COBAS AmpliScreen HBV Test

Summary of Basis for Approval

TABLE OF CONTENTS

I. INTENDED USE 4
II. BRIEF DESCRIPTION OF DEVICE AND PRINCIPLES 5
A. Summary and Explanation of the Test 5
B. Description of Kit and Component Formulations 8
III. MANUFACTURING AND CONTROLS 10
A. Description of Manufacturing Facilities 10
B. Stability Program 11
C. Methods of Validation 12
D. Labeling 13
E. Establishment Inspection 13
F. Environmental Impact Analysis, Claims for a Categorical Exclusion 13
IV. PERFORMANCE CHARACTERISTICS 14
A. Pre-clinical Studies Summary 14
1. Assay Cutoff 14
2. Analytical Sensitivity 16
3. Analytical Specificity 22
4. Potentially Interfering Substances 25
5. Uracil-N-Glycosylase (UNG) Performance 25
B. Clinical Trials Summary — Whole Blood 26
1. Reproducibility 26
2. Pool Reactivity in Whole Blood 29
3. Single Donation Testing Performance 31
4. Detection of HBV DNA in Seropositive Specimens 32
5. High Risk Population 34

6. Sensitivity in Chronic HBV Patients 35
7. Detection of Window Period Cases 36
C. Clinical Trials Summary — Source Plasma 36
1. Pool Reactivity in Source Plasma 36
2. Seroconversion Panels 37

LIST OF TABLES

Table 1: COBAS AmpliScreen HBV Test Results for HBV Sero-Negative Specimens 15

Table 2: COBAS AmpliScreen HBV Test, Standard Specimen Processing Procedure (Undiluted) and Multiprep Specimen Processing Procedure (1:24 Dilution), Summary for HBV Seropositive Specimens 15

Table 3: Cutoff Summary and Test Result Validity Criteria 16

Table 4: HBV WHO International Standard: Dilution Testing Summary for Multiprep Method — Combined Input Values with 95% Confidence Limits 17

Table 5: HBV WHO International Standard: Dilution Testing Summary for Standard Method — Combined Input Values with 95% Confidence Limits 17

Table 6: HBV Genotypes Tested 19

Table 7: Seroconversion Panel Summary — Detection of HBV DNA Prior to HBsAg..... 20

Table 8: Summary of COBAS AmpliScreen HBV Test — Pre-Seroconversion Detection of HBV DNA vs. HBsAg 21

Table 9: COBAS AmpliScreen HBV Test, Microorganism Exclusivity Study using Multiprep Specimen Processing Procedure 22

Table 10: Reproducibility Results — Multiprep Specimen Processing Procedure 27

Table 11: Reproducibility Results — Standard Specimen Processing Procedure 27

Table 12: Operator Variability Data — Multiprep Specimen Processing Procedure 28

Table 13: Operator Variability Data — Standard Specimen Processing Procedure..... 28

Table 14: Pool Reactivity in Whole Blood 29

Table 15: HBV Seropositive Specimens — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24) 30

Table 16: HBV Seropositive Specimens — Results for Standard Specimen Processing Procedure (Specimens Undiluted) 31

Table 17: Paired Specimen Results for Individual Samples with Assigned HBV Status 32

Table 18: HBV Seropositive Specimens (Including 49 Acute Patients) from Commencial Vendors — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24) 33

Table 19: HBV Seropositive Specimens (Including 49 Acute Patients) from Commencial Vendors — Results for Standard Specimen Processing Procedure (Specimens Undiluted) 33

Table 20: HBV Seropositive Specimens from Patients at High Risk for Liver Disease — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24) 34

Table 21: HBV Seropositive Specimens from Patients at High Risk for Liver Disease — Results for Standard Specimen Processing Procedure (Specimens Undiluted)..... 35

Table 22: HBV Seropositive Specimens from Chronic HBV Patients — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24) 35

Table 23: Total HBV Seropositive Specimens from Chronic HBV Patients — Results for Standard Specimen Processing Procedure (Specimens Undiluted)..... 36

Table 24: Pool Reactivity in Source Plasma 37

Table 25: Summary of Pre-Seroconversion Detection of HBV DNA vs. FDA Licensed Serology Tests — Multiprep Specimen Processing Procedure (Specimen Diluted 1:96)..... 38

COBAS AMPLISCREEN HBV TEST
SUMMARY OF BASIS FOR APPROVAL

Trade Name	COBAS AmpliScreen HBV Test
Proper Name (Licensed Name)	Hepatitis B Virus/Polymerase Chain Reaction/Blood Cell Derived [COBAS AmpliScreen™]
Applicant/Manufacturer	Roche Molecular Systems, Inc. 4300 Hacienda Drive Pleasanton, CA 94588-2722 FDA Registration No: 3004141078
Biological License Application (BLA) Reference Number(s)	BL125090/0
Report Date	

I. INTENDED USE

The COBAS AmpliScreen HBV Test is a qualitative *in vitro* test for the direct detection of Hepatitis B Virus (HBV) DNA in human plasma.

The COBAS AmpliScreen HBV Test is intended to be used to screen donors for HBV DNA in addition to the currently recommended serology tests. This product is intended for use as a donor screening test to detect HBV DNA in plasma samples from individual human donors, including donors of whole blood and blood components, source plasma and other living donors. It is also intended for use to screen organ donors when specimens are obtained while the donor's heart is still beating. This test is not intended for use on specimens from cadaveric (non-heart-beating) donors. This test is not intended for use on samples of cord blood.

Plasma from all donors may be screened as individual samples. For donations of whole blood and blood components, plasma samples of the donations may be tested in pools comprised of equal aliquots of not more than 24 individual samples in conjunction with licensed tests for detecting hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc). For donations of Source Plasma, plasma samples of

the donations may be tested in pools comprised of equal aliquots of not more than 96 individual samples in conjunction with licensed tests for detecting HBsAg.

This assay is not intended for use as an aid in diagnosis.

II. BRIEF DESCRIPTION OF DEVICE AND PRINCIPLES

A. Summary and Explanation of the Test

The COBAS AmpliScreen HBV Test uses a generic sample preparation technique in a mini-pool testing format along with automated amplification and detection using PCR on the COBAS AMPLICOR™ Analyzer for the detection of HBV DNA in blood donations. The assay incorporates an Internal Control for monitoring assay performance in each individual test as well as the AmpErase® enzyme (uracil-N-glycosylase) to reduce potential contamination by previously amplified material (amplicon).

The COBAS AmpliScreen HBV Test is based on four major processes:

1. Specimen Processing
2. PCR amplification of target DNA using HBV-specific complementary primers
3. Hybridization of the amplified products to oligonucleotide probes specific to the target(s)
4. Detection of the probe-bound amplified products by colorimetric determination

Specimen Processing

Two specimen processing procedures are used with the COBAS AmpliScreen HBV Test as follows:

- Multiprep Specimen Processing Procedure for preparation of mini-pool specimens
- Standard Specimen Processing Procedure for preparation of individual specimens.

In the Standard Specimen Processing Procedure, HBV DNA is isolated directly from plasma by lysis of the virus particles with Multiprep Lysis Reagent followed by precipitation of the DNA with alcohol. In the Multiprep Specimen Processing Procedure, HBV viral particles are first pelleted from the plasma sample by high speed centrifugation, followed by lysis of the pelleted virus with a chaotropic agent and precipitation of the DNA with alcohol.

The Multiprep Internal Control (MP IC), containing the HBV Internal Control, is introduced into each sample with the Multiprep Lysis Reagent and serves as an extraction and amplification control for each processed specimen and control. The HBV Internal Control is a DNA plasmid with primer binding regions identical to those of the HBV target sequence, a randomized internal sequence of similar length and base composition as the HBV target sequence, and a unique probe binding region that differentiates the HBV Internal Control amplicon from target amplicon. These features were selected to ensure equivalent amplification of the HBV Internal Control and the HBV target DNA.

PCR Amplification

The amplification reactions are performed with the thermostable recombinant enzyme Thermus aquaticus DNA Polymerase (Taq pol). The reaction mixture is heated to separate double-stranded DNA. The Taq pol extends the annealed primers along the target templates to produce a double-stranded DNA molecule termed an amplicon. The COBAS AMPLICOR Analyzer automatically repeats this process for a designated number of cycles, each cycle effectively doubling the amount of amplicon DNA.

To ensure selective amplification of nucleic acid target in the sample and prevent amplification of pre-existing amplicon, the AmpErase® enzyme (uracil-N-glycosylase, UNG) is added to the COBAS AmpliScreen HBV Test. The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon because of the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix

reagent. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme before amplification of the target DNA. AmpErase is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. Following amplification, any residual enzyme is denatured by the addition of the Denaturation Solution, thereby preventing the degradation of any target amplicon.

Hybridization Reaction

Following PCR amplification, the COBAS AMPLICOR Analyzer automatically adds Denaturation Solution to chemically denature the HBV amplicon and the HBV Multiprep Internal Control amplicon to form single-stranded DNA. A suspension of magnetic particles coated with an oligonucleotide probe specific for HBV amplicon or HBV Internal Control amplicon is added. The biotin-labeled HBV and HBV Internal Control amplicon are hybridized to the target-specific oligonucleotide probes bound to the magnetic particles.

Detection Reaction

Following the hybridization reaction, the COBAS AMPLICOR Analyzer washes the magnetic particles to remove unbound material and then adds Avidin-Horseradish Peroxidase Conjugate. The Avidin-Horseradish Peroxidase conjugate binds to the biotin-labeled amplicon. The COBAS AMPLICOR Analyzer removes unbound conjugate by washing the magnetic particles and then adds a substrate solution containing hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB). In the presence of hydrogen peroxide, the particle-bound horseradish peroxidase catalyzes the oxidation of TMB to form a colored complex. The absorbance is measured by the COBAS AMPLICOR Analyzer at a wavelength of 660 nm.

B. Description of Kit and Component Formulations

The COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit and the COBAS AMPLICOR™ Wash Buffer kit are provided as stand-alone kits to be used in conjunction with the COBAS AmpliScreen HBV Test.

COBAS AmpliScreen HBV Test — 96 Tests

Amplification Reagents

HBV MMX (HBV Master Mix) — 8 x 0.7 mL
Tris buffered solution with Ammonium Sulfate, glycerol, Tween 20, AmpliTaq DNA Polymerase, primers, dNTPs, AmpErase and sodium azide as a preservative

HBV Mg^{2+} (HBV Magnesium Solution) — 8 x 0.1 mL
Magnesium Chloride solution with indicator dye and sodium azide as a preservative

COBAS AmpliScreen HBV Detection Reagents

DN4 (Denaturation Solution) — 1 x 100 Tests
EDTA
Thymol blue
Sodium hydroxide

BH PS1 (HBV Probe Suspension 1) — 1 x 100 Tests
MES buffer solution containing capture oligonucleotides and magnetic microparticles with sodium aside as a preservative

BH4 (HBV Probe Suspension 2) — 1 x 100 Tests
Sodium phosphate buffer
Sodium thiocyanate
Solubilizer

BI PS1 (HBV IC Probe Suspension 1) — 1 x 100 Tests
MES buffer solution containing magnetic microparticles with capture oligonucleotides and sodium azide as a preservative

BI4 (HBV IC Probe Suspension 2) — 1 x 100 Tests
Sodium phosphate buffer containing sodium thiocyanate

CN4 (Avidin-Horseradish Peroxidase Conjugate) — 2 x 100 Tests
Tris-HCl buffer solution containing Avidin-horseradish peroxidase conjugate, bovine serum albumin, Emulsit 25 and phenol with ProClin® 150 as a preservative

SB3	(Substrate A)	10 x 75 Tests
Citrate solution containing hydrogen peroxide with ProClin® 150 as a preservative		
SB	(Substrate B)	10 x 75 Test
3,3′,5,5′-Tetramethylbenzidine (TMB) Dimethylformamide (DMF)		

COBAS AmpliScreen Multiprep Specimen Preparation and Control Kit — 96 Tests

MP (+) C	(Multiprep Positive (+) Control)	8 x 0.1 mL
Tris-HCl buffered solution containing noninfectious RNA transcripts for HCV and HIV-1 and noninfectious HBV DNA plasmid with EDTA and sodium azide as a preservative.		
MP LYS	(Multiprep Lysis Reagent)	8 x 9.0 mL
Tris-HCl buffered solution with Dithiothreitol, Glycogen and Guanidine thiocyanate		
MP DIL	(Multiprep Specimen Diluent)	8 x 4.8 mL
Tris-HCl buffered solution with EDTA and sodium azide as a preservative		
MP IC	(Multiprep Internal Control)	8 x 0.1 mL
Tris-HCl buffered solution with non-infectious internal control RNA transcripts for HCV and HIV-1 and DNA plasmid for HBV, Poly rA RNA, EDTA, indicator dye and sodium azide as a preservative.		
MP (–) C	(Multiprep Negative (-) Control)	8 x 0.1 mL
Poly rA RNA, EDTA and sodium azide as a preservative		
NHP	(Negative Plasma (Human))	16 x 1.6 mL
Human plasma, non-reactive by US FDA licensed tests for antibody to HIV-1/2, antibody to HCV and HBsAg, HCV RNA, HIV-1 RNA and HVB DNA with ProClin® 300 as a preservative.		

COBAS AMPLICOR Wash Buffer — 500 Tests

WB	(10X-Wash Concentrate)	2 x 250 Tests
Phosphate buffer solution containing detergent with ProClin® 300 as a preservative		

III. MANUFACTURING AND CONTROLS

A. Description of Manufacturing Facilities

The COBAS AmpliScreen HBV Test is manufactured by Roche Molecular Systems, Inc. (RMS) and prepared under U.S. License 1636. The corporate headquarters is located at 4300 Hacienda Drive, Pleasanton, California. The primary RMS manufacturing facility is located at 11 Franklin Avenue, Belleville, New Jersey. One component of the COBAS AmpliScreen HBV Test (the COBAS AmpliScreen Multiprep Internal Control) is produced in manufacturing laboratories in the RMS facility located at 1080 US Highway 202, Branchburg Township, Somerville, New Jersey (referred to as the Branchburg Facility). The Multiprep Positive Control is one of the Positive Controls manufactured for RMS by Lampire Biological Laboratories, Inc., located at 5185 Applebutter Road, Pipersville, Pennsylvania. The buffers used in the manufacturing of the Positive Controls are supplied by the RMS Belleville facility and RMS Alameda facility (1145 Atlantic Avenue, Alameda, California). Final product is stored and distributed from the RMS warehouse located at 2 Millenium Way, Branchburg Township, Somerville, New Jersey. Finished, approved product is distributed in the United States from the Roche Diagnostic distribution center located in Indianapolis, Indiana.

The DNA oligonucleotide primers (5′-Bio-HBV-104UB, 5′-Bio-HBV-104D and 5′-Bio-HBV-HW016TTBI) and probes (5′-NH_2-HBV-DET/I and 5′-NH_2-SK-535) used in the COBAS AmpliScreen HBV Test are manufactured synthetically on a DNA synthesizer and purified by high pressure liquid chromatography (HPLC) at RMS.

The AmpliTaq DNA Polymerase and Uracil-N-Gylcosylase (rUNG) enzymes used in the Test are manufactured at RMS. Both enzymes are grown in *E. coli* and purified by first chemically disrupting the cells and purifying the enzyme by HPLC.

The RNA Multiprep Positive and Internal Controls include purified RNA transcripts and linearized DNA fragments derived from plasmids grown in *E. coli*. The HIV and HCV RNA Multiprep Positive Controls (pSYC35 HIV and pHCVIIA HCV) and Internal Controls (pSDL150 HIV and pSYC52 HCV), and the HBV Plasmid Multiprep Positive Control (pCABN HBV) and Internal Control DNA (pTMN1 HBV), are purified from the

E. Coli host by first disrupting the cells, then purifying the plasmid by extraction, gradient centrifugation, and alcohol precipitation. RNA transcript is prepared by incubating the purified plasmid DNA with RNA polymerase and extracting the newly transcribed RNA by precipitating the RNA with alcohol. The RNA is further purified by column chromatography.

The linearized plasmid DNA is prepared by cutting the purified plasmid DNA with restriction digest enzymes followed by extraction, and alcohol precipitation.

The Negative Control is prepared from Human Plasma pools, which are tested and found to be negative for anti-HCV, HBV and HIV.

The raw materials used in this product are subjected to appropriate quality control evaluations before they are accepted for use in manufacturing. Acceptance criteria and performance specifications have been established for all test kit components. Components are assembled into test kits, each lot of which is subjected to a final performance test.

Each COBAS AmpliScreen HBV Test kit lot is tested with in-house panels of samples with varying HBV copy numbers/mL and must meet the performance requirements.

B. Stability Program

Components of the COBAS AmpliScreen HBV Test were entered into the stability program in order to define the recommended storage conditions and to establish the expiration dating period (i.e., shelf-life) for each component. The expiration date of the complete Test kit is defined on a lot-by-lot basis as the expiration date of the component lot with the shortest expiration date. In addition, components from the reserve and stability inventory that were at, or beyond shelf life were assembled into “virtual kits” and functional testing was performed.

The results of stability studies completed to date support the shelf-life claims summarized in the table below.

COBAS AmpliScreen. HBV Test
Quality Control Component Shelf-Life, Storage Temperature, and Stability Tests

Component	Proposed Shelf Life (Months)	Storage Temperature	Real Time Stability Tests
AmpliScreen Multiprep Lysis Reagent	18	2-8°C	Appearance, Color, pH, Assay (dithiothreitol)
AmpliScreen Multiprep Internal Control	18	2-8°C	Appearance, Color, Poisson Analysis
AmpliScreen Multiprep Specimen Diluent	24	2-8°C	Appearance, Color, Performance Test
AmpliScreen HBV Master Mix	12	2-8°C	Appearance, Color, Performance Tests
AmpliScreen HBV Magnesium Solution	12	2-8°C	Appearance, Color, Assay (magnesium), % Amaranth
AmpliScreen Multiprep Negative Control	24	2-8°C	Appearance, Color, Performance Test
AmpliScreen Multiprep Positive Control	18	2-8°C	Appearance, Color, Poisson Analysis (HIV, HCV, HBV)
Negative Plasma (Human)	24	2-8°C	Appearance, Color, Performance Tests
COBAS AMPLICOR Denaturation Solution	24	2-25°C	Appearance, Color, pH
COBAS AmpliScreen HBV Probe Suspension 1	12	2-8°C	Appearance, Color, Performance Test
COBAS AmpliScreen HBV Probe Suspension 2,	24	2-8°C	Appearance, Color, pH, Buffering Capacity, Assay (sodium thiocyanate)
COBAS AmpliScreen HBV IC Probe Suspension 1	12	2-8°C	Appearance, Color, Performance Test
COBAS AmpliScreen HBV IC Probe Suspension 2	24	2-8°C	Appearance, Color, pH, Buffering Capacity, Assay (sodium thiocyanate)
COBAS AMPLICOR Avidin-HRP Conjugate	21	2-8°C	Appearance, Color, pH, Performance Test
COBAS AMPLICOR Substrate A	24	2-8°C	Appearance, Color, pH, Assay (hydrogen peroxide)
COBAS AMPLICOR Substrate B	24	2-8°C	Appearance, Color, Assay (TMB)
COBAS AMPLICOR 10XWash Concentrate	24	2-25°C	Appearance, Color, pH, Working Reagent pH, Assay (sodium chloride)

C. Methods of Validation

All test kit components are monitored by in-process testing. Product purity and potency are assured through the evaluation of the product appearance, chemical testing, and performance testing. Product performance is assessed through quality release evaluations of the final test kit against an in-house panel containing negative control specimens and specimens that are known to be positive for HBV virus and the test kit must meet all performance requirements. The COBAS AmpliScreen HBV Test meets the FDA release requirements.

D. Labeling

The product labeling, including immediate container labels, box or package labels, and package insert have been reviewed for compliance with 21 CFR§610.60, 610.61, 610.62 and 809.10 and were found acceptable. The package insert clearly states the intended use of the COBAS AmpliScreen HBV Test as a qualitative *in vitro* test for the direct detection of Hepatitis B Virus DNA in human plasma.

Thistest is intended to be used for detection of HBV DNA in conjunction with licensed tests for detecting HBsAg and antibodies to hepatitis B core antigen (anti-HBc). This product is intended for use as a donor screening test to detect HBV DNA in plasma samples from individual human donors, including donors of whole blood and blood components, Source Plasma and other living donors. It is also intended for use to screen organ donors when specimens are obtained while the donor's heart is still beating. This test is not intended for use on specimens from cadaveric (non-heart-beating) donors. This test is not intended for use on samples of cord blood.

Plasma from all donors may be screened as individual samples. For donations of whole blood and blood components, plasma may be tested in pools comprised of equal aliquots of not more than 24 individual donations. For donations of Source Plasma, plasma may be tested in pools comprised of equal aliquots of not more than 96 individual donations. The product tradename, COBAS AmpliScreen HBV Test is not known to conflict with any other biologic or device tradename.

E. Establishment Inspection

An FDA inspection of the manufacturing facilities where the COBAS AmpliScreen product lines are manufactured, tested, stored and shipped was conducted from September 21, 2004 through September 28, 2004.

F. Environmental Impact Analysis, Claims for a Categorical Exclusion

Roche Molecular Systems, Inc. claimed a Categorical Exclusion from the submission of an Environmental Impact Statement with the COBAS AmpliScreen HBV Test, Biologics License Application. This claim for a Categorical Exclusion was made pursuant to

21 CFR 25.31 (j). The manufacture of the COBAS AmpliScreen HBV Test, is performed under controlled conditions and in compliance with the appropriate federal, state, and local environmental regulations. The disposal of waste from the use of this product is performed in compliance with appropriate federal, state, and local environmental regulations. Based on the materials, concentration, volumes used in this product, the method(s) of product disposal, it is unlikely that the release of any of the substances of this product at the expected level of exposure will be harmful to the environment or toxic to organisms in the environment.

IV. PERFORMANCE CHARACTERISTICS

A. Pre-clinical Studies Summary

1. Assay Cutoff

Study Description. The cut-off value for the COBAS AmpliScreen HBV Test using the Multiprep and the Standard Specimen Processing Procedures was determined by testing 506 seronegative plasma specimens drawn from blood donors and 201 HBV seropositive plasma specimens (collected from chronic HBV patients, BioClinical Partners, Inc. Franklin, MA). There were 2 and 4 seronegative specimens respectively for the Multiprep and Standard Specimen Processing Procedures with invalid IC results that were not included in the calculations. In addition, 10 HBV Genotype specimens whose DNA concentrations were determined by the COBAS AMPLICOR HBV MONITOR Test were diluted to a level near the assay's limit of detection and tested. The cut-off value for the COBAS AmpliScreen HBV Test was determined by creating a Cumulative Distribution Table of the clinical sensitivity and specificity vs. the proposed cut-off values for the test results of the positive and negative specimens used in the study. The complete description of all specimens included in the determination of the cutoff value is provided in the Table 1, Table 2, and Table 3.

Table 1: COBAS AmpliScreen HBV Test Results for HBV Sero-Negative Specimens

	Multiprep Specimen Processing Procedure	Standard Specimen Processing Procedure
Number Specimens Tested	504	502
A_{660} maximum	0.043	0.062
A_{660} minimum	0.001	0.000
Mean A_{660}	0.007	0.007
Standard Deviation	0.006	0.007

Table 2: COBAS AmpliScreen HBV Test, Standard Specimen Processing Procedure (Undiluted) and Multiprep Specimen Processing Procedure (1:24 Dilution), Summary for HBV Seropositive Specimens

	Standard Specimen Processing Procedure	Multiprep Specimen Processing Procedure	
Dilution Factor	Undiluted	1:24	
Number Specimens Tested	211	211	
Number of Positive Results	211	204	
		HBV Negative Results Excluded	HBV Negative Results Included
A_{660} maximum	4.000	4.000	4.000
A_{660} minimum	2.726	3.068	0.002
Mean A_{660}	3.700	3.799	3.673
Standard Deviation	0.323	0.246	0.722

Results: The data show a bimodal distribution of the A_{660} values for true positive and true negative specimens for both the Multiprep and Standard Specimen Processing procedures. This is expected for amplified nucleic acid tests where the goal of the procedure is to achieve large absorbance signals in the presence of low target levels. A cutoff value of ≥ 0.20 A_{660} was determined appropriate for the COBAS AmpliScreen HBV Test for both the Multiprep and Standard Specimen Processing Procedures.

Based on study results, a cutoff of ≥0.20 A_{660} would provide optimal clinical performance for either specimen processing procedure. The following table summarizes the test result validity criteria for Primary Pools, Secondary Pools, and Individual Specimens.

Table 3: Cutoff Summary and Test Result Validity Criteria

HBV Result		IC Result		Interpretation
A_{660}	Comment	A_{660}	Comment	
< 0.2	NEGATIVE	≥ 0.2	VALID	Specimen is negative for HBV DNA.
< 0.2	NEGATIVE	< 0.2	INVALID	Invalid result. Repeat entire test procedure for invalid specimen.
≥ 0.2	POSITIVE	ANY	VALID	Specimen is positive for HBV DNA

2. Analytical Sensitivity

a. Determination of Limit of Detection (LOD) Using the WHO HBV International Standard, 97/746

The Limit of Detection of the COBAS AmpliScreen HBV Test, was determined using the WHO HBV International Standard (97/746). The WHO HBV International Standard was diluted in HBV negative plasma to final concentrations of 300, 100, 30, 20, 15 and 10 IU/mL for the Standard Specimen Processing Procedure and 100, 30, 10, 5, 4 and 3 IU/mL for the Multiprep Specimen Processing Procedure. Each dilution was tested using two lots of COBAS AmpliScreen HBV Test at 60 replicates per lot.

When evaluated using PROBIT analysis, the combined data from all specimens using the Multiprep Specimen Processing Procedure indicate an average 95% LOD of 4.41 IU/mL, with lower and upper 95% confidence limits of 3.56 IU/mL and 6.13 IU/mL, respectively. The LOD of 4.41 IU/mL corresponds to approximately 22 copies/mL.

When evaluated using PROBIT analysis, the combined data from all specimens tested using the Standard Specimen Processing Procedure indicate an average 95% LOD of

15.99 IU/mL, with lower and upper 95% confidence limits of 13.78 IU/mL and 20.06 IU/mL, respectively. The LOD of 15.99 IU/mL corresponds to approximately 80 copies/mL.

Table 4 and Table 5, shown below, summarize the overall results for the Multiprep and Standard Specimen Processing Procedures, respectively.

Table 4: HBV WHO International Standard: Dilution Testing Summary for Multiprep Method — Combined Input Values with 95% Confidence Limits

HBV DNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – Two Tailed (One-Tailed)	95% Upper Confidence Limit – Two Tailed
100	120	120	100.0%	97.0% (97.5%)	100.0%
30	120	120	100.0%	97.0% (97.5%)	100.0%
10	120	120	100.0%	97.0% (97.5%)	100.0%
5	**115**	**120**	**95.8%**	**90.5% (91.4%)**	**98.6%**
4	108	120	90.0%	83.2% (84.3%)	94.7%
3	112	120	93.3%	87.3% (88.3%)	97.1%

Table 5: HBV WHO International Standard: Dilution Testing Summary for Standard Method — Combined Input Values with 95% Confidence Limits

HBV DNA Concentration (IU/mL)	Number of Positives	Number of Individual Trials	% Positive	95% Lower Confidence Limit – Two Tailed (One-Tailed)	95% Upper Confidence Limit – Two Tailed
300	120	120	100.0%	97.0% (97.5%)	100.0%
100	120	120	100.0%	97.0% (97.5%)	100.0%
30	118	119	99.2%	95.4% (96.1%)	100.0%
20	116	120	96.7%	91.7% (92.5%)	99.1%
15	**115**	**120**	**95.8%**	**90.5% (91.4%)**	**98.6%**
10	101	120	84.2%	76.4% (77.6%)	90.2%

b. Sensitivity — Genotype Detectability

One hundred fifteen specimens were diluted to 60 copies/mL and 200 copies/mL with HBV Negative Human Plasma (16 Genotype A, 22 Genotype B, 16 Genotype C, 8 Genotype D, 16 Genotype E, 22 Genotype F, 1 Genotype G, 3 Genotype A/C, 2 Genotype A/D, 2 Genotype C/D, 2 Genotype D/E, and 1 each of Genotypes A/E, B/C, C/E, D/F, and E/F). All the genotypes tested positive by the COBAS AmpliScreen HBV Test at 60 copies/mL with the Multiprep Specimen Processing Procedure, and at 200 copies/mL with the Standard Specimen Processing Procedure. An additional 13 specimens (2 Genotype A, 2 Genotype B, 2 Genotype C, 2 Genotype D, 2 Genotype E and 3 Genotype F) were diluted in HBV Negative Human Plasma and tested in replicates of 22 with the COBAS AmpliScreen HBV Test at a level which resulted in a ≥ 95% detection rate (2 to 100 copies/mL for the Multiprep Specimen Processing Procedure and 15 to 400 copies/mL for the Standard Specimen Processing Procedure). All of the genotypes tested positive by the COBAS AmpliScreen HBV Test. As a result of limited availability, only one Genotype G was evaluated. Therefore, performance of the COBAS AmpliScreen HBV Test with this one Genotype G specimen may not be representative of all Genotype G specimens. Data are provided in Table 6.

Table 6: HBV Genotypes Tested

Genotypes	Quantity	Reactive Total (Multiprep)	Reactive Total (Standard Prep)
A	18	18/18	18/18
B	24	24/24	24/24
C	18	18/18	18/18
D	10	10/10	10/10
E	18	18/18	18/18
F	25	25/25	25/25
G	1	1/1	1/1
A/C	3	3/3	3/3
A/D	2	2/2	2/2
C/D	2	2/2	2/2
D/E	2	2/2	2/2
A/E	1	1/1	1/1
B/C	1	1/1	1/1
C/E	1	1/1	1/1
D/F	1	1/1	1/1
E/F	1	1/1	1/1

Genotype Sensitivity with Plasmid DNA Clones

To evaluate the analytical sensitivity of the COBAS AmpliScreen HBV Test, two plasmid isolates for each of six HBV genotypes (genotypes A, B, C, D, E, and F) were diluted in Multiprep Specimen Diluent to concentrations of 120, 80, 40, and 20 copies per mL. Aliquots of each dilution (equivalent to 6, 4, 2 and 1 copies per PCR reaction) were added directly to the COBAS AMPLICOR Analyzer A-tubes, without prior specimen processing, for amplification and detection. Twenty-two replicates were tested for each dilution. A result was considered positive if the HBV A_{660} was ≥ 0.2.

The COBAS AmpliScreen HBV Test detected all genotypes with a positivity rate of >95% at 4 copies per PCR reaction (equivalent to 80 copies/mL in the original dilution), except for Genotype C, clone p11549-1, which had a positivity rate of 100% at 2 copies per PCR and 68% at 1 copy per PCR. For the other 11 genotype plasmid specimens, the positivity rate at 2 copies per PCR ranged from 73% to 91%.

This study, evaluating the analytical sensitivity of the COBAS AmpliScreen HBV Test, demonstrates equivalent detection of 12 plasmid DNA clones representing HBV Genotypes A, B, C, D, E, and F, with a ≥ 95% positivity rate of 2 to 4 copies per PCR reaction (40 to 80 copies/mL)

c. Sensitivity with Seroconversion Panels

Forty commercially available HBV seroconversion panels were tested using the COBAS AmpliScreen HBV Test. Each panel member (specimen) was tested undiluted, using the Standard Specimen Processing Procedure to simulate single unit testing, and diluted 1:24 with HBV negative human plasma, using the Multiprep Specimen Processing Procedure to simulate 24-specimen mini-pool testing. The COBAS AmpliScreen HBV Test results were then compared to the results from a FDA licensed HBV surface antigen (HBsAg) test (Ortho HBsAg ELISA Test System 3) and an unlicensed HBsAg Test (Abbott PRISM HBsAg test) to determine if the COBAS AmpliScreen HBV Test detected the presence of HBV DNA prior to detection of HBsAg.

For all seroconversion panels, HBV DNA was detected earlier, or at the same bleed as HBsAg by the HBsAg test (Table 7 and Table 8). This was true for both the Multiprep Specimen Processing Procedure, and for the Standard Specimen Processing Procedure.

Table 7: Seroconversion Panel Summary — Detection of HBV DNA Prior to HBsAg

	COBAS AmpliScreen Multiprep Procedure (Specimens diluted 1:24 before testing)		**COBAS AmpliScreen Standard Procedure (Specimens not diluted before testing)**	
	Prior to Ortho HBsAg System 3	**Prior to Abbott PRISM HBsAg**	**Prior to Ortho HBsAg System 3**	**Prior to Abbott PRISM HBsAg**
# Panels w/ PCR Earlier Detection	38	34	39	38
# Specimens w/ PCR Earlier Detection	140	105	190	155
# of Informative Panels	40	40	40	40

Table 8: Summary of COBAS AmpliScreen HBV Test — Pre-Seroconversion Detection of HBV DNA vs. HBsAg

	COBAS AmpliScreen Multiprep Procedure (Specimens diluted 1:24 before testing)		COBAS AmpliScreen Standard Procedure (Specimens not diluted before testing)	
	Days before Ortho HBsAg System 3	Days before Abbott PRISM HBsAg	Days before Ortho HBsAg System 3	Days before Abbott PRISM HBsAg
Mean	15	14	20	19
Median	14	11	18	15
Minimum	0	0	0	0
Maximum	30	94	53	94

** One seroconversion panel was not included in the calculations which was detected by the COBAS AmpliScreen HBV Test 108+ days prior to the Ortho HBsAg ELISA Test System 3. In addition, the calculations do not include the results for five seroconversion panels tested with the Multiprep Specimen Processing Procedure and three seroconversion panels tested by the Standard Specimen Processing Procedure that were intermittently HBV DNA positive up to 100+ days prior to the Ortho test.*

Each of these seroconversion panels represents serial collections derived from HBV infected individuals who provided these specimens before and during the "window period" of HBV infection.

In no instance, was there a specimen that showed HBsAg reactivity, yet was negative for HBV DNA. Using the Multiprep Specimen Processing Procedure, the COBAS AmpliScreen HBV Test detected HBV DNA an average of at least 15 and 14 days prior to the Ortho System 3 and Abbott PRISM HBsAg assays, respectively. Using the Standard Specimen Processing Procedure, the COBAS AmpliScreen HBV Test detected HBV DNA an average of at least 20 and 19 days prior to detection of HBsAg by the Ortho System 3 and Abbott PRISM HBsAg assays, respectively.

The results of these studies provide objective evidence that the COBAS AmpliScreen HBV Test, using either the Multiprep or Standard Specimen Processing Procedures, demonstrates greater sensitivity than observed with current HBsAg serology assays in detecting early HBV infection.

3. Analytical Specificity

a. Analytical Specificity — Potentially Cross Reactive and Interfering Microorganisms

The analytical specificity of the COBAS AmpliScreen HBV Test was evaluated in two separate studies. In the first study a panel of 38 microorganisms, including 33 viral isolates and 5 bacterial strains, were evaluated. The microorganism concentrations tested are shown in Table 9 Viral and bacterial isolates were extracted from a background of negative human plasma using the Multiprep Specimen Processing Procedure. A 30 µL to 1000 µL aliquot of each stock isolate was processed, representing 790 to approximately 1.58×10^8 TCID[50] units or copies per PCR test.

Table 9: COBAS AmpliScreen HBV Test, Microorganism Exclusivity Study using Multiprep Specimen Processing Procedure

Specimen Description	ID#	Strain/Lot #	Titer	Units/PCR	A_{660}	
					HBV	IC
Adenovirus, Human Type 2	VR-846	7D	1.58E7 $TCID_{50}$/mL	3.95E5	0.004	3.046
Adenovirus, Human Type 3	VR-3	8W	1.58E5 $TCID_{50}$/mL	7.90E3	0.004	3.568
Adenovirus, Human Type 7	VR-7	9W	1.58E5 $TCID_{50}$/mL	7.90E3	0.004	4.000
Cytomegalovirus	VR-538	24W	2.81E4 $TCID_{50}$/mL	1.41E3	0.004	4.000
Cytomegalovirus	VR-807	Davis	2.81E4 $TCID_{50}$/mL	1.41E3	0.004	4.000
Cytomegalovirus	VR-977	Towne	8.89E4 $TCID_{50}$/mL	1.56E4	0.004	4.000
Herpes Simplex type 1	VR-260	27W	1.58E8 $TCID_{50}$/mL	7.90E6	0.004	4.000
Herpes Simplex type 2	VR-540	10D	8.89E7 $TCID_{50}$/mL	8.89E6	0.004	4.000
Hepatitis A	VR-1358	2W	1.58E7 $TCID_{50}$/mL	1.98E5	0.004	4.000
Human Papilloma Virus, Type 6a	45150	97-12	Plasmid	Plasmid	0.004	4.000
Human Papilloma Virus, Type 16	45113	97-11	Plasmid	Plasmid	0.003	4.000
Human Papilloma Virus, Type 18	45152	1391460	Plasmid	Plasmid	0.003	4.000
HTLV-I	CRL-8293	-	4.5E6 copies/mL	1.13E5	0.004	4.000
HTLV-II	CRL-8066	1417209	1.1E7 copies/mL	1.10E6	0.004	4.000
Chlamydia trachomatis	VR-348B	Bour	5E4 $TCID_{50}$/mL	5.00E3	0.004	4.000
Neisseria gonorrhoeae	19424	-	3E6 copies/mL	1.20E5	0.004	4.000
Epstein Barr Virus (Burkitt's lymphoma)	VR-602	P-3	Viable cell	Viable cell	0.003	4.000

Specimen Description	ID#	Strain/Lot #	Titer	Units/PCR	A_{660}	
					HBV	IC
Epstein Barr Virus (RAJI Human Burkitt's lymphoma)	CCL-86	Raji	7.1E6 copies/mL	1.78E5	0.004	3.745
Echovirus 1	VR-31	Farouk, 5D	5.6E4 $TCID_{50}$/mL	1.40E3	0.004	4.000
Echovirus 5	VR-35	Noyce, 4W	1.58E8 $TCID_{50}$/mL	4.94E6	0.004	4.000
Coxsackievirus B1	VR-28	Conn-5	1.58E9 $TCID_{50}$/mL	1.58E8	0.003	4.000
Varicella-Zoster	VR-586	Ellen, Lot 24W	1.58E4 $TCID_{50}$/mL	7.90E2	0.004	3.745
Varicella	VR-795	Lot 7W	1.58E4 $TCID_{50}$/mL	7.90E2	0.004	3.746
Propionibacterium acnes	6919	1375853	2.3E8 copies/mL	2.30E7	0.004	4.000
Staphylococcus aureus	12598	1671261	3.3E9 copies/mL	2.50E7	0.003	4.000
Staphylococcus epidermidis	14990	1423966	2E7 copies/mL	1.15E6	0.004	4.000
HCV 1a	MB#	3438	8E5 IU/mL	2.00E5	0.003	4.000
HCV 1b	MB#	3441	1.4E5 IU/mL	3.50E4	0.003	4.000
HCV 2a/2c	MB#	3453	7.7E6 IU/mL	1.93E6	0.004	4.000
HCV 2b	MB#	3452	3E6 IU/mL	7.50E5	0.003	4.000
HCV 3a	MB#	3448	9.5E5 IU/mL	2.38E5	0.003	4.000
HIV Subtype A	SBC##	GS003	1.0E6 copies/mL	2.50E5	0.004	4.000
HIV Subtype B	SBC##	GS004	1.0E6 copies/mL	2.50E5	0.005	4.000
HIV Subtype C	SBC##	GS011	1.0E6 copies/mL	2.50E5	0.005	4.000
HIV Subtype D	SBC##	GS018	1.0E6 copies/mL	2.50E5	0.005	3.746
HIV Subtype E	SBC##	GS021	1.0E6 copies/mL	2.50E5	0.005	4.000
HIV Subtype F	SBC##	GS032	1.0E6 copies/mL	2.50E5	0.004	4.000
HIV Subtype G	SBC##	GS029	1.0E6 copies/mL	2.50E5	0.004	4.000

The following categories were assessed in the second study: clinical specimens from patients infected with HIV-1, HIV-2, EBV, CMV, HAV or HCV, specimens derived from patients having autoimmune disease, and negative human plasma specimens spiked with *Candida albicans*. Ten to twenty-five individual patient plasma specimens from each disease category, confirmed to be serologically positive (with the exception of *Candida albicans*) were obtained from commercial vendors. For the *Candida albicans* specimens, a stock culture from Roche Molecular Systems was spiked to 1000 cells/mL in each of ten negative human plasma specimens. For each disease state, all specimens were tested using both the Multiprep and Standard Specimen Processing Procedures.

The COBAS AmpliScreen HBV Test data presented in Table 9 demonstrates that all microorganism specimens tested produced negative results for HBV DNA, with A_{660} values ranging from 0.003 to 0.005 using the Multiprep Specimen Processing Procedure. In addition, the Internal Control A_{660} values were all positive, indicating that no inhibition had occurred.

No reactivity was observed in the COBAS AmpliScreen HBV Test for all disease category specimens tested, using both the Multiprep and Standard Specimen Processing Procedures.

The results of these specificity studies demonstrate that the COBAS AmpliScreen HBV Test does not cross-react with the 38 microorganisms tested using the Multiprep Specimen Processing Procedure. In addition, using the Multiprep or Standard Specimen Processing Procedures, the COBAS AmpliScreen HBV Test does not cross-react with HBV negative patient specimens that are serologically positive for HAV, HCV, HIV-1, HIV-2, EBV, CMV, autoimmune markers, or with negative human plasma specimens spiked with *Candida albicans.*

b. Analytical Specificity — Co-Infections

Clinical specimens from patients infected with HAV, HCV, HIV-1, HIV-2, EBV or CMV, specimens derived from patients having autoimmune disease, and negative human plasma specimens spiked with *Candida albicans* were tested to determine the potential for non-HBV infectious organisms, and autoimmune disease, in specimens also containing low levels of HBV, to interfere with the sensitivity of the COBAS AmpliScreen HBV Test. Ten to twenty-five individual patient plasma specimens from each disease category were tested. A HBV positive plasma specimen (quantitated using the COBAS AMPLICOR HBV MONITOR Test) was used to spike each specimen, representing each of the disease categories described above, to a final concentration of 90 copies/mL for the Multiprep Specimen Processing Procedure and 300 copies/mL for Standard Specimen Processing Procedure.

For all specimens in the various disease categories that were spiked with HBV, each of the specimens tested were positive when using both the Multiprep and Standard Specimen Processing Procedures.

The results of these interference studies demonstrate that clinical specimens serologically positive for HAV, HCV, HIV-1, HIV-2, EBV, CMV, autoimmune markers or *Candida albicans*, when spiked with low levels of HBV, did not interfere with the sensitivity of the COBAS AmpliScreen HBV Test, using either specimen processing procedure.

4. Potentially Interfering Substances

a. Endogenous Interfering Substances

HBV spiked and non-spiked plasma specimens derived from whole blood containing abnormally high concentrations of bilirubin (up to 20 mg/mL), triglycerides (up to 3000 mg/dL), hemoglobin (up to 1.0 g/dL), and albumin (up to 6 g/dL) were tested. These endogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HBV Test using either the Multiprep or Standard Specimen Processing Procedures.

b. Exogenous Interfering Substances

HBV spiked and non-spiked plasma specimens derived from whole blood containing abnormally high concentrations of aspirin (up to 50 mg/mL), pseudoephedrine-HCl (up to 3 mg/dL), ascorbic acid (up to 20 mg/dL), acetaminophen (up to 40 mg/dL), or ibuprofen (up to 40 mg/dL) were tested. These exogenous substances did not interfere with the sensitivity or specificity of the COBAS AmpliScreen HBV Test using either the Multiprep or Standard Specimen Processing Procedures.

5. Uracil-N-Glycosylase (UNG) Performance

AmpErase (uracil-N-glycosylase, UNG) catalyzes the degradation of DNA containing deoxyuridine, but not DNA containing thymidine or RNA containing uridine. Deoxyuridine is not a constituent of the HBV Target DNA, but is always present in amplicon. In the AmpliScreen HBV Master Mix reagent, deoxyuridine triphosphate

replaces thymidine triphosphate as one of the dNTPs. Only target amplicon containing deoxyuridine is susceptible to UNG-mediated degradation prior to amplification of the target DNA. Therefore, AmpErase is an effective countermeasure against inadvertent amplicon carryover.

B. Clinical Trials Summary — Whole Blood

1. Reproducibility

The reproducibility of the COBAS AmpliScreen HBV Test was established by testing two 6-member EDTA plasma panels with known concentrations of HBV. Panel One, which was tested by using the Multiprep Specimen Processing Procedure, was composed of HBV DNA positive specimens at concentrations of 25, 90, 150, and 25,000 copies/mL and two HBV negative specimens. Panel Two, which was tested by using the Standard Specimen Processing Procedure was composed of HBV positive specimens at concentrations of 75, 300, 500 and 25,000 copies/mL and two HBV negative specimens.

Testing was performed at three sites with two operators at each site using three COBAS AmpliScreen HBV Test kit lots and analyzed in 5 different days. Each operator used a dedicated COBAS AMPLICOR Analyzer throughout the study. Each operator was provided panel sets that had been randomized and labeled in blinded fashion.

All valid reproducibility data were evaluated by calculating the percentage of correct results for each panel member. The data were analyzed by site, lot, testing day, run, and operator for each Specimen Processing Procedure (Multiprep and Standard).

The reproducibility study for the COBAS AmpliScreen HBV Test demonstrated consistency by lot and site for both the Multiprep and Standard Specimen Processing Procedures as seen in Table 10 and Table 11. The reproducibility by operator is shown in Table 12 and Table 13.

Table 10: Reproducibility Results — Multiprep Specimen Processing Procedure

Results By Lot (# Positive / # Tested)					
	Negative	**25 c/mL**	**90 c/mL**	**150 c/mL**	**25,000 c/mL**
Lot #1 (%)	0/180 (0%)	75/90 (83%)	89/90 (99%)	89/90 (99%)	90/90 (100%)
Lot #2 (%)	0/178 (0%)	75/90 (83%)	87/88 (99%)	89/90 (99%)	90/90 (100%)
Lot #3 (%)	1/179 (1%)	76/89 (85%)	88/89 (99%)	90/90 (100%)	90/90 (100%)
Results By Site (# Positive / # Tested)					
Site #1 (%)	0/180 (0%)	80/89 (90%)	90/90 (100%)	90/90 (100%)	90/90 (100%)
Site #2 (%)	0/177 (0%)	76/90 (84%)	84/87 (97%)	88/90 (98%)	90/90 (100%)
Site #3 (%)	1/180 (1%)	70/90 (78%)	90/90 (100%)	90/90 (100%)	90/90 (100%)

Table 11: Reproducibility Results — Standard Specimen Processing Procedure

Results By Lot (# Positive / # Tested)					
	Negative	**75 c/mL**	**300 c/mL**	**500 c/mL**	**25,000 c/mL**
Lot #1 (%)	0/179 (0%)	76/89 (85%)	89/89 (100%)	90/90 (100%)	90/90 (100%)
Lot #2 (%)	1/179 (1%)	73/90 (81%)	88/89 (99%)	88/90 (98%)	90/90 (100%)
Lot #3 (%)	0/180 (0%)	78/90 (87%)	90/90 (100%)	90/90 (100%)	90/90 (100%)
Results By Site (# Positive / # Tested)					
Site #1 (%)	0/180 (0%)	72/89 (81%)	89/89 (100%)	90/90 (100%)	90/90 (100%)
Site #2 (%)	0/179 (1%)	76/90 (4%)	88/89 (99%)	88/90 (98%)	90/90 (100%)
Site #3 (%)	0/179 (0%)	79/90 (88%)	90/90 (100%)	90/90 (100%)	90/90 (100%)

Table 12: Operator Variability Data — Multiprep Specimen Processing Procedure

Results by Operator / Instrument (# Positive / # Tested)					
Operator	**Negative**	**25 c/mL**	**90 c/mL**	**150 c/mL**	**25,000 c/mL**
1	0/90 (0%)	39/44 (88.6%)	45/45 (100%)	45/45 (100%)	45/45 (100%)
2	0/90 (0%)	41/45 (91.1%)	45/45 (100%)	45/45 (100%)	45/45 (100%)
3	0/90 (0%)	44/45 (97.8%)	45/45 (100%)	45/45 (100%)	45/45 (100%)
4	0/90 (0%)	32/45 (71.1%)	39/42 (92.2%)	43/45 (95.6%)	45/45 (100%)
5	0/90 (0%)	35/45 (77.8%)	45/45 (100%)	45/45 (100%)	45/45 (100%)
6	1/90 (1.1%)	35/45 (77.8%)	45/45 (100%)	45/45 (100%)	45/45 (100%)

Table 13: Operator Variability Data — Standard Specimen Processing Procedure

Results by Operator / Instrument (# Positive / # Tested)					
Operator	**Negative**	**75 c/mL**	**300 c/mL**	**500 c/mL**	**25,000 c/mL**
1	0/90 (0%)	39/44 (88.6%)	45/45 (100%)	45/45 (100%)	45/45 (100%)
2	0/90 (0%)	33/45 (73.3%)	44/44 (100%)	45/45 (100%)	45/45 (100%)
3	0/90 (0%)	41/45 (91.1%)	45/45 (100%)	45/45 (100%)	45/45 (100%)
4	1/89 (1.1%)	35/45 (77.8%)	43/44 (97.7%)	43/45 (95.6%)	45/45 (100%)
5	0/89 (0%)	41/45 (91.1%)	45/45 (100%)	45/45 (100%)	45/45 (100%)
6	0/90 (0%)	38/45 (84.4%)	45/45 (100%)	45/45 (100%)	45/45 (100%)

2. Pool Reactivity in Whole Blood

Of the 25,845 pools tested, 25,695 were negative for HBV DNA and 150 (0.58%) were initially reactive. Of the 150 initially reactive pools, 85 pools resolved to a positive HBV DNA individual specimen with concordant serology, and 2 positive pools were due to window cases. There were 51 pools that were initially reactive but determined to be HBV DNA negative upon resolution testing. A total of 9 pools were found positive but were not confirmed positive by serology or by subsequent testing of individual specimens by the COBAS AmpliScreen HBV Test. There were 3 pools with two positive units, one with concordant serology and one without concordant serology. Pool reactivity data from volunteer blood donors is summarized in Table 14.

Table 14: Pool Reactivity in Whole Blood

Category	Pools	Percentage
Pools Tested	25,845	100%
Non-reactive pools	25,695	99.42%
Initially reactive pools	150	0.58%
Initial pools with concordant positive serology	85	0.33%
Positive pools due to window period case	2	0.008%
Initially reactive pools with negative serology and negative individual specimen AmpliScreen testing (false positive)	51	0.2%
Initial pools with positive COBAS resolution and without concordant serology	9	0.03%
Initial pools with 2 positive COBAS resolutions; one concordant with serology and one without concordant serology	3	0.01%

A total of 581,790 specimens were tested from 5 geographically divergent sites. The results from these specimens were used to determine the specificity and sensitivity of COBAS AmpliScreen HBV Test. The HBV serology status of each specimen was determined using each specimens antigen and antibody results. HBV serology status-negative included specimens that were HBsAg and anti-HBc negative unless the subject was enrolled in the follow-up study and had test results that changed this assessment.

HBV serology status positive included those specimens that were HBsAg positive regardless of the anti-HBc results unless the subject was enrolled in the follow-up study and had test results that changed this assessment. HBV serology status unknown included those specimens that were anti-HBc positive and HBsAg negative.

There were 578,694 specimens that were determined to be HBV serology status negative 2 HBV MP NAT positive serology negative). Of these, 578,673 were also HBV DNA negative (21 specimens were false positive). The specificity of the COBAS AmpliScreen HBV Test in this study was 578,673/578,694 or 99.9964% with 95% confidence limits of 99.9948% to 99.9979%.

During the clinical studies, there were 105 HBV specimens confirmed positive for HBsAg which were also tested with the COBAS AmpliScreen HBV Test. There were 87 specimens (82.9%) HBV DNA positive and 18 specimens (17.1%) HBV DNA negative using the Multiprep Specimen Processing Procedure. Of these 105 HBsAg positive specimens, 104 specimens were tested individually using the Standard Specimen Processing Procedure, and 97 specimens (93.3%) tested DNA positive and 7 specimens (6.7%) tested DNA negative. The specimens discordant with serology when tested individually using the Standard Specimen Processing Procedure were a subset of the specimens which were discordant with serology when tested using the Multiprep Specimen Processing Procedure. *See* Table 15 and Table 16.

Table 15: HBV Seropositive Specimens — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24)

		HBV DNA Results
Multiprep Procedure	**+**	87 (82.9%)
	–	18*
Total		105

** 6 of 18 specimens were negative for HBV DNA by alternate qualitative NAT and specimens from 3 of 18 were not available for testing by alternate qualitative NAT. 11 of 18 were quantitated for HBV DNA by alternate quantitative NAT and 7 of these contained < 300 copies/mL HBV DNA.*

Table 16: HBV Seropositive Specimens — Results for Standard Specimen Processing Procedure (Specimens Undiluted)

		HBV DNA Results
Standard Procedure	**+**	97 (93.3%)
	–	7*
Total		104

** 4 of 7 specimens were negative for HBV DNA by alternate NAT 1 was not tested by alternate NAT and the remaining 2 quantified by alternate NAT had HBV DNA < 100 copies/mL).*

It should be noted that 3 of the specimens with 1200 copies/mL, 2600 copies/mL and 5900 copies/mL of DNA that were also tested positive for HBsAg and anti-HBc were negative on mini-pool NAT.

3. Single Donation Testing Performance

A total of 1754 specimens for which serology results were available were tested individually in the COBAS AmpliScreen HBV Test clinical trial. The results are shown in Table 17. There were 89/1754 classified as HBV status positive (87 were HBsAg, anti-HBc and HBV DNA positive and 2 were HBV window period cases based on follow-up testing). In the absence of follow-up testing 13/1754 were classified as HBV status unknown (12 were HBsAg negative, anti-HBc positive and HBV DNA negative, and 1 was HBsAg negative, anti-HBc positive and HBV DNA positive). There were 1652/1754 classified as HBV status negative and of these, 1625/1652 were COBAS AmpliScreen HBV Test negative. Of the remaining 27 specimens that tested positive by the COBAS AmpliScreen HBV Test and negative by serology testing, 12 were enrolled in the follow-up study and determined to be false positives. Based on these results, all 27 were presumed to be false positives on the COBAS AmpliScreen HBV Test. The specificity of the COBAS AmpliScreen HBV Test in this study was 98.4% (1625/1652) with a 95% confidence interval of 97.6% to 98.9%.

Table 17: Paired Specimen Results for Individual Samples with Assigned HBV Status

HBsAg Result	Anti-HBc Result	COBAS AmpliScreen DNA Result	Status	Total
Negative	NR	Negative	Negative	1625
Negative	RR	Negative	Unknown	12
Negative	NR	Positive	Negative	27
Negative	NR	Positive	Positive*	2
Negative	RR	Positive	Unknown	1
Positive	Any	Negative	Positive	0
Positive	Any	Positive	Positive	87
			Total	1754

** Status Positive reclassified due to follow-up results.*

4. Detection of HBV DNA in Seropositive Specimens

A total of 1177 known HBV seropositive (HBsAg positive) specimens were tested by the COBAS AmpliScreen HBV Test. These HBV seropositive specimens included the following specimens and sources: 723 HBV seropositive specimens, including 49 acute patients (HBsAg and HBeAg positive) obtained from commercial vendors and blood banks in the US, Japan and China; 100 chronic HBV patient specimens (HBsAg positive for at least 6 months) obtained from a commercial vendor; 60 HBsAg seropositive specimens collected from patients at high risk for hepatitis, and 189 HBsAg positive specimens from 40 seroconversion panels. These specimens were tested using both the Multiprep Specimen Processing Procedure (specimens diluted 1:24 in negative human plasma) and Standard Specimen Processing Procedure (specimens tested undiluted). An additional 105 HBsAg positive specimens were tested in the Clinical Performance study. These specimens were initially tested in primary mini-pools of 24 specimens using the Multiprep Specimen Processing Procedure, and tested individually using the Standard Specimen Processing Procedure.

Seropositive Specimens (Including 49 Acute Patients) from Commercial Vendors

A total of 723 HBV seropositive specimens, including 49 acute patients (HBsAg and HBeAg positive) obtained from commercial vendors and blood banks in the US, Japan, and China were tested with the COBAS AmpliScreen HBV Test.

Of the 723 HBsAg positive specimens that were tested using the Multiprep Specimen Processing Procedure, 694 specimens (96.0%) tested HBV DNA positive and 29 specimens (4.0%) were HBV DNA negative. Of these 723 HBsAg positive specimens that were tested individually using the Standard Specimen Processing Procedure, 708 specimens (97.9%) tested HBV DNA positive and 15 specimens (2.1%) tested HBV DNA negative. The specimens discordant with serology when tested individually using the Standard Specimen Processing Procedure were a subset of the specimens which were discordant with serology when tested using the Multiprep Specimen Processing Procedure. *See* Table 18 and Table 19.

Table 18: HBV Seropositive Specimens (Including 49 Acute Patients) from Commencial Vendors — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24)

		HBV DNA Results
Multiprep Procedure	**+**	694 (96.0%)
	–	29*
Total		723

** 22 of the 29 HBV DNA negative specimens were tested by alternate HBV DNA tests and were negative (< 300 copies/mL). Includes 7 Specimens which were not retested by alternate HBV DNA tests due to insufficient volume.*

Table 19: HBV Seropositive Specimens (Including 49 Acute Patients) from Commencial Vendors — Results for Standard Specimen Processing Procedure (Specimens Undiluted)

		HBV DNA Results
Standard Procedure	**+**	708 (97.9%)
	–	15*
Total		723

All HBV DNA negative specimens were tested by alternate HBV DNA tests and contained < 300 copies/mL HBV DNA.

5. High Risk Population

Specimens were collected from patients who were being evaluated in hematology clinics for biochemical, clinical and/or histological evidence for liver disease and/or evidence of hepatitis virus infection. Specimens were excluded from the study if the patient had received anti-viral therapy within 6 months of being screened. Specimens were tested blindly until 50 specimens tested positive by the COBAS AmpliScreen HBV Test using the Standard Specimen Processing Procedure.

A total of 80 specimens, 60 HBsAg-positive and 20 HBsAg-negative, from patients at high-risk for liver disease were tested using the COBAS AmpliScreen HBV Test. Of the 60 HBsAg-positive specimens tested, 59 specimens (98.3%) were concordant and one specimen (1.7%) was discordant with HBsAg serologic results using the Multiprep Specimen Processing Procedure, and all 60 specimens (100%) were concordant using the Standard Specimen Processing Procedure. The 20 HBsAg-negative specimens were also negative for HBV DNA when tested using the Standard Specimen Processing Procedure and the Multiprep Specimen Processing Procedure.

The HBV DNA concentration in the one discordant specimen was determined using the COBAS AMPLICOR HBV MONITOR Test, which has a limit of quantitation (LOQ) of 300 HBV DNA copies/mL. HBV DNA was not detected in this specimen indicating that the HBV DNA level in this specimen was below 300 HBV DNA copies/mL. Data are shown in Table 20 and Table 21.

Table 20: HBV Seropositive Specimens from Patients at High Risk for Liver Disease — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24)

		HBV DNA Results
Multiprep Procedure	**+**	59 (98.3%)
	–	1*
Total		60

** The HBV DNA negative specimen was tested by alternate HBVDNA tests and contained < 300 copies/mL HBV DNA*

Table 21: HBV Seropositive Specimens from Patients at High Risk for Liver Disease — Results for Standard Specimen Processing Procedure (Specimens Undiluted)

		HBV DNA Results
Standard Procedure	**+**	60 (100%)
	–	0
Total		60

6. Sensitivity in Chronic HBV Patients

A total of 100 HBV seropositive specimens from chronic HBV patients were tested with the COBAS AmpliScreen HBV Test. Of the 100 HBsAg positive specimens, there were 78 specimens (78.0%) HBV DNA positive and 22 specimens (22.0%) HBV DNA negative with the Multiprep Specimen Processing Procedure, and 95 specimens (95.0%) HBV DNA positive and 5 specimens (5.0%) HBV DNA negative with the Standard Specimen Processing Procedure. The specimens discordant with serology when tested individually using the Standard Specimen Processing Procedure were a subset of the specimens which were discordant with serology when tested using the Multiprep Specimen Processing Procedure. *See* Table 22 and Table 23.

Table 22: HBV Seropositive Specimens from Chronic HBV Patients — Results for Multiprep Specimen Processing Procedure (Specimens Diluted 1:24)

		HBV DNA Results
Multiprep Procedure	**+**	78 (78.0%)
	–	22*
Total		100

** All HBV DNA negative specimens were tested by alternate HBV DNA tests and contained < 300 copies/mL HBV DNA*

Table 23: Total HBV Seropositive Specimens from Chronic HBV Patients — Results for Standard Specimen Processing Procedure (Specimens Undiluted)

		HBV DNA Results
Standard Procedure	**+**	95 (97.9%)
	–	5*
Total		100

** All HBV DNA negative specimens were tested by alternate HBV DNA tests and contained < 300 copies/mL HBV DNA*

7. Detection of Window Period Cases

A confirmed window period case is defined as an individual from whom the index donation was positive in the COBAS AmpliScreen HBV Test but tested negative by HBsAg and anti-HBc and a follow-up specimen tested positive either by COBAS AmpliScreen HBV Test, HBsAg, or anti-HBc. Two window period cases were detected during the clinical trial for a detection rate of 1:290,895 with exact 95% confidence limits of 1:11,497,826 to 1:52,083.

C. Clinical Trials Summary — Source Plasma

1. Pool Reactivity in Source Plasma

A total of 1,080 primary pools tested in the 96-member mini-pool format representing 103,680 specimens from 40,230 donors revealed that 8 pools were reactive with the COBAS AmpliScreen HBV Test for an initial reactive rate of 0.74%. Of the 8 reactive pools, there were 3 identified HBV DNA positive pools and 2 pools were positive due to apparent window period cases. The remaining 3 pools were reactive but were not confirmed. The data are presented in Table 24.

Table 24: Pool Reactivity in Source Plasma

Category	No. of Pools	Percentage
Pools tested	1080	100%
Non-reactive pools	1072	99.26%
Initially reactive pools	8	0.74%
Initial pools containing a reactive individual specimen with concordant serology	3	0.28%
Positive pools due to window period case[1]	2	0.18%
Initially reactive pools with negative resolution COBAS AmpliScreen Testing (false positive)	3	0.28%

[1] *Two HBsAg negative specimens were in one 96-member mini-pool*

There were 1075 pools used to determine the specificity of the COBAS AmpliScreen HBV Test. Of these pools, 1072 were HBV DNA negative and 3 were Initially Reactive with negative Resolution COBAS AmpliScreen Testing (false positive). The specificity of the COBAS AmpliScreen HBV Test in this study was 1072/1075 or 99.7209% with 95% confidence limits of 99.19% to 99.94.

2. Seroconversion Panels

Ten commercially available HBV seroconversion panels, characterized by HBsAg were tested using the Multiprep Specimen Processing Procedure. Each panel member was diluted 1:96 with normal human plasma and tested with the COBAS AmpliScreen HBV Test. Results were compared with test results from U.S. FDA licensed tests for HBsAg to determine whether the COBAS AmpliScreen HBV Test would detect HBV at the same time or earlier than tests routinely used in standard plasma screening practices.

In two panels, COBAS AmpliScreen HBV Test detected HBV DNA on the same day as HBsAg was detected by the Ortho Antibody to HBsAg ELISA Test System 2. In three panels, COBAS AmpliScreen HBV Test detected HBV DNA on the same day as HBsAg was detected by the Ortho HBsAg Test System 3. In two panels, COBAS AmpliScreen

HBV Test detected HBV DNA on the same day as HBsAg was detected by the Abbott Auszyme Test. Data are presented in Table 25.

Table 25: Summary of Pre-Seroconversion Detection of HBV DNA vs. FDA Licensed Serology Tests — Multiprep Specimen Processing Procedure (Specimen Diluted 1:96)

	Days Before Ortho HBsAg ELISA Test System 2 (8 panels tested*)	Days BeforeOrtho HBsAg ELISA Test System 3 (9 panels tested*)	Days Before Abbott Auszyme overnight for HBsAg (9 panels tested*)
Mean	8.8	8.3	11.0
Median	8	7	9
Maximum	27	27	27
Minimum	0	0	0

** One seroconversion panel was not included in the calculations which was detected by the COBAS AmpliScreen HBV Test 108+ days prior to detection of HBsAg by the Ortho HBsAg ELISA Test System 2, 101 days prior to the Ortho HBsAg ELISA Test System 3, and 87 days prior to the Abbott Auszyme test.*

The seroconversion study demonstrates the COBAS AmpliScreen HBV Test used with the Multiprep Specimen Processing Procedures and pools of 96 specimens, identifies HBV infected specimens at the same time or earlier than the U.S. FDA licensed HBsAg tests.