# ABBOTT PRISM® HBsAg
# ABBOTT PRISM® HBsAg Confirmatory
# Summary Basis for Approval

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| **Product Trade Names** | ABBOTT PRISM® HBsAg<br>ABBOTT PRISM® HBsAg Confirmatory |
| **Proper Names** | Antibody to Hepatitis B Surface Antigen (Mouse Monoclonal IgM)<br>Antibody to Hepatitis B Surface Antigen (Human) |
| **Applicant** | Abbott Laboratories<br>Dept. 49C, Bldg. AP6C<br>100 Abbott Park Road<br>Abbott Park, IL 60064 |
| **Submission Tracking Number** | BL 103766 |
| **Report Date** | July 2006 |

## I. Intended Use

The ABBOTT PRISM HBsAg assay is an *in vitro* chemiluminescent immunoassay (ChLIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma specimens. The ABBOTT PRISM HBsAg (ChLIA) is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of HBsAg. It is also intended for use in testing blood and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

The ABBOTT PRISM HBsAg Confirmatory assay is an *in vitro* qualitative chemiluminescent immunoassay (ChLIA) used to confirm the presence of hepatitis B surface antigen (HBsAg) in human serum and plasma by means of specific antibody neutralization. The ABBOTT PRISM HBsAg Confirmatory assay is intended to be used for confirmation of samples found to be repeatedly reactive by the ABBOTT PRISM HBsAg assay.

## II. Brief Description of the Test

### A. Description of the Test

The ABBOTT PRISM HBsAg assay utilizes microparticles coated with mouse monoclonal anti-HBs as the solid phase, to bind HBsAg present in human serum or plasma. A complex consisting of acridinium-labeled goat polyclonal anti-HBs conjugate is then incubated with the microparticles.

A chemiluminescent signal is generated by addition of an alkaline hydrogen peroxide solution. The amount of light emitted is proportional to the amount of HBsAg in the sample.

The ABBOTT PRISM HBsAg Confirmatory assay uses the principle of specific antibody neutralization to confirm the presence of HBsAg in specimens found to be repeatedly reactive by the ABBOTT PRISM HBsAg assay. The ABBOTT PRISM HBsAg Confirmatory Reagent A, Antibody to Hepatitis B Surface Antigen (anti-HBs, human), is pre-incubated with the specimen in solution. If HBsAg is present in the specimen, it will be bound by Reagent A. The neutralized HBsAg is subsequently blocked from binding to the antibody-coated microparticles. This results in a reduction of signal when compared to the non-neutralized specimen in which ABBOTT PRISM HBsAg Confirmatory Reagent B (recalcified human plasma nonreactive for HBsAg and negative for anti-HBs) is used in place of Reagent A. A specimen is confirmed positive if the signal emitted by the non-neutralized specimen (the specimen with Reagent B added) is greater than or equal to the ABBOTT PRISM HBsAg Confirmatory cutoff value, and if the percent neutralization is 50% or greater.

## B. Reagents

1. The ABBOTT PRISM HBsAg Assay Kit (No. 6D19-68) contains the following components:

   a. 1 Bottle (333 mL) Antibody to Hepatitis B Surface Antigen (Mouse Monoclonal IgM) Coated Microparticles in phosphate buffered saline with bovine serum albumin, Tween®* 20, and protein stabilizers. Minimum concentration: 0.03% solids. Preservative: 0.1% sodium azide. (Symbol: ●)

   b. 1 Bottle (328 mL) Antibody to Hepatitis B Surface Antigen (Goat Polyclonal): Acridinium Conjugate in phosphate buffered saline with calf serum and recalcified, human plasma, nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV and anti-HIV-1/HIV-2. Minimum concentration: 0.025 µg/mL. Preservative: 0.1% sodium azide. (Symbol: ▲)

   c. 3 Bottles (10.4 mL each) Negative Calibrator (Human). Recalcified plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV and anti-HIV-1/HIV-2. Preservative: 0.1% sodium azide. (Symbol: **NC**)

   *Tween is a registered trademark of ICI Americas.

d. 3 Bottles (10.4 mL each) Positive Calibrator (Human). Recalcified, inactivated plasma reactive for HBsAg and nonreactive for HIV-1 Ag or HIV-1 NAT, anti-HCV and anti-HIV-1/HIV-2. HBsAg concentration: 0.25-0.65 ng/mL. Preservative: 0.1% sodium azide. (Symbol: **PC**)

2. The ABBOTT PRISM HBsAg Confirmatory Kit, 10 Tests (No. 6E51-68) contains the following components:

a. 1 Bottle (2 mL) Reagent A. Antibody to Hepatitis B Surface Antigen (Human) and recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV and anti-HIV-1/HIV-2. Minimum concentration: 0.01 mg/mL. Contains Red Dye D&C. Preservative: 0.1% sodium azide. (Symbol: **RGT A**)

b. 1 Bottle (2 mL) Reagent B. Recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV and anti-HIV-1/HIV-2. Contains bromophenol blue. Preservative: 0.1% sodium azide. (Symbol: **RGT B**)

c. 1 Bottle (4 mL) Reagent C. Specimen treatment reagent with 20 mM citrate buffer. (Symbol: **RGT C**)

d. 1 Bottle (18 mL) Diluent. Recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV and anti-HIV-1/HIV-2. Preservative: 0.1% sodium azide. (Symbol: **DIL**)

e. 1 Package (100 units) ABBOTT PRISM Sample Cups.

f. 1 Package (10 count) ABBOTT PRISM HBsAg Confirmatory Bar Code Labels.

## C. Other Reagents Required

1. The ABBOTT PRISM HBsAg Wash Kit (No. 6D19-58) contains the following components:

a. 1 Bottle (3393 mL) Transfer Wash. Phosphate buffered saline. Preservative: 0.1% sodium azide. (Symbol: ~)

b. 1 Bottle (2811 mL) Conjugate Wash. Borate buffered saline. Preservative: 0.1% sodium azide. (Symbol: ★)

2. The ABBOTT PRISM Activator Concentrate (No. 1A75-02) contains the following component:

   a. 4 Bottles (900 mL each) Activator Concentrate. 0.4% hydrogen peroxide/0.06% diethylenetriaminepentaacetic acid.

3. The ABBOTT PRISM Activator Diluent (No. 1A75-01) contains the following component:

   a. 4 Bottles (900 mL each) Activator Diluent. 0.3 *N* sodium hydroxide.

4. The ABBOTT PRISM Run Control Kit (No. 3E60-10) contains the following components:

   a. 2 Bottles (10 mL each) Positive Control (Human). Purified anti-HBc IgG (Concentration: 0.9 - 2.6 PEI* Units/mL) and recalcified, inactivated plasma reactive for HBsAg (Concentration: 0.10 - 0.40 ng/mL), anti-HCV, anti-HIV-1 and anti-HTLV-I. Plasma is also tested for HIV-1 by either HIV-1 Ag and is nonreactive, or by HIV-1 NAT, and may be reactive. Positive Control may be cross-reactive with antibody to HTLV-II. Preservative: 0.1% sodium azide. (Symbol: **POS**)

   b. 1 Bottle (10 mL) Supplemental Positive Control (Human). Recalcified, inactivated plasma reactive for anti-HIV-2 and anti-HTLV-II, nonreactive for HBsAg, anti-HCV and HIV-1 Ag or HIV-1 NAT. Supplemental Positive Control may be cross-reactive with antibody to HTLV-I. Preservative: 0.1% sodium azide. (Symbol: **SUP**)

   c. 2 Bottles (10 mL each) Negative Control (Human). Recalcified plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV, anti-HIV-1/HIV-2, anti-HBc, anti-HBs and anti-HTLV-I/HTLV-II. Preservative: 0.1% sodium azide. (Symbol: **NEG**)

   * Concentration standardized against the reference standard of the Paul Ehrlich Institute (PEI), Langen, Germany.

5. The ABBOTT PRISM Positive Run Control Kit (No. 3E60-11) contains the following component:

   a. 6 Bottles (10 mL each) Positive Control (Human). Purified anti-HBc IgG (Concentration: 0.9 - 2.6 PEI* Units/mL) and recalcified, inactivated plasma reactive for HBsAg (Concentration: 0.10 - 0.40 ng/mL), anti-HCV, anti-HIV-1 and anti-HTLV-I. Plasma is also tested for HIV-1 by either HIV-1 Ag and is nonreactive, or by HIV-1 NAT, and may be reactive. Positive Control may be cross-reactive with antibody to HTLV-II. Preservative: 0.1% sodium azide. (Symbol: **POS**).

   * Concentration standardized against the reference standard of the Paul Ehrlich Institute (PEI), Langen, Germany.

## III. Manufacturing and Controls

### A. Manufacturing and Controls

The ABBOTT PRISM HBsAg assay is manufactured by Abbott Laboratories and prepared under U.S. License Number 43.

Mouse Monoclonal IgM Anti-HBs is used in the ABBOTT PRISM HBsAg assay. (b)(4)

(b)(4)

(b)(4)

(b)(4)

Raw materials intended for use in the product are subjected to quality control evaluations before they are accepted for use in manufacturing. All components have established acceptance criteria and performance specifications. Final components are subjected to performance testing and assembled into kits. The final ABBOTT PRISM HBsAg and ABBOTT PRISM HBsAg Confirmatory assay kits are then further subjected to final performance testing.

Each lot of ABBOTT PRISM HBsAg and ABBOTT PRISM HBsAg Confirmatory assay kits is tested with in-house panels of samples with varying levels of HBsAg reactivity, as well as the CBER HBsAg Reference Panel, and must meet the performance requirements of both panels.

## B. Stability Studies

Components of the ABBOTT PRISM HBsAg assay kit, ABBOTT PRISM HBsAg Confirmatory assay kit, ABBOTT PRISM Run Control kit, ABBOTT PRISM Positive Run Control kit, ABBOTT PRISM HBsAg Wash kit, as well as the ABBOTT PRISM Activator Concentrate and ABBOTT PRISM Activator Diluent were entered into a stability program to establish the recommended storage conditions and the expiration dating. Three different lots of each component were manufactured, tested, assembled into kits and evaluated during storage. The studies support a (b)(4) month dating period for the wash kit components and a [redacted] month dating period for the components of both assay kits, both Run Control kits, the Activator Concentrate and the Activator Diluent. The expiration date of the kit lot is the same as that of the shortest dated kit component.

## C. Methods of Validation

Production of components is monitored by in-process testing. Product potency is assured through evaluation of (b)(4) testing and performance testing. Product consistency is assured through lot uniformity testing of components.

Product performance is assessed through laboratory evaluations of each test kit lot against in-house panels and the CBER HBsAg Reference Panel. Each lot of product and protocols summarizing pertinent product testing are submitted for evaluation and approval by FDA prior to release for distribution.

## D. Labeling

The product labeling, including immediate container, package labels, and package insert (directions for use), are in compliance with 21 CFR 610 Subpart G, 21 CFR 801 Subpart A and 21 CFR 809.10. The product trade names, ABBOTT PRISM HBsAg and ABBOTT PRISM HBsAg Confirmatory, are not known to conflict with any other biologic or device trade name.

## E. Establishment Inspection

A pre-licensing inspection of the areas where product is manufactured, tested, stored and shipped was most recently conducted in April 2006. Facilities and procedures for this product were found to be in substantial conformity with the Quality System Regulation.

## F. Environmental Impact Analysis Report (EIAR)

Abbott Laboratories has filed a detailed EIAR. This product has no significant environmental impact.

# IV. Biological Principles of the Procedure

The ABBOTT PRISM HBsAg assay is a two-step sandwich ChLIA. The reactions occur within the ABBOTT PRISM System in the following sequence:

- Microparticles coated with mouse monoclonal anti-HBs are incubated with sample (either plasma, serum, calibrator, or control) in the incubation well of the reaction tray. During incubation, HBsAg present in the sample binds to the antibody on the Microparticles.

- After this first incubation is complete, the reaction mixture is transferred to the glass fiber matrix (matrix) of the reaction tray using the Transfer Wash. The Microparticles are captured by the matrix while the remaining mixture flows through to the absorbent blotter.

- The Acridinium-Labeled Goat Polyclonal Anti-HBs Conjugate is added to the Microparticles on the matrix and incubated. After this second incubation, the unbound Conjugate is washed into the blotter with the Conjugate Wash.

- The chemiluminescent signal is generated by addition of an alkaline hydrogen peroxide solution. The resultant photons are counted.

The amount of light emitted is proportional to the amount of HBsAg in the sample. The presence or absence of HBsAg in the sample is determined by comparing the number of photons collected from the sample to a cutoff value determined from a calibration performed in the same batch. If the number of photons collected from a test sample is less than the cutoff value, the sample is considered nonreactive for HBsAg by the criteria of the ABBOTT PRISM HBsAg assay. These specimens need not be further tested. If the number of photons collected from a test sample is greater than or equal to the cutoff value, the sample is considered reactive for HBsAg by the criteria of the ABBOTT PRISM HBsAg assay.

Specimens that are initially reactive must be handled according to the package insert instructions and retested in duplicate. A specimen that is repeatedly reactive must be confirmed by the ABBOTT PRISM HBsAg Confirmatory assay, a licensed neutralizing confirmatory test. Only the specimens that are confirmed by specific neutralization with anti-HBs are considered positive for HBsAg.

The ABBOTT PRISM HBsAg Confirmatory assay involves two steps: an off-line specimen dilution and neutralization, and the automated processing of the ABBOTT PRISM HBsAg assay. The reactions occur within the ABBOTT PRISM System in the following sequence:

Off-line Dilution and Neutralization Procedure

- Each specimen is diluted using the ABBOTT PRISM HBsAg Confirmatory Diluent.

- Each sample (including ABBOTT PRISM Positive Control, ABBOTT PRISM Negative Control, and undiluted and diluted specimens) is precision pipetted into a set of ABBOTT PRISM Sample Cups. ABBOTT PRISM HBsAg Confirmatory Reagent C is added to each cup. Reagent A is added to one sample cup and Reagent B is added to the other sample cup.

- Following an off-line pre-incubation period, samples are tested using the ABBOTT PRISM HBsAg assay.

ABBOTT PRISM HBsAg Procedure

- Microparticles coated with mouse monoclonal anti-HBs are incubated with the sample/Confirmatory Reagent mixture (mixture) in the incubation well of the reaction tray. During incubation, HBsAg present in the mixture binds to the antibody on the Microparticles (HBsAg neutralized by Reagent A will not bind to the anti-HBs on the Microparticles).

- After the first incubation is complete, the reaction mixture is transferred to the glass fiber matrix (matrix) of the reaction tray using the Transfer Wash. The Microparticles are captured by the matrix, while the remaining mixture flows through to the absorbent blotter.

- The Acridinium-Labeled Goat Polyclonal Anti-HBs Conjugate is added to the Microparticles on the matrix and incubated. After the second incubation, the unbound Conjugate is washed into the blotter with the Conjugate Wash.

- The chemiluminescent signal is generated by addition of an alkaline hydrogen peroxide solution. The resultant photons are counted.

The amount of light emitted by a non-neutralized sample (sample with Reagent B) is proportional to the amount of HBsAg in the sample. If the sample contains HBsAg, the same sample neutralized by Reagent A will emit less light. This resulting reduction in signal is used to calculate the percent neutralization of the sample. The presence or absence of HBsAg in the sample is determined by comparing the number of photons collected from the sample with Reagent B to the ABBOTT PRISM HBsAg Confirmatory assay cutoff value determined from an ABBOTT PRISM HBsAg calibration performed in the same batch. In addition, the percent neutralization of the sample is evaluated. If the number of photons collected from the test sample with Reagent B added is greater than or equal to the ABBOTT PRISM HBsAg Confirmatory assay cutoff value and the calculated percent neutralization is greater than or equal to 50%, the sample is confirmed positive for HBsAg by the criteria of the ABBOTT PRISM HBsAg Confirmatory assay.

## V. Performance Characteristics

### A. Summary of Non-Clinical Studies

The following studies were performed: 1) specimen collection (including anticoagulant studies, segment vs. tubes and matched serum and plasma evaluation); 2) specimen handling (including off the blood cell, on the blood cell, and on the clot storage conditions, heat inactivation, microbial contamination and specimen freeze-thaw effects); 3) effects of potentially interfering substances (including triglycerides, bilirubin, hemoglobin, protein and red blood cells); 4) assay detectability studies (including fresh serum vs. plasma specimens and HBsAg subtype and mutant detectability); 5) reagent studies (including microbial challenge of kit reagents, ABBOTT PRISM Positive Control analyte cross-reactivity and evaluation of within-run variability and validation of batch size); and 6) cadaveric serum specimens.

## 1. Specimen Collection

Results of in-house studies showed that the following specimen collection containers and anticoagulants are suitable for use in the ABBOTT PRISM HBsAg and ABBOTT PRISM HBsAg Confirmatory assays: serum, serum separator tubes, EDTA, potassium oxalate, sodium citrate, ACD-A, ACD-B, CP2D, CPD, CPDA-1, heparin or segmented tubing. Since the ABBOTT PRISM System pipettes samples simultaneously for all assays and due to the anticoagulant testing limitations of the ABBOTT PRISM HCV assay, the ABBOTT PRISM HBsAg and HBsAg Confirmatory package inserts state: "CAUTION: Do not use specimens collected in heparin. Use of heparin as an anticoagulant may cause a reduction in Sample Net Counts and in Sample Net Counts/Cutoff Value (S/CO) for ABBOTT PRISM HCV; therefore, heparin is not recommended for any ABBOTT PRISM assay."

No qualitative differences in results were observed between matched donor serum and EDTA plasma specimens.

## 2. Specimen Handling

The results of in-house studies showed no significant differences when using HBsAg nonreactive or reactive specimens that have been stored on and off the red blood cells/clot for up to days at 2 to 8 °C. However, as a precautionary measure, the package insert states: "Specimens may be stored for up to 14 days at 2 - 8 °C."

Results from heat inactivation studies indicated that HBsAg nonreactive and reactive serum and plasma specimens showed no qualitative differences in results after heat treatment. However, as a precautionary measure, the package insert states: "Do not use heat-inactivated specimens."

Studies were conducted to evaluate the effect of microbial contamination of specimens on assay performance in ABBOTT PRISM HBsAg. These studies showed that no significant assay performance differences were observed when HBsAg nonreactive and reactive specimens were inoculated with elevated levels of

. However, as a precautionary measure, the package insert states: "Do not use specimens with obvious microbial contamination."

Results from the freeze-thaw studies showed that specimens could be frozen and thawed times with no qualitative performance differences. However, as a precautionary measure, the package insert states: "Some specimens that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may give erroneous or inconsistent test results."

**3. Potentially Interfering Substances**

No qualitative performance differences were observed for the ABBOTT PRISM HBsAg assay in controlled studies using HBsAg nonreactive and reactive specimens when testing the following potentially interfering substances at the specified levels: bilirubin (≤ 20 mg/dL), hemoglobin (≤ 500 mg/dL), red blood cells (≤ 0.4% v/v), triglycerides (≤ 3,000 mg/dL) or protein (≤ 12 g/dL).

**4. Detectability Studies**

In order to evaluate whether the ABBOTT PRISM HBsAg assay is susceptible to the fresh serum effects observed in some assays, freshly collected serum and EDTA plasma specimens were spiked with HBsAg reactive plasma and analyzed
There were no significant differences observed between the fresh vs. stored serum, or between fresh serum vs. plasma specimens.

The detectability of known variants of hepatitis B surface antigen was evaluated using the ABBOTT PRISM HBsAg assay. A HBsAg subtype panel

**5. Reagent Studies**

The data for the microbial challenge of kit components indicate that the preservative used in the ABBOTT PRISM HBsAg assay (sodium azide) is effective in preventing microbial growth of

A study was performed to demonstrate the absence of cross-reactivity between analytes (HBsAg, anti-HCV, anti-HIV-1, anti-HTLV-1, and anti-HBc) used in the multiconstituent ABBOTT PRISM Positive Control. No significant differences were observed between single analyte and multi-analyte solutions indicating that a reactive result with each of the five ABBOTT PRISM assays is due to the corresponding assay specific analyte.

No significant within-run variability in the S/CO of the ABBOTT PRISM Positive Run Control was observed during in-house investigations. There is minimal variation in the ABBOTT PRISM Positive Run Control S/CO with time over the duration of an -hour ABBOTT PRISM batch.

## 6. Performance Characteristics of Cadaveric Serum Testing

### a. Reproducibility

Inter-assay reproducibility of the ABBOTT PRISM HBsAg assay was assessed using 10 postmortem donor sera. These sera specimens were spiked with human plasma positive for HBsAg to create low-level reactive specimens. Each of the specimens was tested in triplicate on three different days on each of three reagent lots of ABBOTT PRISM HBsAg at one site for a total of 270 replicates. Three replicates generated dispense errors and 16 replicates generated drain time errors and were excluded from the analysis. For intra-assay reproducibility, the %CV ranged from 2.9 to 5.5 for the low level reactive specimens. For inter-assay reproducibility over all lots, the percent coefficient of variation (%CV) ranged from 4.4 to 8.7 for the low-level reactive specimens. The total reproducibility ranged from 5.3 to 9.7 for the low level reactive specimens. Note: Inter-assay reproducibility includes intra-assay and inter-assay variation. Total reproducibility includes intra-assay, inter-assay and inter-lot variations.

### b. Specificity

Specificity was evaluated using 51 postmortem donor specimens and 54 normal donor specimens. Each of the specimens was tested once on each of three reagent lots of ABBOTT PRISM HBsAg. The mean sample to cutoff (S/CO) ratio for the 136 nonreactive postmortem replicates (51 specimens with three reagent lots; see Table I, footnotes a and b) was 0.35, and the mean S/CO for 162 normal donor replicates (54 specimens with three reagent lots) was 0.24. Results are presented in Table I.

Assuming the specimen with the initial reactive result would have a reactive result upon retest, the ABBOTT PRISM HBsAg assay has an estimated specificity of 99.27% (136/137) (binomial confidence interval = [96.00%, 99.98%]) in these studies of postmortem serum specimens collected up to a maximum of 16.1 hours after death.

### c. Sensitivity

Sensitivity was evaluated using 51 postmortem specimens and 54 normal donor specimens that were pre-screened for anti-HBs and HBsAg and found to be negative. The 105 specimens were spiked with human plasma positive for HBsAg to create low-level reactive specimens. Each of the specimens was tested once on each of three reagent lots of ABBOTT PRISM HBsAg. The mean sample to cutoff (S/CO) for the 142 postmortem replicates (51 specimens, with three reagent lots; see Table II, footnote a) was 2.05, and the mean S/CO ratio for the 162 normal donor replicates (54 specimens, with three reagent lots) was 2.07. Results are presented in Table II.

The ABBOTT PRISM HBsAg assay has an estimated sensitivity of 100.00% (142/142) (binomial confidence interval = [97.44%, 100.00%]) in these studies of postmortem serum specimens collected up to 16.1 hours after death.

## B. Summary of Clinical Studies

ABBOTT PRISM HBsAg Assay

### 1. Assay Reproducibility

Assay reproducibility was determined by testing a seven-member panel consisting of three diluted specimens reactive for HBsAg *ad* subtype (panel members 1, 2 and 3), three diluted specimens reactive for HBsAg *ay* subtype (panel members 4, 5 and 6) and one specimen nonreactive for HBsAg (panel member 7). Panel members were prepared in recalcified human plasma. Each panel member was tested in replicates of four in five runs over five days with each of three reagent lots at six sites. In addition, each panel member was tested in replicates of four in five runs over five days with one of the three reagent lots at four of the six sites.

The Negative and Positive Controls were tested once at the beginning and end of each run on each subchannel. The Negative and Positive Calibrators were automatically tested in triplicate at the beginning of each run on each subchannel. The intra-assay and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) were determined with a variance component analysis for a mixed model (Table III).

## 2. Assay Specificity

A total of 25,238 fresh serum and plasma specimens from volunteer whole blood donors and plasmapheresis donors were collected and tested at six geographically distinct blood centers (Table IV). Two sites tested a total of 8,246 serum specimens with initial and repeat reactive rates of 0.06% (5/8,246) and 0.04% (3/8,246), respectively.

Three sites tested a total of 13,911 plasma specimens with initial and repeat reactive rates of 0.06% (8/13,911) and 0.04% (5/13,911), respectively. One site tested a total of 3,081 plasmapheresis donor specimens with initial and repeat reactive rates of 0.03% (1/3,081) and 0.00% (0/3,081), respectively. A total of eight specimens were repeatedly reactive. In six of the eight specimens (75.00%), the presence of HBsAg was confirmed by specific neutralization with anti-HBs. Two of the eight specimens were not confirmed as positive.

Specificity based on assumed zero prevalence of HBsAg in whole blood and plasmapheresis donors was estimated in these studies to be 99.99% (25,230/25,232) with a 95% confidence interval (CI) of 99.97% to 100.00%. The six repeatedly reactive specimens that confirmed positive for HBsAg were excluded from these calculations.

Three sites evaluated 870 serum and plasma specimens either collected from individuals with medical conditions unrelated to HBV infection or containing potentially interfering substances (Table IV). Fifty-nine of the 870 specimens (6.78%) were initially reactive and 50 of the 870 specimens (5.75%) were repeatedly reactive. Forty of the 50 specimens (80.00%) confirmed positive for HBsAg, and ten specimens did not confirm by specific antibody neutralization. The ten specimens included one anti-EBV positive (12 tested), one anti-HSV positive (12 tested), one rubella antibody positive (12 tested), one anti-nuclear antibody positive (12 tested), one elevated triglycerides (10 tested) and five pregnant females (555 tested). The estimated specificity in this population was 98.80% (820/830).

## 3. Assay Sensitivity

A total of 1,212 serum and plasma specimens from 514 individuals known to be positive for HBsAg, 98 individuals with acute HBV infection, 101 individuals with chronic HBV infection, 47 individuals who have recovered from HBV infection, and 452 individuals at increased risk for HBV infection were tested with the ABBOTT PRISM HBsAg assay.

A total of 767 specimens (63.28%) were repeatedly reactive, of which 754 (98.31%) were confirmed positive by specific antibody neutralization (Table V). The overall sensitivity was estimated in these studies to be 100.00% (754/754) with a 95% CI of 99.51% to 100.00%.

The sensitivity of the ABBOTT PRISM HBsAg assay was evaluated using a seven-member panel comprised of specimens from an Abbott Laboratories HBsAg Sensitivity Panel. Panel members were prepared in recalcified human plasma. Three panel members were reactive for HBsAg *ad* subtype, three members were reactive for HBsAg *ay* subtype and one member was nonreactive for HBsAg. The detection of HBsAg *ad* and *ay* subtypes is presented in Tables VI and VII, respectively.

The ability of the ABBOTT PRISM HBsAg assay to detect HBsAg was evaluated by testing 12 HBV seroconversion panels from blood and plasmapheresis donors who seroconverted over the course of their donation history. All specimens were also tested by a FDA licensed assay. The ABBOTT PRISM HBsAg assay detected HBsAg three to 13 days (one to three bleeds) earlier in ten of the 12 panels and five to 48 days (one to three bleeds) longer in four of the 12 panels when compared to the licensed assay. Both assays detected HBsAg in the first available bleed for two of the 12 panels.

ABBOTT PRISM HBsAg Confirmatory Assay

**1. Assay Reproducibility**

Assay reproducibility was determined by testing a seven-member panel consisting of three specimens reactive for HBsAg *ad* subtype (panel members 1, 2 and 3), three specimens reactive for HBsAg *ay* subtype (panel members 4, 5 and 6) and one specimen nonreactive for HBsAg (panel member 7). Panel members were prepared in recalcified human plasma. Each undiluted panel member was tested in duplicate in five runs over five days with each of three reagent lots at four sites. The Negative and Positive Controls were tested in replicates of seven in five runs over five days with each of three reagent lots at four sites. The intra-assay and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) of the S/CO and percent neutralization (%Neut) were determined with a variance component analysis for a mixed model (Table VIII).

### 2. Confirmation of HBsAg Reactive Specimens

Specimens from the following categories were evaluated with the ABBOTT PRISM HBsAg assay: volunteer whole blood donors (8,246 serum and 13,911 plasma), medical conditions unrelated to HBV infection and potentially interfering substances (870), preselected HBsAg positive (199), acute HBV infection (98), chronic HBV infection (101) and increased risk for HBV infection (452). Specimens that were repeatedly reactive by the ABBOTT PRISM HBsAg assay were evaluated with the ABBOTT PRISM HBsAg Confirmatory assay. The percentage of HBsAg repeatedly reactive specimens that confirmed positive ranged from 66.67 to 100.00 (Table IX).

## VI. Package Insert

See the package inserts (directions for use) for the ABBOTT PRISM HBsAg assay and the ABBOTT PRISM HBsAg Confirmatory assay.

**TABLE I**

**Reactivity with ABBOTT PRISM HBsAg**
**Cadaveric Serum Testing**

| Population | Number of Specimens | Number of Replicates | Mean S/CO | Nonreactive | Initial Reactive |
|---|---|---|---|---|---|
| Postmortem | 51 | 137[a] | 0.35 | 136 (99.27%) | 1[b] (0.73%) |
| Normal Donor | 54 | 162 | 0.24 | 162 (100.0%) | 0 (0.0%) |

[a] No results were obtained for 15 specimens on one lot due to drain time errors and one specimen on one lot due to an invalid result.
[b] Specimen was not retested due to insufficient specimen volume.

**TABLE II**

**Reactivity with ABBOTT PRISM HBsAg**
**Cadaveric Serum Testing**

| Population | Number of Specimens | Number of Replicates | Mean S/CO | Nonreactive | Initial Reactive |
|---|---|---|---|---|---|
| Postmortem | 51 | 142[a] | 2.05 | 0 (0.0%) | 142 (100.0%) |
| Normal Donor | 54 | 162 | 2.07 | 0 (0.0%) | 162 (100.0%) |

[a] No results were obtained for 7 unique specimens, and 2 specimens using 2 reagent lots due to drain time errors.

## TABLE III

## ABBOTT PRISM HBsAg Assay Reproducibility

| Panel Member or Control | Number of Replicates | Mean S/CO* | Intra-assay SD | Intra-assay %CV | Inter-assay[a] SD | Inter-assay[a] %CV |
|---|---|---|---|---|---|---|
| 1 | 440 | 6.98 | 0.283 | 4.1 | 0.390 | 5.6 |
| 2 | 440 | 4.06 | 0.160 | 3.9 | 0.222 | 5.5 |
| 3 | 440 | 1.39 | 0.068 | 4.9 | 0.077 | 5.6 |
| 4 | 439[b] | 8.86 | 0.513 | 5.8 | 0.596 | 6.7 |
| 5 | 438[c] | 4.62 | 0.162 | 3.5 | 0.244 | 5.3 |
| 6 | 439[b] | 1.37 | 0.078 | 5.7 | 0.083 | 6.1 |
| 7 | 440 | 0.34 | 0.036 | 10.6 | 0.039 | 11.6 |
| Negative Control | 439[b] | 0.26 | 0.038 | 14.6 | 0.041 | 15.6 |
| Positive Control | 440 | 2.63 | 0.138 | 5.2 | 0.204 | 7.8 |

* Cutoff Value = Mean Negative Calibrator Net Counts + (0.19 x Mean Positive Calibrator Net Counts)

| Calibrator | Number of Replicates | Mean Net Counts | Intra-assay SD | Intra-assay %CV | Inter-assay[a] SD | Inter-assay[a] %CV |
|---|---|---|---|---|---|---|
| Negative | 660 | 89 | 9.6 | 10.8 | 13.1 | 14.7 |
| Positive | 660 | 1299 | 73.3 | 5.6 | 73.3 | 5.6 |

[a] Inter-assay variability contains intra-assay variability.

[b] One replicate was invalid due to instrument detection of sample drain time error.

[c] Two replicates were invalid due to instrument detection of sample dispense errors.

## TABLE IV

**Reactivity of the ABBOTT PRISM HBsAg Assay in Whole Blood and Plasmapheresis Donors, in Specimens from Individuals with Medical Conditions Unrelated to HBV Infection and in Specimens Containing Potentially Interfering Substances**

| Category | Number Tested | IR (% of Total) (95% CI) | RR (% of Total) (95% CI) | Number Confirmed Positive[a] (% of RR) |
|---|---|---|---|---|
| Volunteer Blood Donors | | | | |
| Serum | 8,246 | 5 (0.06) (0.02 – 0.14) | 3 (0.04) (0.01 – 0.11) | 2 (66.67) |
| Plasma | 13,911 | 8 (0.06) (0.02 – 0.11) | 5 (0.04) (0.01 – 0.08) | 4 (80.00) |
| Plasmapheresis Donors | 3,081 | 1 (0.03) (0.00 – 0.18) | 0 (0.00) (0.00 – 0.12) | |
| Total Donors | 25,238 | 14 (0.06) (0.03 – 0.09) | 8 (0.03) (0.01 – 0.06) | 6 (75.00) |
| Medical Conditions Unrelated to HBV Infection and Potentially Interfering Substances[b] | 870 | 59 (6.78) | 50[c] (5.75) | 40[d] (80.00) |

IR = Initial Reactive; RR = Repeat Reactive; CI = Confidence Interval

[a] A specimen was confirmed positive for HBsAg if the non-neutralized specimen (with ABBOTT PRISM HBsAg Confirmatory assay Reagent B added) exhibited a net count greater than or equal to the ABBOTT PRISM HBsAg Confirmatory assay cutoff value and if the neutralization with anti-HBs (Reagent A) was 50% or greater.

[b] Specimens from individuals with medical conditions unrelated to HBV infection and specimens containing potentially interfering substances included the following categories: anti-CMV positive (11), anti-EBV positive (12), anti-HSV positive (12), anti-HAV positive (12), anti-HCV positive (12), anti-HIV-1 positive (12), anti-HIV-2 positive (5), anti-HTLV-I positive (12), anti-HTLV-II positive (12), non-viral liver diseases (42), rubella antibody positive (12), toxoplasma antibody positive (11), *E. coli* infections (5), syphilis serology positive (12), anti-nuclear antibody positive (12), rheumatoid factor positive (12), influenza vaccine recipients (52), elevated IgG (12), elevated IgM (12), elevated triglycerides (10), elevated bilirubin (12), elevated hemoglobin (11) and pregnant females (555).

[c] The 50 repeatedly reactive specimens included the following: anti-EBV positive (1), anti-HSV positive (1), anti-HCV positive (1), anti-HIV-1 positive (5), anti-HIV-2 positive (1), non-viral liver diseases (5), rubella antibody positive (1), anti-nuclear antibody positive (1), influenza vaccine recipients (1), elevated triglycerides (1) and pregnant females (32).

[d] The following 40 specimens confirmed positive for HBsAg: anti-HCV positive (1), anti-HIV-1 positive (5), anti-HIV-2 positive (1), non-viral liver diseases (5), influenza vaccine recipients (1) and pregnant females (27).

## TABLE V

**Reactivity of the ABBOTT PRISM HBsAg Assay in Selected Populations with HBV Infection and at Increased Risk for HBV Infection**

| Category | Number Tested | Number Repeatedly Reactive (% of Total) | Number Confirmed Positive (% of Repeatedly Reactive) |
|---|---|---|---|
| Preselected HBsAg Positive | 514 | 514[a] (100.00) | 514[b] (100.00) |
| Acute HBV Infection | 98 | 98 (100.00) | 98 (100.00) |
| Chronic HBV Infection | 101 | 101 (100.00) | 101 (100.00) |
| Recovered HBV Infection | 47 | 0 (0.00) | |
| Increased Risk for HBV Infection[c] | 452 | 54[d] (11.95) | 41[e] (75.93) |
| Total | 1,212 | 767 (63.28) | 754 (98.31) |

[a] Specimens from the preselected HBsAg positive category were tested only once.

[b] Preselected HBsAg positive specimens were previously confirmed positive by specific antibody neutralization.

[c] Individuals at increased risk for HBV infection included the following categories: intravenous drug users (204), hemodialysis patients (50), hemophilia patients (50) and STD clinic patients (148).

[d] The 54 repeatedly reactive specimens included the following: intravenous drug users (25), hemodialysis patients (6), hemophilia patients (4) and STD clinic patients (19).

[e] The 41 specimens that confirmed positive for HBsAg included the following: intravenous drug users (15), hemodialysis patients (5), hemophilia patients (3) and STD clinic patients (18). Of these 41 specimens, 32 were confirmed positive by a licensed reference HBsAg test. The PRISM assay confirmed an additional 9 specimens. In addition, there were no specimens in this category (452 specimens) that were confirmed positive by the licensed reference HBsAg test that were not confirmed positive by the PRISM assay.

**TABLE VI**

**Detection of Purified HBsAg *ad*
by the ABBOTT PRISM HBsAg Assay**

| HBsAg Concentration (ng/mL) | Mean S/CO Value | Result |
|---|---|---|
| 0.917 | 6.98 | + |
| 0.525 | 4.06 | + |
| 0.124 | 1.39 | + |
| 0.000 | 0.34 | - |

**TABLE VII**

**Detection of Purified HBsAg *ay*
by the ABBOTT PRISM HBsAg Assay**

| HBsAg Concentration (ng/mL) | Mean S/CO Value | Result |
|---|---|---|
| 1.002 | 8.86 | + |
| 0.485 | 4.62 | + |
| 0.131 | 1.37 | + |
| 0.000 | 0.34 | - |

## TABLE VIII

## ABBOTT PRISM HBsAg Confirmatory Assay S/CO and %Neut Reproducibility

| Panel Member or Control | Number of Replicates[a] | Mean S/CO[b] | Intra-assay SD | Intra-assay %CV | Inter-assay[c] SD | Inter-assay[c] %CV |
|---|---|---|---|---|---|---|
| 1 | 118 | 8.80 | 0.526 | 6.0 | 0.772 | 8.8 |
| 2 | 116 | 5.14 | 0.246 | 4.8 | 0.314 | 6.1 |
| 3 | 118 | 1.81 | 0.109 | 6.0 | 0.148 | 8.1 |
| 4 | 118 | 10.94 | 0.275 | 2.5 | 0.514 | 4.7 |
| 5 | 120 | 5.75 | 0.165 | 2.9 | 0.288 | 5.0 |
| 6 | 120 | 1.75 | 0.071 | 4.1 | 0.089 | 5.1 |
| 7 | 120 | 0.46 | 0.046 | 10.1 | 0.054 | 11.8 |
| Negative Control | 415 | 0.38 | 0.053 | 13.9 | 0.060 | 15.9 |
| Positive Control | 415 | 3.74 | 0.162 | 4.3 | 0.228 | 6.1 |

| Panel Member or Control | Number of Replicates[a] | Mean %Neut[d] | Intra-assay SD | Intra-assay %CV | Inter-assay[c] SD | Inter-assay[c] %CV |
|---|---|---|---|---|---|---|
| 1 | 118 | 98.00 | 0.699 | 0.7 | 1.015 | 1.0 |
| 2 | 116 | 97.36 | 2.447 | 2.5 | 3.015 | 3.1 |
| 3 | 118 | 94.92 | 3.776 | 4.0 | 5.327 | 5.6 |
| 4 | 118 | 98.27 | 0.372 | 0.4 | 0.809 | 0.8 |
| 5 | 120 | 97.57 | 0.702 | 0.7 | 1.194 | 1.2 |
| 6 | 120 | 93.14 | 3.902 | 4.2 | 4.824 | 5.2 |
| Positive Control | 415 | 98.41 | 2.194 | 2.2 | 2.334 | 2.4 |

[a] Several panel member replicates were not obtained due to instrument detection of three control aspiration or dispense errors and two invalid control results.

[b] Mean S/CO = mean of all replicate S/CO values for each panel member or control tested.

[c] Inter-assay variability contains intra-assay variability.

[d] %Neut = [Sample with Reagent B Net Counts – Sample with Reagent A Net Counts] ÷ [Sample with Reagent B Net Counts – Negative Control with Reagent B Net Counts] × 100. This value is not reported for specimens that are nonreactive in the confirmatory assay.

## TABLE IX

## Confirmation of ABBOTT PRISM HBsAg Reactive Specimens

| Category | Number of Specimens Tested | HBsAg Assay Repeatedly Reactive (% of Total) | HBsAg Confirmatory Positive[a] (% of Repeatedly Reactive) |
|---|---|---|---|
| Volunteer Blood Donors | | | |
| Serum | 8,246 | 3 (0.04) | 2 (66.67) |
| Plasma | 13,911 | 5 (0.04) | 4 (80.00) |
| Medical Conditions Unrelated to HBV Infection and Potentially Interfering Substances[b] | 870 | 50 (5.75) | 40[c] (80.00) |
| Preselected HBsAg Positive | 199 | 199[d] (100.00) | 199 (100.00) |
| Acute HBV Infection | 98 | 98 (100.00) | 98 (100.00) |
| Chronic HBV Infection | 101 | 101 (100.00) | 101 (100.00) |
| Increased Risk for HBV Infection[e] | 452 | 54 (11.95) | 41[f] (75.93) |

[a] A specimen was confirmed positive for HBsAg if the non-neutralized specimen (with ABBOTT PRISM HBsAg Confirmatory assay Reagent B added) exhibited a net count greater than or equal to the ABBOTT PRISM HBsAg Confirmatory assay cutoff value and if the neutralization with anti-HBs (Reagent A) was 50% or greater.

[b] Specimens from individuals with medical conditions unrelated to HBV infection and specimens containing potentially interfering substances included the following categories: anti-CMV positive (11), anti-EBV positive (12), anti-HSV positive (12), anti-HAV positive (12), anti-HCV positive (12), anti-HIV-1 positive (12), anti-HIV-2 positive (5), anti-HTLV-I positive (12), anti-HTLV-II positive (12), non-viral liver diseases (42), rubella antibody positive (12), toxoplasma antibody positive (11), *E. coli* infections (5), syphilis serology positive (12), anti-nuclear antibody positive (12), rheumatoid factor positive (12), influenza vaccine recipients (52), elevated IgG (12), elevated IgM (12), elevated triglycerides (10), elevated bilirubin (12), elevated hemoglobin (11) and pregnant females (555).

[c] The 40 specimens that confirmed positive for HBsAg included the following: anti-HCV positive (1), anti-HIV-1 positive (5), anti-HIV-2 positive (1), non-viral liver diseases (5), influenza vaccine recipients (1) and pregnant females (27).

[d] Specimens from the preselected HBsAg positive category were tested only once.

[e] Individuals at increased risk for HBV infection included the following categories: intravenous drug users (204), hemodialysis patients (50), hemophilia patients (50) and STD clinic patients (148).

[f] The 41 specimens that confirmed positive for HBsAg included the following: intravenous drug users (15), hemodialysis patients (5), hemophilia patients (3) and STD clinic patients (18). Of these 41 specimens, 32 were confirmed positive by a licensed reference HBsAg test. The PRISM assay confirmed an additional 9 specimens. In addition, there were no specimens in this category (452 specimens) that were confirmed positive by the licensed reference HBsAg test that were not confirmed positive by the PRISM assay.