

SUMMARY OF SAFETY AND EFFECTIVENESS

I. GENERAL INFORMATION:

Device Generic Name: Antibody to Hepatitis B Surface Antigen (anti-HBs)

Device Trade Name: AxSYM[®] HBsAg
AxSYM[®] HBsAg Confirmatory
AxSYM[®] HBsAg Controls

Name and Address of Applicant: Abbott Laboratories
Abbott Diagnostics Division
100 Abbott Park Road
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Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P050049

Date of Notice of Approval to the Applicant: June 1, 2006

II. INDICATIONS FOR USE:

AxSYM[®] HBsAg

AxSYM HBsAg is an in vitro microparticle enzyme immunoassay (MEIA) intended for the qualitative detection of hepatitis B surface antigen (HBsAg) in neonatal serum, and adult and pediatric serum (including serum collected in serum separator tubes) or plasma (collected in potassium EDTA, sodium citrate, sodium heparin, lithium heparin, or plasma separator tubes). The assay is used as an aid in the diagnosis of acute or chronic hepatitis B virus (HBV) infection in conjunction with other laboratory results and clinical information. The assay may be used to test for HBV infection in pregnant women.

AxSYM[®] HBsAg Confirmatory

AxSYM HBsAg Confirmatory is an in vitro MEIA intended for the confirmation of the presence of HBsAg in neonatal serum, and adult and pediatric serum (including serum collected in serum separator tubes) or plasma (collected in potassium EDTA, sodium citrate, sodium heparin, lithium heparin, or plasma separator tubes). The assay is used for confirmation of samples found to be repeatedly reactive by the AxSYM HBsAg assay. The assay may be used to confirm HBV infection in pregnant women.

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AxSYM[®] HBsAg Controls

The AxSYM HBsAg Positive and Negative Controls are for use in monitoring the performance of the AxSYM HBsAg Reagent Kit and the AxSYM HBsAg Confirmatory Kit. The performance of the AxSYM HBsAg Controls has not been established with any other HBsAg assays.

III. CONTRAINDICATIONS: None known.

IV. WARNINGS AND PRECAUTIONS:

Warnings and precautions for the AxSYM HBsAg Reagent Kit, AxSYM HBsAg Confirmatory Kit, and AxSYM HBsAg Controls are stated in the respective product labeling.

Both AxSYM HBsAg and AxSYM HBsAg Confirmatory are for in vitro diagnostic use only. Both assays are not intended for use in screening blood, plasma, or tissue donors. The effectiveness of AxSYM HBsAg and AxSYM HBsAg Confirmatory for use in screening blood, plasma, or tissue donors has not been established.

V. DEVICE DESCRIPTION:

The AxSYM HBsAg Reagent Kit is composed of the following components:

1 Bottle (15.5 mL) Anti-biotin (Rabbit): Alkaline Phosphatase Conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.05 μ g/mL. Preservative: 0.1% Sodium Azide.

1 Bottle (5.1 mL) Anti-HBs (Mouse, Monoclonal, IgM) Coated Microparticles in sodium phosphate buffer with protein (1.0% bovine) stabilizer. Minimum concentration: 0.2% solids. Preservative: 0.1% Sodium Azide.

1 Bottle (17.5 mL) Biotinylated Anti-HBs (Goat, IgG) in TRIS buffer containing animal sera. Minimum concentration: 1.25 μ g/mL. Preservatives: 0.1% Sodium Azide and Antimicrobial Agents.

1 Bottle (49.2 mL) Probe Wash Solution prepared in TRIS buffer. Preservative: Antimicrobial Agent.

1 Bottle (6 mL) AxSYM HBsAg Index Calibrator. Recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs. Preservative: 0.1% Sodium Azide. Dye: Green (Acid Yellow No. 23 and Acid Blue No. 9).

The AxSYM HBsAg Control Kit is composed of:

2 Bottles (8 mL each) of AxSYM HBsAg Controls are prepared with recalcified human plasma. Preservative: 0.1% Sodium Azide.

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The Negative Control is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.

The Positive Control is reactive for HBsAg, and nonreactive for HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs. Reactive plasma is heat-inactivated.

The AxSYM HBsAg Confirmatory Kit is composed of the following components:

1 Bottle (1 mL) Reagent A. Antibody to Hepatitis B Surface Antigen (Human) nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservative: 0.1% Sodium Azide. Dye: Violet (FD&C Red No. 33 and Acid Blue No. 9).

1 Bottle (1.5 mL) Reagent B. Recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs. Preservative: 0.1% Sodium Azide. Dye: Yellow (Acid Yellow No. 23).

1 Bottle (18 mL) Dilution Reagent. Recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs. Preservative: 0.1% Sodium Azide.

In addition, the following components are required for performing the AxSYM HBsAg assays:

2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammonium Hydroxide (TEAH).

4 Bottles (230 mL each) Solution 1 (MUP) containing 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: 0.1% Sodium Azide.

4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M Sodium Chloride in TRIS buffer. Preservatives: 0.1% Sodium Azide and Antimicrobial Agents.

1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M Phosphate buffer. Preservatives: 0.1% Sodium Azide and Antimicrobial Agent.

Assay Principle and Format

AxSYM HBsAg

AxSYM HBsAg is based on MEIA technology and utilizes the principle of direct binding of the HBsAg in the sample to anti-HBs coated microparticles and indirect detection of the HBsAg by biotinylated anti-HBs followed by anti-biotin:alkaline phosphatase conjugate. The Matrix Cell is washed with Matrix Cell Wash to remove materials not bound to the microparticles. The substrate (MUP) is added and the fluorescent product formed is measured by the MEIA optical assembly on the AxSYM System.

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The presence or absence of HBsAg in the sample is determined by comparing the rate of formation of fluorescent product (S) to the cutoff rate (CO), which is calculated from a previous AxSYM HBsAg Index Calibration. Samples with S/CO values greater than or equal to 1.00 are considered reactive for HBsAg. Samples with S/CO values less than 1.00 are considered negative for HBsAg.

AxSYM HBsAg Confirmatory

AxSYM HBsAg Confirmatory is based on MEIA technology. In addition to the AxSYM HBsAg Confirmatory Kit, this assay requires the use of the AxSYM HBsAg Reagent Kit.

AxSYM HBsAg Confirmatory utilizes the principle of specific antibody neutralization to confirm the presence of HBsAg in samples found to be repeatedly reactive by AxSYM HBsAg. The assay principle involves two steps: treatment of the sample with Reagent A or Reagent B, and HBsAg testing.

Antibody to hepatitis B surface antigen (human anti-HBs) (Reagent A) is incubated with a sample. If HBsAg is present in the sample, it will be neutralized by the antibody. The neutralized HBsAg is subsequently blocked from binding to the anti-HBs coated microparticles. This results in a reduction of signal when compared to the signal of a paired sample that has not been treated with the antibody reagent (Reagent B). A sample is considered confirmed positive for HBsAg if its reactivity in the AxSYM HBsAg Confirmatory assay is neutralized by the addition of antibody reagent and the reduction in signal (% neutralization) is greater than or equal to 50%. A sample is considered repeat reactive and nonconfirming for HBsAg if it is reactive and not neutralized in the AxSYM HBsAg Confirmatory assay.

The presence of nonneutralized HBsAg in the sample is determined by comparing the rate of formation of fluorescent product (S) to a cutoff rate (CO), which is calculated from a previous AxSYM HBsAg Confirmatory Index Calibration. In the undiluted or diluted sample, if the rate of the nonneutralized sample (incubated with Reagent B) is greater than or equal to the cutoff rate (S/CO \geq 1.00), and the S/CO of the neutralized sample (incubated with Reagent A) is reduced by at least 50% compared to the nonneutralized sample, the sample is considered confirmed positive for HBsAg.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

The patient's medical history and thorough physical examination, including hepatitis serology, determination of liver enzyme levels, and biopsy of the liver, will provide further information on the status of a HBV infection. Alternative procedures for the detection of HBV in human serum and plasma depend on the detection of HBV deoxyribonucleic acid (DNA) by research polymerase chain reaction (PCR) assays or nucleic acid testing (NAT), or the detection of HBV antigens and antibodies by commercially-available assays that are licensed or approved in the United States.

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VII. MARKETING HISTORY

These products (AxSYM HBsAg, List No. 9B01-20 and AxSYM HBsAg Confirmatory, List No. 9B01-60) have not been marketed in any other country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

It is recognized that presently available methods for the detection of HBsAg may not detect all potentially infected individuals. HBsAg reactivity as determined by AxSYM HBsAg and AxSYM HBsAg Confirmatory should be correlated with patient history and the presence or absence of other hepatitis markers for the diagnosis of hepatitis B viral infection. Reactive results do not discriminate between acute or chronic HBV infections. Other than the circumstances mentioned above, there are no known potential adverse effects on the health of the patient or user if these in vitro devices are used according to the instructions in the package inserts. However, a failure of the assays to perform as indicated or human error during performance of the assays may lead to a false diagnosis and improper patient management.

IX. SUMMARY OF PRECLINICAL STUDIES

Summary of the Nonclinical Laboratory Studies

Nonclinical laboratory studies were performed at Abbott Laboratories to evaluate the performance characteristics of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays. The studies are summarized below.

Cutoff Rationale

Studies were conducted to determine an appropriate assay cutoff that would result in optimal specificity while maintaining acceptable sensitivity for the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays. For AxSYM HBsAg, assay sensitivity was evaluated by testing a panel of known concentrations of HBsAg subtype *ad* and HBsAg subtype *ay*. Assay specificity was evaluated by testing specimens from random hospital patients.

The data support the following cutoff rate and sample result calculations for AxSYM HBsAg: Cutoff Rate (CO) = Index Calibrator Mean Rate \times 2 S/CO = Sample Rate/(Index Calibrator Mean Rate \times 2)

For AxSYM HBsAg Confirmatory, assay sensitivity was evaluated by testing a panel of known concentrations of HBsAg subtype *ad* and HBsAg subtype *ay*.

The data support the following cutoff rate and sample result calculations for AxSYM HBsAg Confirmatory: Cutoff Rate (CO) = Index Calibrator Mean Rate \times 1.5 S/CO = Sample Rate/(Index Calibrator Mean Rate \times 1.5)

Sample Handling and Collection

Sample Types (Serum and Plasma)

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A study was conducted to evaluate which specimen collection tube types are acceptable for use with the AxSYM HBsAg assay. Sets of specimens were collected in the control specimen collection tube type (serum in glass) and the specimen collection tube types selected for evaluation. Tubes from each set were spiked with human plasma positive for HBsAg to prepare high negative samples (0.80 S/CO target) and low positive samples (1.2 S/CO target), and the samples were tested.

On average, the lower one-sided 95% confidence interval of % bias for the 25 low positive samples was less than 11% compared to the control sample type (serum in glass). The results for the low positive samples are summarized in Table 1 on page 10.

The data support the use of the AxSYM HBsAg assay with serum specimens, specimens collected in serum separator tubes (SST[®]) or plasma separator tubes (PST), and specimens collected in tubes containing the following anticoagulants: potassium ethylenediaminetetraacetic acid (EDTA), sodium citrate, sodium heparin, lithium heparin.

Table 1
AxSYM HBsAg
Sample Types (Serum and Plasma) Study
Summary of Results

Evaluation Tube Type	% Bias Samples Targeted to 1.2 S/CO	
	Mean	Lower One-sided 95% Confidence Interval
Plastic Serum	-6.04	-8.33
Glass Serum Separator	-5.40	-7.57
Plastic Serum Separator	-4.35 ^a	-6.28
Plastic Plasma Separator	-0.84	-2.60
Plastic Potassium EDTA	-8.23	-10.51
Plastic Sodium Citrate	-7.83	-10.07
Plastic Sodium Heparin	0.55	-1.80
Plastic Lithium Heparin	0.74	-1.81

Value shown is a median; %bias values were not normally distributed.

NOTE: Potassium EDTA plasma and sodium citrate have been shown to lower the S/CO values in some HBsAg reactive samples. High nonreactive results (0.80-0.99 S/CO) obtained on samples collected with these anticoagulants should be interpreted accordingly.

Interference

Analytical Specificity

Studies were conducted to characterize the performance of the AxSYM HBsAg assay when used to test specimens containing potentially interfering substances.

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Specimens from individuals with various medical conditions unrelated to HBV were tested using the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays. The results are summarized in Table 2.

In addition, high negative (0.8 S/CO target) and low positive (1.2 S/CO target) serum samples were spiked with bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) and viral or parasitic antigens (cytomegalovirus, Epstein-Barr virus, herpes simplex virus, rubella, *Toxoplasma gondii*, and varicella-zoster virus). The bacteria were spiked to 10^{2-3} , 10^{3-4} , and 10^{5-6} colony-forming units per mL (CFU/mL). The viral or parasitic antigens were spiked to 1 µg/mL and 1 ng/mL. All high negative samples (0.8 S/CO target) remained nonreactive and all low positive samples (1.2 S/CO target) remained reactive.

TABLE 2

**Cross-reactivity of AxSYM HBsAg
in Specimens from Individuals with Medical Conditions Unrelated to HBV**

Specimen Category ^a	Number of Specimens Tested	AxSYM HBsAg Nonreactive	AxSYM HBsAg Reactive	AxSYM HBsAg Confirmatory Positive
Hepatitis A Virus	15	15	0	0
Hepatitis C Virus	10	10	0	0
Human Immunodeficiency Virus	10	10	0	0
Herpes Simplex Virus	15	15	0	0
Cytomegalovirus	15	15	0	0
Epstein-Barr Virus	14	14	0	0
Rubella	15	15	0	0
Syphilis	15	15	0	0
Parvovirus B19 Infection	9	7	2	0
Systemic Lupus Erythematosus	15	15	0	0
Rheumatoid Factor Positive	38	35	3	1 ^b
Human Anti-mouse Antibodies	15	15	0	0
Toxoplasmosis	8	8	0	0
Alcoholic Liver Disease	15	15	0	0
Obstructive Jaundice	15	15	0	0
Hepatocellular Carcinoma	5	5	0	0
Total (%)	229	224/229 (97.8%)	5/229 (2.2%)	1/229 (0.4%)

Information about age and gender of the individuals is not available.

This specimen was confirmed positive by an FDA-licensed HBsAg confirmatory assay.

Potentially Interfering Substances – Triglycerides, Total Protein, Bilirubin, and Hemoglobin

Studies were conducted to evaluate the performance of the AxSYM HBsAg assay when used to test specimens containing a high level of triglycerides, total protein, bilirubin (unconjugated), and hemoglobin. Human serum nonreactive for HBsAg was spiked with human plasma positive for HBsAg to prepare high negative samples (0.80 S/CO target) and low positive samples (1.2 S/CO target). A triglyceride test sample was prepared by supplementing the high negative and low positive samples with LIPOSYN[®] III to a minimum triglyceride concentration of 3,000 mg/dL. A total protein test sample was prepared by supplementing the high negative and low positive samples with human albumin powder to a minimum concentration of 12 g/dL. A bilirubin test sample was prepared by supplementing the high negative and low positive samples with unconjugated bilirubin stock solution to a minimum concentration of 20 mg/dL. A hemoglobin test sample was prepared by supplementing the high negative and low positive samples with a hemoglobin stock solution to a minimum concentration of 500 mg/dL. Control samples were prepared for each test sample representing endogenous levels of each interferent. The control and test samples were tested.

The difference for the samples targeted at 0.8 S/CO was less than or equal to 0.1 S/CO and the %bias for the samples targeted at 1.2 S/CO was less than or equal to 10%, indicating that no interference was observed. In conclusion, the data support the use of the AxSYM HBsAg assay with specimens that contain up to 3,000 mg/dL of triglycerides, up to 12 g/dL of total protein, up to 20 mg/dL of bilirubin (unconjugated), and up to 500 mg/dL of hemoglobin.

Within- and Between-assay Sample Carryover

Studies were conducted to evaluate the susceptibility of the AxSYM HBsAg assay to sample carryover within the assay or from other AxSYM assays when processing samples containing high levels of HBsAg. Carryover events were modeled by testing human plasma nonreactive for HBsAg to mimic a sample that was not exposed to potential sample carryover (protected negative), followed by a human plasma sample containing a high concentration of HBsAg, followed again by human plasma nonreactive for HBsAg to mimic a sample exposed to potential sample carryover (unprotected negative).

For the within-assay carryover study, the difference between the protected negative and unprotected negative mean S/CO values was 0.01 S/CO, indicating that no sample carryover was present within the AxSYM HBsAg assay.

For the between-assay carryover study, the difference between the protected negative and unprotected negative mean S/CO values ranged from –0.00 to 0.02 S/CO in the Sampling Center, and the difference between the protected negative and unprotected negative mean or median S/CO values ranged from –0.12 to 0.14 S/CO in the Processing Center. The data indicate that no

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between-assay sample carryover was present between the AxSYM HBsAg assay and any of the potential contaminator assays evaluated.

High Dose Hook Effect

A study was conducted to characterize the performance of the AxSYM HBsAg assay when used to test specimens containing extremely high concentrations of HBsAg that have the potential to cause a high dose hook effect.

High-titer HBsAg reactive human plasma (0.0817 mg/mL) and recombinant hepatitis B surface antigen (15.2 mg/mL) were each serially diluted and tested. Suppressed S/CO results indicative of high dose hook effect were observed at HBsAg concentrations above 0.015 mg/mL (approximately 3,800 IU/mL); however, all results remained reactive. The mean result for the undiluted HBsAg reactive human plasma sample was 84.54 S/CO and the mean result for the undiluted recombinant HBsAg sample was 40.83 S/CO.

The data demonstrate that no high dose hook effect was observed in the AxSYM HBsAg assay with samples containing less than 3,800 IU/mL HBsAg. When the high dose hook effect was observed, samples containing up to approximately 3,800,000 IU/mL of HBsAg remained reactive, i.e., no qualitative change in the assay results occurred.

Analytical Sensitivity

Studies were conducted to evaluate the analytical sensitivity of the AxSYM HBsAg assay. One study was conducted using a panel of known concentrations of HBsAg subtype *ad* and HBsAg subtype *ay*, and the AxSYM HBsAg Index Calibrator as a nonreactive (0 ng/mL) sample. The HBsAg concentration at the assay cutoff (ng/mL sensitivity) was estimated using a linear regression analysis. The mean analytical sensitivity was determined to be 0.15 ng/mL (approximately 0.04 IU/mL) for HBsAg subtype *ad* and 0.12 ng/mL (approximately 0.03 IU/mL) for HBsAg subtype *ay*. This data supports an expected analytical sensitivity for the AxSYM HBsAg assay of less than or equal to 0.6 ng/mL for both HBsAg subtypes *ad* and *ay*.

Table 3

Analytical Sensitivity (ng/mL) of AxSYM HBsAg

Sample	Mean Sensitivity (ng/mL)	Mean Sensitivity (Approximate IU/mL)	Upper One-sided 95% Confidence Limit
HBsAg Sensitivity Panel (subtype <i>ad</i>)	0.15	0.04	0.18
HBsAg Sensitivity Panel (subtype <i>ay</i>)	0.12	0.03	0.15

Another study was conducted using serial dilutions of the World Health Organization (WHO) 1st International HBsAg Standard. Human plasma nonreactive for HBsAg was used to represent the 0 International Units (IU)/mL sample. The HBsAg concentration at the assay cutoff (IU/mL sensitivity) was estimated using a linear regression analysis. The mean analytical sensitivity was determined to be 0.03 IU/mL. The data are summarized in Table 4.

Table 4

Analytical Sensitivity (IU/mL) of AxSYM HBsAg

Sample	Mean Sensitivity (IU/mL)	Upper One-sided 95% Confidence Limit
Dilutions of WHO HBsAg Standard	0.03	0.04

Seroconversion Detectability

A study was conducted to demonstrate the ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect HBsAg when used to test serial bleed specimens from HBV-infected individuals.

Fifteen seroconversion panels (a total of 175 serial bleed specimens from 15 HBV-infected individuals) were obtained from three commercial vendors and tested. The AxSYM HBsAg/HBsAg Confirmatory results were compared to results from an FDA-licensed HBsAg assay (reference).

HBsAg was detected by AxSYM HBsAg and confirmed positive by AxSYM HBsAg Confirmatory 3 to 7 days earlier than the reference HBsAg assay in five panels, and coincident with the reference HBsAg assay in ten panels. The results are summarized in Table 5.

The data demonstrate acceptable HBsAg seroconversion detectability by the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays when used to test serial bleed specimens from HBV-infected individuals.

Table 5

AxSYM HBsAg Seroconversion Detectability Study

Seroconversion Panel Identification	Days to HBsAg Reactivity from First Bleed		Difference in Days to HBsAg Reactivity (Reference – AxSYM)
	Reference HBsAg Assay	AxSYM HBsAg/ AxSYM HBsAg Confirmatory	
PHM903	14	10	4
PHM909	9	9	0
PHM915	33	26	7
PHM916	65	62	3
PHM917	50	50	0
PHM920	26	26	0
PHM923	15	15	0
0994/3457	11	4	7
26982/14399	0	0	0
43527/3453	0	0	0
6271	12	12	0
6272	94	94	0
6273	25	25	0
6274	4	0	4
6275	22	22	0

HBsAg Genotype Detectability

A study was conducted to demonstrate the ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect HBsAg genotypes. Thirty-four samples from a HBsAg genotype panel containing genotypes A through G were obtained from a commercial vendor and tested.

The data in Table 6 demonstrate the ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect HBsAg genotypes A through G. The conformational epitope used by AxSYM HBsAg to detect HBsAg is present in genotypes A through H^{1,2,3}. Therefore, detection of genotype H by AxSYM HBsAg and AxSYM HBsAg Confirmatory is expected to be similar to the detection of genotypes A through G demonstrated in this study.

Table 5

AxSYM HBsAg Seroconversion Detectability Study

Seroconversion Panel Identification	Days to HBsAg Reactivity from First Bleed		Difference in Days to HBsAg Reactivity (Reference – AxSYM)
	Reference HBsAg Assay	AxSYM HBsAg/ AxSYM HBsAg Confirmatory	
PHM903	14	10	4
PHM909	9	9	0
PHM915	33	26	7
PHM916	65	62	3
PHM917	50	50	0
PHM920	26	26	0
PHM923	15	15	0
0994/3457	11	4	7
26982/14399	0	0	0
43527/3453	0	0	0
6271	12	12	0
6272	94	94	0
6273	25	25	0
6274	4	0	4
6275	22	22	0

HBsAg Genotype Detectability

A study was conducted to demonstrate the ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect HBsAg genotypes. Thirty-four samples from a HBsAg genotype panel containing genotypes A through G were obtained from a commercial vendor and tested.

The data in Table 6 demonstrate the ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect HBsAg genotypes A through G. The conformational epitope used by AxSYM HBsAg to detect HBsAg is present in genotypes A through H^{1,2,3}. Therefore, detection of genotype H by AxSYM HBsAg and AxSYM HBsAg Confirmatory is expected to be similar to the detection of genotypes A through G demonstrated in this study.

Table 6
AxSYM HBsAg
HBsAg Genotype Detectability Study

Genotype	Number Tested	Number AxSYM HBsAg Reactive/ AxSYM HBsAg Confirmatory Positive
A	5	5
B	1	1
C	7	7
D	3	3
E	6	5 ^a
F	11	11
G	1	1
Total	34	33

^aOne Genotype E sample was nonreactive by AxSYM HBsAg and an FDA-licensed HBsAg assay. The sample contained 200 copies/mL by one NAT test method and less than 200 copies/mL by another NAT test method.

HBsAg Mutant Detectability

A study was conducted to demonstrate the ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect multiple HBsAg mutants. A panel of 27 recombinant HBsAg mutant samples were prepared as described by Coleman et al.⁴ Each sample was prepared to a concentration of approximately 1 ng/mL and tested by AxSYM HBsAg. Three of the 27 samples were nonreactive by AxSYM HBsAg; these three samples contained antigen with various mutations at amino acid position 123. All of the remaining 24 samples were reactive by AxSYM HBsAg. These reactive samples included seven samples with mutations surrounding the amino acid 123 site (amino acids 115–120 and 126–133) and ten samples with mutations in the second loop (amino acids 139–147). The data in Table 7 demonstrate the ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect HBsAg in samples with mutations outside of the region of amino acids 121 to 124, including the glycine to arginine mutation at amino acid position 145 (representing the most frequent and stable HBsAg mutation reported in the literature).⁵ Samples that contain HBsAg mutations in the region of amino acids 121 to 124 may be nonreactive by AxSYM HBsAg.

Table 7

Detection of Recombinant HBsAg Mutants by AxSYM HBsAg/AxSYM HBsAg Confirmatory

Sample Description	AxSYM HBsAg		AxSYM HBsAg Confirmatory
	S/CO	Interpretation	Interpretation
Control			
<i>adw2</i> wild type sequence	3.46	Reactive	Positive
Mutants			
Asn 40 to Ser	3.77	Reactive	Positive
Pro 111 to Thr	3.22	Reactive	Positive
Thr Thr 115, 116 to Ile Ile	3.78	Reactive	Positive
Thr 118 to Ser	4.19	Reactive	Positive
Pro 120 to Gln	7.92	Reactive	Positive
Thr 123 to Ala	0.58	Nonreactive	Not Tested
<i>adw2</i> sequence with (123 Arg – Ala insert)	0.50	Nonreactive	Not Tested
PreS2/S <i>ayw1</i> sequence with (123 Asp – Thr insert)	0.69	Nonreactive	Not Tested
Thr 126 to Ser	2.01	Reactive	Positive
Gln 129 to His	2.17	Reactive	Positive
Thr 131 to Ile	5.31	Reactive	Positive
Met 133 to Leu	2.84	Reactive	Positive
Pro 135 to Ser	7.27	Reactive	Positive
Lys 141 to Glu	6.89	Reactive	Positive
Pro 142 to Leu	6.27	Reactive	Positive
Pro 142 to Ser	6.35	Reactive	Positive
Asp 144 to Ala	3.46	Reactive	Positive
Gly 145 to Ala	3.19	Reactive	Positive
Gly 145 to Arg	2.66	Reactive	Positive
Thr 126 to Ser + Gly 145 to Arg	2.82	Reactive	Positive
Pro 142 to Leu + Gly 145 to Arg	3.84	Reactive	Positive
Pro 142 to Ser + Gly 145 to Arg	7.14	Reactive	Positive
Asp 144 to Ala + Gly 145 to Arg	5.25	Reactive	Positive
Gly 145 to Lys	3.29	Reactive	Positive
Thr 148 to His	4.54	Reactive	Positive
Ser 154 to Trp	2.26	Reactive	Positive
Met Met Met 197, 198, 199 to Ser Ser Ser	4.08	Reactive	Positive

^a One Genotype E sample was nonreactive by AxSYM HBsAg and an FDA-licensed HBsAg assay. The sample contained 200 copies/mL by one NAT test method and less than 200 copies/mL by another NAT test method.

Cord Blood (Neonate) Evaluation

A study was conducted to characterize the performance of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays when used to test cord blood (neonate) specimens. A total of 20 cord blood specimens were obtained from a commercial vendor. Thirteen of the specimens were spiked

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with human plasma positive for HBsAg to prepare low positive samples (1.2 S/CO target). Each sample (spiked and unspiked) was tested using AxSYM HBsAg/HBsAg Confirmatory.

As shown in Table 8, one unspiked cord blood sample was repeatedly reactive by AxSYM HBsAg but was not confirmed by AxSYM HBsAg Confirmatory. The remaining 19 unspiked cord blood samples were nonreactive. All spiked cord blood samples were reactive by AxSYM HBsAg and were confirmed positive by AxSYM HBsAg Confirmatory.

Table 8

Evaluation of Neonatal (Cord Blood) Specimens by AxSYM HBsAg/AxSYM HBsAg Confirmatory

Sample Description	Number Tested	AxSYM HBsAg Nonreactive	AxSYM HBsAg Reactive	AxSYM HBsAg Confirmatory Positive
Cord Blood	20	19	1	0
Cord Blood (low positive [1.2 S/CO target])	13	0	13	13

Within-Laboratory (20-day) Precision

A within-laboratory (20-day) precision study was conducted based on guidance from Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) document EP5-A2⁶ to evaluate the precision performance of the AxSYM HBsAg assay. Testing was performed using two AxSYM HBsAg reagent lots, one control lot, and two AxSYM instruments. Testing included two precision runs per day (a minimum of two hours apart) for each reagent lot, on each instrument, on each of 20 days. Each precision run included two replicates of each of three HBsAg subtype *ad* and three HBsAg subtype *ay* panel members, and the AxSYM HBsAg Index Calibrator, Negative Control, and Positive Control. Panel members were prepared by adding purified HBsAg to recalcified nonreactive human plasma. The panel members selected for testing were those with S/CO values closest to the target S/CO values of 0.8, 1.2, and 4.0. The data demonstrate the acceptable precision of the AxSYM HBsAg assay. The results are summarized in the Table 9 below.

Table 9

AxSYM HBsAg Within- Laboratory Precision

Sample	Total No. Reps	Grand Mean S/CO	Within-run		Within-Day		Within-Laboratory Precision (Total)			Precision with additional component of Between-lot		Precision with additional component of Between-instrument	
			SD	%C V	SD	%C V	SD	%C V	CL	SD	%C V	SD	%C V
Panel adH	320	0.71	0.035	4.9	0.038	5.3	0.038	5.3	5.7	0.047	6.6	0.041	5.8
Panel adF	320	1.19	0.044	3.7	0.050	4.2	0.056	4.7	5.1	0.093	7.8	0.058	4.9
Panel adC	320	3.16	0.133	4.2	0.138	4.4	0.152	4.8	5.2	0.328	10.4	0.180	5.7
Panel ayH	320	0.69	0.032	4.6	0.036	5.2	0.038	5.5	5.9	0.047	6.9	0.040	5.8
Panel ayF	320	1.40	0.061	4.4	0.066	4.7	0.069	4.9	5.3	0.132	9.4	0.072	5.1
Panel ayC	320	3.82	0.142	3.7	0.142	3.7	0.170	4.4	4.8	0.411	10.7	0.207	5.4
NC	320	0.50	0.031	6.2	0.035	7.0	0.036	7.2	7.8	0.036	7.3	0.044	8.9
PC	320	2.45	0.097	4.0	0.109	4.4	0.122	5.0	5.4	0.245	10.0	0.136	5.6

Microbial Challenge

Studies were conducted to establish the level of antimicrobial protection provided by the preservative system used in the AxSYM HBsAg/AxSYM HBsAg Confirmatory kit components, and to determine the effect of bioburden and/or its by-products on assay performance.

Components of the AxSYM HBsAg Reagent Kit and Controls and AxSYM HBsAg Confirmatory Reagent Kit were inoculated with the following microorganisms at concentrations between 10^2 and 10^6 colony forming units/mL (CFU/mL): *Bacillus subtilis*, *Candida albicans*, *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Psuedomonas fluorescens*. The inoculated materials were evaluated for microbial growth over a period of 15 months and for assay performance for a minimum of 35 days.

No growth of the challenge organisms was observed during the study. Assay performance was acceptable at bioburden levels up to 10^{5-6} CFU/mL for *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Psuedomonas fluorescens*, up to 10^{3-4} CFU/mL for *Bacillus subtilis* and *Candida albicans*, and up to 10^{2-3} CFU/mL for *Aspergillus niger*. The data demonstrate that the AxSYM HBsAg Reagent Kit and Controls and the AxSYM HBsAg Confirmatory Reagent Kit are adequately protected by the preservative system used.

Stability

Recommended Storage Stability

Real-time stability studies were conducted to demonstrate the shelf-life integrity of the AxSYM HBsAg Reagent Kit, AxSYM HBsAg Confirmatory Kit, and AxSYM HBsAg Controls at the recommended storage condition of 2 to 8°C. Three lots each of the AxSYM HBsAg Reagent Kit,

Summary of Safety and Effectiveness Data

AxSYM HBsAg Confirmatory Kit, and AxSYM HBsAg Controls were stored at 2 to 8°C (recommended storage). The AxSYM HBsAg Reagent Kits were tested at Month 0 and monthly thereafter through Month 19. The AxSYM HBsAg Confirmatory Kits and AxSYM HBsAg Controls were tested at Months 0, 0.5, 1, and monthly thereafter through Month 19.

The data support a recommended storage shelf life of 18 months for the AxSYM HBsAg Reagent Kit, AxSYM HBsAg Confirmatory Kit, and AxSYM HBsAg Controls.

Onboard Reagent Pack Stability

A study was conducted to determine how long the AxSYM HBsAg Reagent Pack can be stored on board the AxSYM System. Three lots of AxSYM HBsAg Reagent Packs were stored at 2 to 8°C (recommended storage) and at 31°C (simulated onboard storage) and tested at 24-hour intervals up to a total of 360 hours. The AxSYM HBsAg Index Calibrator and the AxSYM HBsAg Confirmatory Kit were not evaluated for onboard stability because these reagents are not left on board the AxSYM System. The data support the storage of the AxSYM HBsAg Reagent Pack on board the AxSYM System for 360 hours.

Calibration Stability and Control Frequency

An analysis was performed to determine if an AxSYM HBsAg calibration that is stored on the AxSYM System for a minimum of 14 days can be used to generate valid results (calibration stability), and to support a minimum control requirement to test controls once every 24 hours (control frequency). The validity data generated in the Within-Laboratory (20-day) Precision Study were used for this analysis. The study was conducted using two AxSYM instruments and two AxSYM HBsAg Reagent Kit lots for 20 days. A calibration was performed on the first day of testing for each instrument and reagent kit lot combination. The AxSYM HBsAg Negative Control and Positive Control were each tested for validity purposes, once per run, twice daily (a minimum of two hours apart), on each of 20 days, using each instrument and reagent kit lot combination.

The data demonstrate that an AxSYM HBsAg calibration may be stored on the AxSYM System and used to generate valid results for a minimum of 14 days. The data also support the testing of controls once every 24 hours.

X. Summary of the Clinical Studies

A multi-center study was conducted to demonstrate that the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays perform as intended in a diagnostic population. The study was designed to measure the precision of the AxSYM HBsAg assay, and determine the percent agreement between AxSYM HBsAg and the FDA-approved HBsAg reference method.

Reproducibility (5-day Precision)

The precision of the AxSYM HBsAg assay was evaluated by testing three AxSYM HBsAg reagent and control master lots at three clinical testing sites for five days. Testing included two precision runs per day (a minimum of two hours apart) for each of three reagent master lots, on

Summary of Safety and Effectiveness Data

each of five days. Each precision run included four replicates of each precision panel member and four replicates each of AxSYM HBsAg Index Calibrator, Negative Control, and Positive Control. The precision panel was composed of three members, two of which were near the assay cutoff. Panel members were prepared by adding purified HBsAg to nonreactive human serum. The analysis method was based on CLSI EP15-A2⁷. The results are presented in Table 9.

Table 9 AxSYM HBsAg System Reproducibility: Overall Precision

Panel members/ controls	Total No. Repts	Grand Mean S/CO	Within-Run		Within-Day		Within-Laboratory Precision (Total)			Precision With Additional Component of Between-Lot		Precision With Additional Component of Between-Site		Precision with Additional Component of Site and Lot (Overall)	
			SD	%CV	SD	%CV	SD	%CV	CL	SD	%CV	SD	%CV	SD	%CV
	360	0.76	0.039	5.2	0.041	5.4	0.043	5.6	6.0	0.065	8.5	0.065	8.5	0.065	8.5
	360	1.24	0.047	3.8	0.052	4.2	0.054	4.3	4.7	0.094	7.6	0.090	7.2	0.094	7.6
	360	3.55	0.197	5.5	0.207	5.8	0.213	6.0	6.4	0.342	9.6	0.317	8.9	0.342	9.6
	360	0.51	0.032	6.3	0.034	6.7	0.037	7.2	7.8	0.048	9.4	0.048	9.4	0.048	9.4
	360	2.50	0.089	3.6	0.100	4.0	0.105	4.2	4.5	0.211	8.4	0.196	7.8	0.211	8.4

Index Calibrator	Total No. Repts	Grand Mean Rate	Within-Run		Within-Day		Within-Laboratory Precision (Total)			Precision With Additional Component of Between-Lot		Precision With Additional Component of Between-Site		Precision with Additional Component of Site and Lot (Overall)	
			SD	%CV	SD	%CV	SD	%CV	CL	SD	%CV	SD	%CV	SD	%CV
	360	6.75	0.411	6.1	0.425	6.3	0.472	7.0	7.6	1.122	16.6	0.558	8.3	1.147	17.6

Reps = Replicates; SD = Standard Deviation; CL = Confidence Limit^a Overall variability contains within-run, between-run, between-day, between-lot, between-site, and lot-site interaction variance components.

Description of Clinical Studies

The clinical specimens used in the study were obtained from six specimen collection sites and two specimen vendors. A total of 2,868 linked serum specimens were prospectively collected and tested. In addition, 15 seroconversion panel members and 100 specimens from a surplus pediatric population were obtained.

The specimens included the following categories:

- 1,314 specimens from individuals at increased risk of hepatitis B virus (HBV) infection
- 704 specimens from individuals with signs and symptoms of hepatitis infection
- 49 specimens from individuals diagnosed with acute or chronic HBV infection

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- 60 specimens from Vietnam (53 specimens were determined to be chronic by HBV classification and 7 specimens could not be classified as acute or chronic and were not included in the final analysis)
- 741 specimens from pregnant females (This population included a combination of individuals at low risk and increased risk for HBV infection.)
- 15 seroconversion panel members classified as acute by four-marker HBV reference testing
- 100 specimens were from a pediatric population (This population included specimens from children > 2 to 12 years of age and adolescents > 12 to 18 years of age.)

Three AxSYM HBsAg reagent and control master lots, and three AxSYM HBsAg Confirmatory reagent master lots were used in the percent agreement evaluation. Three clinical testing sites performed the AxSYM HBsAg and AxSYM HBsAg Confirmatory testing, with the exception of the 15 seroconversion panel members, which were tested in-house. Specimens were sent to an external reference laboratory for reference HBsAg assay testing, including retesting and confirmatory testing where required, and supplemental testing was performed where required.

A summary of the percent agreement results for all specimen categories is presented in the Table 10 below. The results of the percent agreement evaluation demonstrate that the AxSYM HBsAg assay can be used, in conjunction with other serological and clinical information, as an aid in the diagnosis of acute or chronic HBV infection and may be used to test for HBV infection in pregnant women and in a pediatric population.

Table 10
Summary of Percent Agreement Between
AxSYM HBsAg and the Reference HBsAg Assay

Specimen Category	Number of Specimens Tested	Positive Percent Agreement	Negative Percent Agreement
Individuals at Increased Risk of HBV Infection and Individuals With Signs and Symptoms of Hepatitis Infection	2,018	98.00% (49/50)	99.49% (1,958/1,968)
Individuals With Acute HBV Infection ^a	21	100.00% (21/21)	NA
Individuals With Chronic HBV Infection ^b	96	100.00% (94/94)	50.00% (1/2)
Pregnant Females	741	100.00% (2/2)	100.00% (739/739)
Pediatric Population	100	NA	100.00% (100/100)

NA = Not Applicable

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^a Includes 6 specimens from individuals diagnosed with acute HBV infection and 15 seroconversion panel members classified as acute by four-marker HBV reference testing.

^b Includes 43 specimens collected in the U.S. from individuals diagnosed with chronic infection defined by the presence of HBsAg for ≥ 6 months and 53 specimens collected in Vietnam and classified as chronic by four-marker HBV reference testing.

AxSYM HBsAg assay performance was evaluated by testing 2,018 specimens from individuals at increased risk of HBV infection and individuals with signs and symptoms of hepatitis infection. These specimens were prospectively collected from specimen collection sites located in Galveston, TX (39.35%); Dallas, TX (5.80%); Miami, FL (4.41%); St. Petersburg, FL (4.21%); Chicago, IL (8.23%); and Denver, CO (6.10%), or were obtained from a specimen vendor at the following three locations: Colton, CA (5.85%); Plymouth, MA (16.90%); and High Point, NC (9.17%). The population was Caucasian (52.87%), African American (28.59%), Hispanic (14.62%), Asian (1.98%), and American Indian/Alaska Native (0.45%), with the remaining 1.49% represented by other ethnic groups. The population was 52.58% female and 47.42% male and ranged in age from 18 to 83 years.

AxSYM HBsAg was further evaluated by testing a total of 117 acute and chronic subjects, which included prospectively collected specimens from six individuals diagnosed with acute HBV infection, 15 seroconversion panel members classified as acute by four-marker HBV reference testing, 53 specimens prospectively collected in Vietnam and classified as chronic by four-marker HBV reference testing, and 43 specimens prospectively collected in the U.S. from individuals classified as chronic defined by the presence of HBsAg for ≥ 6 months.

All specimens, with the exception of the 43 specimens classified as chronic, were sent to an external reference laboratory for HBV reference marker testing by FDA-approved reference assays for the detection of HBsAg, anti-HBc IgM, total anti-HBc, and anti-HBs. These specimens were assigned an HBV classification using the results for the four HBV reference markers and the modification of the serological criteria established by the National Center of Infectious Diseases (CDC) for diagnosing HBV infection.

Testing of these specimens occurred at clinical testing sites located in Port Jefferson, NY (44.03%); Dallas, TX (12.88%); and Raritan, NJ (42.39%), and at Abbott Laboratories (0.70%).

HBV Classification for Individuals at Increased Risk of HBV Infection, Individuals With Signs and Symptoms of Hepatitis Infection, and Individuals With Acute and Chronic HBV Infection

Number of Specimens	HBV Reference Markers				HBV Classification ^a
	HBsAg	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
17	+	-	-	-	Early Acute
11	+	+	+	-	Acute
1	+	+	+	I	Chronic
3	+	-	+	+	Chronic
85	+	-	+	-	Chronic
2	+	-	-	+	Chronic
4	+	-	+	I	Chronic ^b

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	Presence of HBsAg ≥ 6 months				Chronic
43					
1	+	+	+	+	Late Acute/Recovering ^b
4	-	+	+	+	Recovering Acute
3	-	+	+	I	Early Recovery ^b
193	-	-	+	+	Immune Due to Natural Infection
31	-	-	+	I	Distantly Immune/Anti-HBs Unknown
107	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
508	-	-	-	+	Immune Due to HBV Vaccination
66	-	-	-	I	Unknown
1,056	-	-	-	-	Susceptible
2,135					Total

I = indeterminate

^a Classification presented is a modification of the CDC Interpretation of Viral Hepatitis B Panel testing, based on the results of four HBV reference markers.

^b Three additional patterns were observed during the clinical investigation.

Comparison of AxSYM HBsAg Results With Reference HBsAg Results by HBV Classification

Individuals at Increased Risk of HBV Infection, Individuals With Signs and Symptoms of Hepatitis Infection, and Individuals With Acute and Chronic HBV Infection

HBV Classification	Reference HBsAg Result ^a				Total
	+		-		
	AxSYM HBsAg Result ^b				
	+	-	+	-	
Early Acute	17	0	0	0	17
Acute	11	0	0	0	11
Chronic	135	1 ^c	1 ^d	1	138
Late Acute/Recovering	1	0	0	0	1
Recovering Acute	0	0	0	4	4
Early Recovery	0	0	0	3	3
Immune Due to Natural Infection	0	0	3 ^e	190	193
Distantly Immune/Anti-HBs Unknown	0	0	0	31	31
Distantly Immune/Anti-HBs Not Detected	0	0	3 ^f	104	107
Immune Due to HBV Vaccination	0	0	1 ^g	507	508
Unknown	0	0	0	66	66
Susceptible	0	0	3 ^h	1,053	1,056
Total	164	1	11	1,959	2,135

^a Includes retesting and confirmatory testing performed according to the package insert.

^b Includes retesting performed according to the clinical brochure, with the exception of the 15 well-characterized seroconversion panel members.

^c This specimen was tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA. An additional aliquot sent for reference HBsAg assay testing was negative.

^d This specimen was tested and determined to be positive for anti-HBe, and negative for HBeAg and HBV DNA.

^e One specimen was nonreactive by AxSYM HBsAg Confirmatory and two specimens were repeat reactive, nonconfirming (RRNC).

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^f Two specimens were tested and determined to be positive for anti-HBe, and negative for HBeAg and HBV DNA; one specimen was AxSYM HBsAg Confirmatory RRNC.

^g This specimen was tested and determined to be negative for HBeAg, anti-HBe, and HBV DNA.

^h One specimen was tested and determined to be negative for HBeAg and anti-HBe and positive for HBV DNA; one specimen was negative for HBeAg, anti-HBe, and HBV DNA; and one specimen was nonreactive by AxSYM HBsAg Confirmatory.

Table 11

**Percent Agreement Between AxSYM HBsAg Results and Reference HBsAg Results
Summarized by HBV Classification
Individuals at Increased Risk of HBV Infection and Individuals With Signs and Symptoms of Hepatitis Infection**

HBV Classification	Positive Percent Agreement	95% Confidence Interval	Negative Percent Agreement	95% Confidence Interval
Early Acute	2/2 (100.00%)	[15.81%, 100.00%]		
Acute	5/5 (100.00%)	[47.82%, 100.00%]		
Chronic	41/42 (97.62%)	[87.43%, 99.94%]		
Late Acute/Recovering	1/1 (100.00%)	[2.50%, 100.00%]		
Recovering Acute			4/4 (100.00%)	[39.76%, 100.00%]
Early Recovery			3/3 (100.00%)	[29.24%, 100.00%]
Immune Due to Natural Infection			190/193 (98.45%)	[95.52%, 99.68%]
Distantly Immune/Anti-HBs Unknown			31/31 (100.00%)	[88.78%, 100.00%]
Distantly Immune/Anti-HBs Not Detected			104/107 (97.20%)	[92.02%, 99.42%]
Immune Due to HBV Vaccination			507/508 (99.80%)	[98.91%, 100.00%]
Unknown			66/66 (100.00%)	[94.56%, 100.00%]
Susceptible			1,053/1,056 (99.72%)	[99.17%, 99.94%]
Overall	49/50 (98.00%)	[89.35%, 99.95%]	1,958/1,968 (99.49%)	[99.07%, 99.76%]

Expected Results

Expected results were determined using the AxSYM HBsAg results for individuals at increased risk of HBV infection. Approximately 45.93% (1,314/2,861) of the prospective subjects participating in the clinical investigation were from individuals at increased risk of HBV infection. All subjects were at risk of HBV infection due to lifestyle, behavior, occupation, or known exposure event but were asymptomatic and reported no current signs or symptoms of hepatitis. The population ranged in age from 18 to 75 years. A demographic summary of this population is presented in the following table:

	Total Number of Specimens (%)
Ethnicity:	
Caucasian	47.56
African American	36.30
Hispanic	12.71
Asian	1.45
American Indian/Alaska Native	0.46
Other	1.52
Gender:	
Female	62.10
Male	37.90

The AxSYM HBsAg assay was reactive in 1.67% of the individuals in this population. The percent of individuals at increased risk of HBV infection enrolled at each location and the percent of AxSYM HBsAg reactive results observed from each location are presented in Table 12 on page 39. The percent AxSYM HBsAg reactive and nonreactive results by age range and gender is presented in Table 13 on page 39.

Table 12

AxSYM HBsAg Reactive Results by Specimen Collection Site or Specimen Vendor for Individuals at Increased Risk of HBV Infection

Specimen Collection Site/ Specimen Vendor	Percent of Individuals at Increased Risk of HBV Infection Enrolled at Each Location (%)	Percent of AxSYM HBsAg Reactive Results Observed From Each Location (%)
Site 1, Galveston, TX	56.54 (743/1,314)	1.48 (11/743)
Site 2, Dallas, TX	4.49 (59/1,314)	5.08 (3/59)
Site 3, Miami, FL	3.96 (52/1,314)	7.69 (4/52)
Site 4, St. Petersburg, FL	4.26 (56/1,314)	3.57 (2/56)
Site 5, Chicago, IL	0.61 (8/1,314)	0.00 (0/8)
Site 6, Denver, CO	2.74 (36/1,314)	0.00 (0/36)
Specimen Vendor Location:		
Colton, CA	5.78 (76/1,314)	0.00 (0/76)
Plymouth, MA	7.53 (99/1,314)	1.01 (1/99)
High Point, NC	14.08 (185/1,314)	0.54 (1/185)

Table 13

AxSYM HBsAg Results by Age Range and Gender for Individuals at Increased Risk of HBV Infection

Age Range	Gender	AxSYM HBsAg Result		Total
		+ Number of Specimens (%)	- Number of Specimens (%)	
10 to 19	Female	0 (0.00)	14 (100.00)	14
	Male	0 (0.00)	11 (100.00)	11
20 to 29	Female	1 (0.54)	183 (99.46)	184
	Male	0 (0.00)	97 (100.00)	97
30 to 39	Female	0 (0.00)	184 (100.00)	184
	Male	5 (4.67)	102 (95.33)	107
40 to 49	Female	5 (1.99)	246 (98.01)	251
	Male	3 (1.89)	156 (98.11)	159
50 to 59	Female	1 (0.73)	136 (99.27)	137
	Male	6 (5.50)	103 (94.50)	109
60 to 69	Female	0 (0.00)	35 (100.00)	35
	Male	1 (8.33)	11 (91.67)	12
70 to 79	Female	0 (0.00)	8 (100.00)	8
	Male	0 (0.00)	3 (100.00)	3
Unknown ^a	Female	0 (0.00)	3 (100.00)	3
Total		22 (1.67)	1,292 (98.33)	1,314

^a Age was not provided for three subjects.

Summary of Safety and Effectiveness Data
AxSYM HBsAg Confirmatory

Specimens that were found to be repeatedly reactive by AxSYM HBsAg were tested at the clinical testing sites with AxSYM HBsAg Confirmatory. The results were compared to the corresponding reference HBsAg assay results. As shown in Table 14 on page 41, of the 2,968 specimens tested in the clinical investigation, a total of 178 specimens were tested by either AxSYM HBsAg Confirmatory or reference HBsAg confirmatory assays or were > 5.00 S/C in the reference assay. One hundred sixty-six specimens were confirmed positive and five specimens were negative for the presence of HBsAg by both methods. Of the 177 specimens that were repeatedly reactive by AxSYM HBsAg, 172 were confirmed positive and five were nonreactive or repeat reactive, nonconfirming by AxSYM HBsAg Confirmatory.

The results of this evaluation demonstrate that the AxSYM HBsAg Confirmatory assay can be used for confirmation of samples found to be repeatedly reactive by the AxSYM HBsAg assay and may be used to confirm HBV infection in pregnant women.

Table 14
Comparison of AxSYM HBsAg Confirmatory Results to
Reference HBsAg Confirmatory Results

Specimen Category	Reference HBsAg Confirmatory Result				Total
	+ ^a		-		
	AxSYM HBsAg Confirmatory Result				
	+	-	+	-	
Individuals at Increased Risk of HBV Infection	18	1 ^b	2 ^c	2	2
Individuals With Signs and Symptoms of Hepatitis Infection	31	0	3 ^d	3	3
Individuals With Acute HBV Infection	21	0	0	0	2
Individuals With Chronic HBV Infection	41	0	1 ^e	0	4
Specimens From Vietnam	53	0	0	0	5
Pregnant Females	2	0	0	0	1
Total	166	1	6	5	1

^a Includes specimens tested by the reference HBsAg assay with S/C results > 5.00.

^b This specimen was not tested by AxSYM HBsAg Confirmatory because it was negative by AxSYM HBsAg. This specimen was tested and determined to be positive for anti-HBs. An additional aliquot of this specimen was sent for reference HBsAg assay testing and was negative.

^c One specimen was tested and determined to be positive for HBV DNA, and one specimen was negative for anti-HBc IgM, anti-HBc, anti-HBs, HBeAg, anti-HBe, and HBV DNA.

^d Two specimens were tested and determined to be positive for anti-HBc and anti-HBe, and one specimen was positive for anti-HBs.

^e This specimen was tested and determined to be positive for anti-HBc, anti-HBs, and anti-HBe.

X. CONCLUSIONS DRAWN FROM THE STUDIES

Multi-centered clinical studies were conducted in the US to evaluate the AxSYM HBsAg assays. The intended use and safety and effectiveness of the AxSYM HBsAg and AxSYM HBsAg

Summary of Safety and Effectiveness Data

Confirmatory assays are substantiated by the data contained in this submission. The data support the use of these assays to detect the presence of hepatitis B surface antigen (HBsAg) in neonatal serum, and adult and pediatric serum and plasma. The data also support the use of these assays to aid in the diagnosis of acute or chronic hepatitis B virus (HBV) infection and to test for HBV infection in pregnant women.

Hepatitis B virus classification using the prospective population showed 16 unique reference marker patterns. The overall positive percent agreement between the AxSYM HBsAg assay and the reference assays was 98.0% (49/50) in the high risk, signs and symptoms. The overall negative percent agreement between the AxSYM HBsAg assay and the reference assays was 99.49% (1958/1968) in the same population.

The ability of the AxSYM HBsAg assays to detect HBsAg was demonstrated in pediatric and neonatal specimen testing.

Precision and reproducibility of the AxSYM HBsAg was established for within-run, within-day, within-lab, and between sites.

Tube Type Interference study results support the use of human serum and plasma (potassium EDTA, sodium citrate, lithium heparin, sodium heparin, and plasma separator tubes) and neonatal serum in the AxSYM HBsAg assay.

The results from both the non-clinical and clinical studies indicate that the AxSYM HBsAg assay can be used safely and effectively for the qualitative *in vitro* determination of Hepatitis B surface antigens in human serum and plasma. The assay may be used with other HBV serological markers to define the clinical status of patients known to be infected with HBV.

RISK BENEFIT ANALYSIS

As a diagnostic test, the AxSYM HBsAg assays involves removal of blood from an individual for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is having their blood drawn for any other diagnostic evaluation. The benefits to HBV-infected individuals tested by the assay outweigh any potential adverse event or risk to the patient or user due to assay malfunction or operator error.

The potential risks encountered with this *in vitro* diagnostic test are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for the device. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

SAFETY

Based on the results of the preclinical and clinical laboratory studies, the AxSYM HBsAg assays, when used according to the provided directions and in conjunction with other serological and clinical information, should be safe and effective and pose minimal risk to the patient due to false test results.

Summary of Safety and Effectiveness Data

EFFECTIVENESS

The effectiveness of the AxSYM HBsAg assay has been demonstrated for use in determining if hepatitis B virus surface antigen virus is present in an individual's serum or plasma. A reasonable determination of effectiveness of the AxSYM HBsAg assay for aiding in the diagnosis of HBV infection and status of HBV infection in suspected individuals has been demonstrated.

XI. PANEL RECOMMENDATIONS

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

XII. CDRH DECISION

FDA issued an approval order on June 1, 2006.

The applicant's manufacturing facility was inspected on 5/8/06 (N. Chicago), 5/16/06 (Abbott Park), & 5/19/04 (Puerto Rico) and found to be in compliance with the Quality Systems Regulation (21 CFR 820).

XIII. APPROVAL SPECIFICATIONS

Directions for use: See the labeling.

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

Postapproval Requirements and Restrictions: See approval order.

Bibliography

1. Chen Y-CJ, Delbrook K, Dealwis C, et al. Discontinuous epitopes of hepatitis B surface antigen derived from a filamentous phage peptide library. *Proc Natl Acad Sci USA* 1996;93:1997-2001.
2. Norder H, Courouce AM, Coursaget P, et al. Genetic diversity of hepatitis B virus strains derived worldwide; genotypes, subgenotypes, and HBsAg subtypes. *Intervir* 2004;47:289-309.
3. Qui X, Schroeder P, Bridon D. Identification and characterization of a C(K/R)TC motif as a common epitope present in all subtypes of hepatitis B surface antigen. *J Immunol* 1996;156:3350-3356.

Summary of Safety and Effectiveness Data

4. Coleman PF, Chen Y-CJ, Mushahwar IK. Immunoassay detection of hepatitis B surface antigen mutants. *J Med Virol* 1999;59:19–24.
5. Zuckerman AJ. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet* 2000;355:1382–4.
6. National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline – Second Edition*. CLSI Document EP5-A2. Wayne, PA: CLSI, 2004.
7. Clinical and Laboratory Standards Institute. *User Verification of Performance for Precision and Trueness: Approved Guideline – Second Edition*. CLSI Document EP15-A2. Wayne, PA: CLSI, 2005.