% (0.00)	16% (4.35)	10% (3.46)	70% (9.09)	0.047	10.91

Note: Results with detectable viral load are included in this table; Within the range of assay are results from 1.0E+01 IU/mL to 1.0E+09 IU/mL.

[a] Calculated using the SAS MIXED procedure.
[b] Number of valid tests with detectable viral load.
[c] Calculated using the total variability from the SAS MIXED procedure.
[d] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100.
CV(%) = percent coefficient of variation; HBV = hepatitis B virus; DNA = deoxyribonucleic acid; No. = number; SD = standard deviation; sqrt = square root.

The NPA was 100% (106/106; 95% CI: 96.58% to 100%) using negative panel member tests on the **cobas**® 6800 System as presented in Table 12 below.

Table 12: Negative Percent Agreement Using the Negative Panel Member (Reproducibility) on the cobas® 6800 System

Expected HBV DNA Concentration	No. of Tests	Positive Results	Negative Results	Negative Percent Agreement[a]	95% CI[b]
Negative	106	0	106	100.00	(96.58, 100.00)

[a] NPA = (number of negative results/total number of valid tests in negative panel member) * 100.
[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.
CI = confidence interval; DNA = deoxyribonucleic acid; HBV = hepatitis B virus; No. = number; NPA = negative percent agreement.

H. Performance with HBV DNA-Negative Samples

The performance of the **cobas**® HBV assay with HBV DNA-negative samples was determined by analyzing HBV negative EDTA plasma and serum samples from individual donors. Three hundred individual EDTA plasma and 300 individual serum samples (600 total results) were tested with two lots of **cobas**® HBV reagents. All samples tested negative for HBV DNA. In the test panel, the results of all specimens tested with **cobas**® HBV was 100% "Target Not Detected" (with a two-sided 95% confidence interval of 99.4% – 100%).

I. Analytical Specificity

The analytical specificity of **cobas**® HBV was evaluated by diluting a panel of microorganisms with HBV DNA positive and HBV DNA negative EDTA plasma (Table 7). The microorganisms were added to negative human EDTA plasma and tested with and without HBV DNA. None of the non-HBV pathogens interfered with test performance. Negative results were obtained with **cobas**® HBV for all microorganism samples without HBV target and positive results were obtained on all of the microorganism samples with HBV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HBV samples containing potentially cross-reacting organisms was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 7: Microorganisms Tested for Cross-Reactivity

Viruses		Bacteria	Yeast
Adenovirus type 5	West Nile Virus	Propionibacterium Acnes	Candida albicans
Cytomegalavirus	St. Louis encephalitis Virus	Staphylococcus aureus	
Hepatitis A Virus	Dengue virus types 1, 2, 3, and 4		
Hepatitis C Virus	FSME virus (strain HYPR)		
Hepatitis D Virus	Yellow Fever Virus		
Human Immunodeficiency Virus-1	Human Papillomavirus		
Human T-Cell Lymphotropic Virus types 1 and 2	Varizella-Zoster Virus		
Human Herpes Virus Type-6	Influenza A		
Herpes Simplex Virus Type-1and 2	Zika Virus		

J. Analytical Specificity – Interfering Substances

EDTA-plasma samples with and without HBV DNA were spiked with elevated levels of triglycerides (34.5 g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) and tested with **cobas**® HBV. The tested potentially interfering substances were shown not to interfere with the test performance of **cobas**® HBV.

Moreover, the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody were tested.

In addition, drug compounds listed in Table 8 below were tested at three times the Cmax in the presence and absence of HBV DNA.

All potentially interfering substances have been shown to not interfere with the test performance. Negative results were obtained with **cobas®** HBV for all samples without HBV target and positive results were obtained on all of the samples with HBV target. Furthermore, the mean $\log_{10}$ titer of each of the positive HBV samples containing potentially interfering substances was within ± 0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 8: Drug Compounds Tested for Interference with the Quantitation of HBV DNA by cobas® HBV

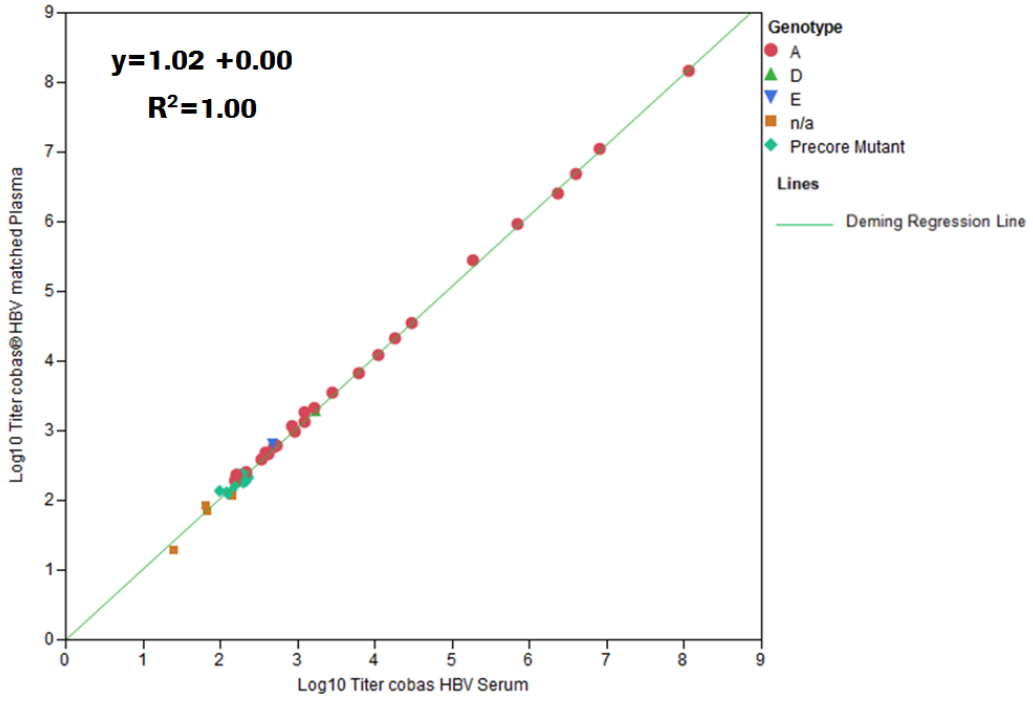
Class of Drug	Generic Drug Name	
Immune Modulator	Peginterferon α-2a Ribavirin	Peginterferon α-2b
HIV entry inhibitor	Maraviroc	
HIV Integrase Inhibitor	Elvitegravir/Cobicistat	Raltegravir
Non-nucleoside HIV Reverse Transcriptase Inhibitor	Efavirenz Etravirine	Nevirapine Rilpivirine
HIV protease inhibitor	Atazanavir	Lopinavir
	Tipranavir	Nelfinavir
	Darunavir	Ritonavir
	Fosamprenavir	Saquinavir
HCV Protease Inhibitor	Boceprevir	Telaprevir
	Simeprevir	
Reverse transcriptase or DNA polymerase inhibitors	Abacavir	Tenofovir
	Emtricitabine	Adefovir dipivoxil
	Entecavir	Telbivudine
	Foscarnet	Zidovudine
	Cidofovir	Aciclovir
	Lamivudine	Valganciclovir
	Ganciclovir	Sofosbuvir
Compounds for Treatment of Opportunistic Infections	Azithromycin	Pyrazinamide
	Clarithromycin	Rifabutin
	Ethambutol	Rifampicin
	Fluconazole	Sulfamethoxazole
	Isoniazid	Trimethoprim

K. Matrix Equivalency – EDTA Plasma Versus Serum

Fifty paired EDTA plasma and serum samples were analyzed for matrix equivalency. The HBV positive samples covered most genotypes and had titers across the entire linear range.

Matrix equivalency was shown in the tested samples with a mean titer deviation of 0.05 $\log_{10}$ (Figure 4).

Figure 4: Matrix Equivalency Performance Between EDTA Plasma and Serum



L. Cross Contamination

The cross-contamination rate for **cobas**® HBV was determined by testing 240 replicates of a normal, virus-negative (HIV, HCV and HBV) human EDTA-plasma sample and 225 replicates of a high titer HBV sample at 1.00E+09 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were non-reactive, resulting in a cross-contamination rate of 0%. The two-sided 95% exact confidence interval was 0% - 1.53%.

M. Specimen Stability

The specimen stability of HBV viral target in whole blood specimens collected in EDTA plasma or serum preparation tubes and/or plasma and serum samples was evaluated using the **cobas**® HBV test for use on the **cobas**® 6800/8800 Systems simulating handling, transportation and storage conditions of patient samples prior to testing in diagnostic laboratories. The specimens were handled and stored under the conditions that simulated the handling, transporting and processing of blood specimens prior to testing separated plasma and serum with the **cobas**® HBV test for use on the **cobas**® 6800/8800 Systems. In addition the freeze/thaw stability of the HBV viral target in plasma and serum samples for up to 4 freeze/thaw cycles was evaluated.

The results of this study demonstrated acceptable specimen stability under the following storage conditions for whole blood and separated EDTA plasma and serum samples:

- Whole blood collected in EDTA plasma or Serum preparation tubes may be stored for up to 24 hours at 2 ºC to 25 ºC before further processing and matrix separation

Additionally plasma and serum samples are stable for:

- up to 6 days at 2°C to 8°C or up to 6 months at -15°C to -80°C
- or 6 days at 2 to 8°C followed by 6 months at -75°C ± 15°C after matrix separation.

Plasma and serum samples are stable for up to 4 freeze/thaw cycles when frozen between - 18°C to -80°C.

N. Real-Time Reagent Stability:

Expiration dating for the cobas® HBV reagents has been established and approved at 16 months when stored at 2-8°C.

X. SUMMARY OF CLINICAL STUDIES

A. Study Design

The study was designed to evaluate the ability of the assay to predict clinical outcome.

Residual specimens were obtained from 396 subjects who were either:

- Participating in a randomized pharmaceutical clinical trial to receive treatment for 100 weeks with entecavir plus tenofovir or entecavir monotherapy, or
- Receiving standard treatment (tenofovir monotherapy) from routine clinical practice

Table 13: Treatment Groups

Clinical Study	HBeAg Status	Treatment	Treatment Arm
Pharmaceutical Clinical Trial[21]	HBeAg (+)	entecavir monotherapy	Arm I
		entecavir + tenofovir	Arm II
	HBeAg (-)	entecavir monotherapy	Arm III (includes up to 17 subjects from clinical practice)
		entecavir + tenofovir	Arm IV
Clinical Practice	HBeAg (-)	tenofovir monotherapy	Arm V

HBeAg = Hepatitis B e antigen.

Testing with **cobas**® HBV was performed at 3 test sites, using 3 kit lots of reagents; each sample was tested with 1 kit lot of reagent. Each test site was equipped with 1 **cobas**® 6800 system.

The samples were labeled to ensure the test site personnel were blinded to any identifying information and reduce bias. Each sample had a unique identification number and a visit number. The samples were evenly distributed among the 3 test sites. Samples from each individual subject were tested at a single test site.

B. Study Population Demographics and Baseline Parameters

The demographic distribution of the subjects in this study was consistent with that of patients with chronic HBV in the US,[2,3] HBeAg (+) and HBeAg (-) subjects were enrolled in this study, and data for these populations were analyzed separately (Table 14).

Table 14: Demographics and Baseline Characteristics of Subjects

Characteristics	Statistics
Total, N	396
Age Category (years), n (%)	
< 40	186 (47.0%)
>= 40	210 (53.0%)
Age (years)	
Mean ± SD	42 ± 15.2
Median	42
Range	17 - 81
Gender, n (%)	
Male	276 (69.7%)
Female	120 (30.3%)
Race, n (%)	
Asian	204 (51.5%)
Black / African American	14 (3.5%)
White / Caucasian	169 (42.7%)
Other	9 (2.3%)
Genotype, n (%)	
A	64 (16.2%)
A & G	1 (0.3%)
B	62 (15.7%)
C	74 (18.7%)
D	105 (26.5%)
E	4 (1.0%)
F	10 (2.5%)

Characteristics	Statistics
Mixed	1 (0.3%)
Unknown	75 (18.9%)
Normal ALT at Baseline, n (%)	
Yes	23 (5.8%)
No	361 (91.2%)
Unknown	12 (3.0%)
Baseline ALT (IU/L)	
Mean ± SD	140 ± 169.9
Median	96
Range	14 - 1583
HBV DNA (log_{10} IU/mL) at Baseline	
Mean ± SD	6.6 ± 2.38
Median	7.4
Range	-0.0 - 10.1
HBV DNA Category, n (%)	
< 2.0 x 10^3 IU/mL	41 (10.4%)
2.0 x 10^3 to 2.0 x 10^4 IU/mL	13 (3.3%)
> 2.0 x 10^4 IU/mL	330 (83.3%)
Unknown	12 (3.0%)

ALT = alanine aminotransferase; HBV = hepatitis B virus; DNA = deoxyribonucleic acid; SD = standard deviation.

C. Safety and Effectiveness Results

Definitions:

- Week 12 virologic response (VR) = HBV DNA 2 log_{10} decrease from baseline
- Week 24 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-))
- Week 48 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-))
- Week 96 VR = HBV DNA < 50 IU/mL (VR endpoint)
- No-VR endpoint = HBV DNA > 50 IU/mL at Week 96
- Biochemical Response (BR) = normalization of ALT compared to baseline; for male ALT < 30 IU/L and for female ALT < 19 IU/L
- HBeAg loss = conversion from HBeAg (+) to HBeAg (-) status during therapy

Predicting Virological Response at Week 96

In this study, baseline HBV DNA concentration and VRs at Weeks 12, 24, and 48 of treatment were used to evaluate the ability to predict outcome (VR, BR, or HBeAg

loss) at Week 96 of therapy. VR96 (HBV DNA < 50 IU/mL) was assessed using HBV DNA results from an approved test.

When **cobas**® HBV was used to measure HBV DNA, a low baseline HBV DNA concentration (< 10^8 IU/mL) and VRs at Weeks 12, 24, and 48 were shown to be highly predictive of VR96 in both the HBeAg (+) and HBeAg (-) populations and for all the treatment arms in this study (PPVs 79.6% to 100%) (Table 15 and Table 16).

Table 15: Probability of Achieving Virological Response at Week 96 Given Baseline HBV DNA <10^8 IU/mL by Treatment Arm

			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Evaluable Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Baseline	Arm I	103	93.5 (82.5, 97.8)	43 / 46	31.6 (21.0, 44.5)	18 / 57	6.62 (1.81, 24.20)
	Arm II	102	96.2 (87.0, 98.9)	50 / 52	4.0 (1.1, 13.5)	2 / 50	1.04 (0.14, 7.69)
	Arm III	49	100.0 (92.1, 100.0)	46 / 46	25.0 (4.6, 69.9)	1 / 4	30.00 (0.83, 1087.42)
	Arm IV	48	97.9 (88.9, 99.6)	46 / 47	100.0 (20.7, 100.0)	1 / 1	92.00 (1.81, 4686.43)
	Arm V	30	90.0 (74.4, 96.5)	27 / 30	NC	0	9.00 (0.15, 541.69)

Table 16: Probability of Achieving Virologic Response at Week 96 Given Virologic Response at a Specific On-Treatment Visit by Treatment Arm

			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Week 12	Arm I	103	79.6 (70.8, 86.3)	82 / 103	NC	0	3.90 (0.08, 202.63)
	Arm II	100	97.0 (91.5, 99.0)	97 / 100	NC	0	32.33 (0.54, 1921.79)
	Arm III	48	97.8 (88.7, 99.6)	45 / 46	0.0 (0.0, 65.8)	0 / 2	11.25 (0.28, 445.33)
	Arm IV	48	95.8 (86.0, 98.8)	46 / 48	NC	0	23.00 (0.36, 1485.21)
	Arm V	21	85.7 (48.7, 97.4)	6 / 7	7.1 (1.3, 31.5)	1 / 14	0.46 (0.02, 8.69)
Week 24	Arm I	103	96.1 (89.2, 98.7)	74 / 77	69.2 (50.0, 83.5)	18 / 26	55.50 (13.37, 230.39)

			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
	Arm II	102	96.7 (90.8, 98.9)	89 / 92	10.0 (1.8, 40.4)	1 / 10	3.30 (0.31, 35.08)
	Arm III	47	100.0 (89.8, 100.0)	34 / 34	7.7 (1.4, 33.3)	1 / 13	5.67 (0.18, 179.94)
	Arm IV	49	97.7 (87.9, 99.6)	42 / 43	16.7 (3.0, 56.4)	1 / 6	8.40 (0.45, 156.19)
	Arm V	20	94.1 (73.0, 99.0)	16 / 17	33.3 (6.1, 79.2)	1 / 3	8.00 (0.35, 184.38)
Week 48	Arm I	101	89.9 (81.9, 94.6)	80 / 89	91.7 (64.6, 98.5)	11 / 12	97.78 (11.28, 847.86)
	Arm II	97	95.9 (89.9, 98.4)	93 / 97	NC	0	23.25 (0.41, 1328.83)
	Arm III	46	100.0 (91.6, 100.0)	42 / 42	25.0 (4.6, 69.9)	1 / 4	28.00 (0.77, 1015.78)
	Arm IV	48	97.8 (88.4, 99.6)	44 / 45	33.3 (6.1, 79.2)	1 / 3	22.00 (0.98, 494.79)
	Arm V	28	92.3 (75.9, 97.9)	24 / 26	50.0 (9.5, 90.5)	1 / 2	12.00 (0.53, 273.05)

Notes (Table 14 & 15): Positive Predictive Value (PPV) = TP / (TP + FP) or the probability of being a VR96 given the subject was a virologic responder at a specific visit.

Negative Predictive Value (NPV) = TN / (FN + TN) or the probability of not being a VR96 given the subject was not a virologic responder at a specific visit.

Odds Ratio (OR) = (TP • TN) / (FP • FN).

95% CIs for PPV and NPV are calculated based on Wilson Score CI.

0.5 was added to empty cells (TP, TN, FP, or FN = 0), prior to calculation of OR and corresponding 95% CI.

VR96 is achieved if the subject has HBV DNA < 50 IU/mL from the COBAS® TaqMan® HBV Test For Use with the High Pure System at Week 96.

Week 12 VR = HBV DNA > 2 $\log_{10}$ decrease from baseline; Week 24 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-)); Week 48 VR = HBV DNA < 2000 IU/mL (HBeAg (+)) or < 50 IU/mL (HBeAg (-)).

Predicting Biochemical Response at Week 96

The probability of achieving a biochemical response at Week 96 given an on-treatment VR at Week 12, Week 24 or Week 48 is summarized in Table 17.

The value of VR at Week 12, Week 24, or Week 48 as a predictor of VR96 varied by VR week, treatment Arm and baseline HBeAg status.

Table 17: Probability of Achieving Biochemical Response at Week 96 Given Virologic Response at a Specific On-Treatment Visit by Treatment Arm

			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Week 12	Arm I	101	62.4 (52.6, 71.2)	63 / 101	NC	0	1.66 (0.03, 85.30)
	Arm II	100	43.0 (33.7, 52.8)	43 / 100	NC	0	0.75 (0.01, 38.79)
	Arm III	49	50.0 (36.1, 63.9)	23 / 46	66.7 (20.8, 93.9)	2 / 3	2.00 (0.17, 23.62)
	Arm IV	49	32.7 (21.2, 46.6)	16 / 49	NC	0	0.48 (0.01, 25.57)
	Arm V	21	40.0 (16.8, 68.7)	4 / 10	90.9 (62.3, 98.4)	10 / 11	6.67 (0.60, 74.51)
Week 24	Arm I	102	66.2 (55.1, 75.8)	51 / 77	60.0 (40.7, 76.6)	15 / 25	2.94 (1.16, 7.45)
	Arm II	103	44.6 (34.8, 54.7)	41 / 92	81.8 (52.3, 94.9)	9 / 11	3.62 (0.74, 17.68)
	Arm III	51	47.2 (32.0, 63.0)	17 / 36	33.3 (15.2, 58.3)	5 / 15	0.45 (0.13, 1.57)
	Arm IV	50	38.6 (25.7, 53.4)	17 / 44	100.0 (61.0, 100.0)	6 / 6	7.56 (0.40, 144.09)
	Arm V	24	42.1 (23.1, 63.7)	8 / 19	80.0 (37.6, 96.4)	4 / 5	2.91 (0.27, 31.22)
Week 48	Arm I	100	65.2 (54.8, 74.3)	58 / 89	81.8 (52.3, 94.9)	9 / 11	8.42 (1.71, 41.41)
	Arm II	97	43.3 (33.9, 53.2)	42 / 97	NC	0	0.76 (0.01, 39.29)
	Arm III	49	52.3 (37.9, 66.2)	23 / 44	40.0 (11.8, 76.9)	2 / 5	0.73 (0.11, 4.81)
	Arm IV	49	37.0 (24.5, 51.4)	17 / 46	100.0 (43.9, 100.0)	3 / 3	3.52 (0.17, 74.51)
	Arm V	28	33.3 (18.0, 53.3)	8 / 24	75.0 (30.1, 95.4)	3 / 4	1.50 (0.13, 16.82)

Predicting HBeAg loss

HBeAg loss could only be evaluated in subjects who were HBeAg (+) at baseline.

Absence of VR at Week 24 was highly predictive of persistence of HBeAg (NPVs were ≥ 80.0% for both Arms I and II), and absence of VR at Week 48 also predicted HBeAg persistence in Arm I (NPV was 100%) (Table 18). As all subjects on the

combination regimen (Arm II) had achieved VRs by Week 48, it was not possible to calculate an NPV at this time point for this group.

Table 18: Probability of HBeAg Loss at Week 96 Given Virologic Response at a Specific On-Treatment Visit by Treatment Arm

			PPV (%)		NPV (%)		OR
On-Treatment Visit	**Treatment Arm**	**Eligible Subjects**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**	**n / N**	**Estimate (95% CI)**
Week 12	Arm I	102	46.1 (36.7, 55.7)	47 / 102	NC	0	0.85 (0.02, 43.91)
	Arm II	101	41.6 (32.5, 51.3)	42 / 101	NC	0	0.71 (0.01, 36.60)
Week 24	Arm I	103	52.6 (41.6, 63.3)	41 / 78	80.0 (60.9, 91.1)	20 / 25	4.43 (1.51, 13.00)
	Arm II	104	44.1 (34.4, 54.2)	41 / 93	81.8 (52.3, 94.9)	9 / 11	3.55 (0.73, 17.33)
Week 48	Arm I	101	51.1 (41.0, 61.2)	46 / 90	100.0 (74.1, 100.0)	11 / 11	23.00 (1.31, 403.28)
	Arm II	98	40.8 (31.6, 50.7)	40 / 98	NC	0	0.69 (0.01, 35.48)

Conclusion

The results demonstrated that **cobas**® HBV is effective for monitoring the HBV DNA viral load in subjects with chronic HBV infection at the start of and during antiviral treatment. This study demonstrated that HBV DNA concentration measurement at baseline, a decrease in HBV DNA concentration at Week 12, or HBV DNA concentrations below specific thresholds at Weeks 24 or 48 during treatment predicted response to therapy; the study identified subjects who achieved Virologic Response, Biochemical Response, or loss of HBeAg at Week 96 of therapy.

D. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included three investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

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

In accordance with the 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.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the **cobas**® HBV has been demonstrated when used for the quantitation of hepatitis B virus (HBV) DNA in human EDTA plasma or serum of HBV-infected individuals. Study results indicate this test is effective when used as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy. The test can be used to measure HBV DNA levels at baseline and during treatment to aid in assessing response to treatment.

B. Safety Conclusions

Based on the results of the analytical and clinical laboratory studies, the **cobas**® HBV, when used according to the provided directions and in conjunction with other laboratory results and clinical information, should be safe and pose minimal risk to the patient due to false test results.

C. Risk/Benefit Analysis

The probable benefits of the device are based on data collected in a clinical study conducted to support PMA approval as described above. As an in vitro nucleic acid amplification test, the **cobas**® HBV involves removal of blood from an individual for testing purposes. 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. Additionally, the direct benefit of the measurement of HBV DNA measurement is to determine response to anti-viral therapy and adjust dosages and durations of these critical medications accordingly.

- Additional factors to be considered in determining probable risks and benefits for the **cobas**® HBV include:
- As a quantitative assay, risks are primarily from inaccurate results rather than false positive or false negative results, although the latter could be considered at the very low end of measurement where levels below 50 IU are considered a virological response. In essence, when used as a quantitative assay, there are several important decisions points, i.e., high levels ($> 10^8$) where combination therapy may be considered, and at very low levels used to confirm complete response. Within this range, effects of inaccurate measurements are likely to have limited clinical consequences; perhaps the greatest potential for an adverse effect would be if a measurement was inaccurately elevated in the setting of treatment such that treatment response (or viral resistance) was inferred. However, in this circumstance, other markers (such as ALT) might suggest inaccuracy, and repeat measurement is likely. There is also substantial biological variability in HBV viral load (in the absence of treatment), and inaccurate measurements will be interpreted in the context of expected variability.

- Inaccurate results would be most significant between 2000 – 20,000 IU/mL, where treatment may be considered under different scenarios; however, serial measurements are common, and clinicians are likely to repeat measurements when there is consideration of treatment but a deciding factor is a low HBV value. The risks from 'overtreatment' (i.e., treatment initiated due to an unexpectedly high HBV viral load) are relatively small absent the cost of medication.
- There are other FDA-approved devices for this specific use. Performance of this device is similar to existing devices. Studies with other devices have arrived at conclusion similar to the clinical performance study contained in this submission.
- Chronic Hepatitis B infection is a significant illness that is potentially fatal over time; approximately 1- 5% of immunocompetent adults with acute Hepatitis B infection develop chronic infection; the rate of chronic infection is far higher for patients exposed at birth by infected mothers or exposed early in childhood. Excluding neonatal exposure, transmission of Hepatitis B is by exposure to infected blood or by sexual contact with a chronically infected partner.
- There are multiple FDA-approved drugs for treatment, primarily nucleoside/nucleotide analogs as well as Interferon. Treatment is generally very effective but less so in patients with greater degrees of cirrhosis and may be complicated by the emergence of viral resistance. Well adopted published treatment guidelines exist.
- In conclusion, given the available information above, the data support that the **cobas**® HBV, when used for the quantitation of hepatitis B virus (HBV) DNA in human EDTA plasma or serum of HBV-infected individuals, the probable benefits outweigh the probable risks.

D. 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 preclinical studies demonstrated acceptable analytical sensitivity, traceability, linearity, precision, and analytical specificity of the **cobas**® HBV 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 usefulness study and the statistical analysis of clinical data in this application has shown that the results obtained with the **cobas**® HBV are informative for use as an aid in the management of patients with chronic HBV infection undergoing anti-viral therapy, and that the test is safe and effective when used according to the directions for use in the labeling.

XIII. CDRH DECISION

CDRH issued an approval order on October 14, 2015. The final conditions of approval can be found in the approval order.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.