ABBOTT PRISM® HBsAg
Antibody to Hepatitis B Surface Antigen
(Mouse Monoclonal IgM)

NOTE: This package insert must be read carefully prior to product use. Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NOTE: If you receive reagents, calibrators, controls or bulk solutions that are in a condition contrary to the package insert or label recommendation, or that are damaged, contact your local customer service organization.

For use with software version 2.1 or higher

Note Changes Highlighted

© 1995, 2005 Abbott / Printed in Germany / ABBOTT PRISM HBsAg
August 2005

Key to symbols used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>List Number</td>
</tr>
<tr>
<td>IVD</td>
<td>For In Vitro Diagnostic Use</td>
</tr>
<tr>
<td>8°C</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>2°C</td>
<td>Store at 15-30°C</td>
</tr>
<tr>
<td>!</td>
<td>CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use.</td>
</tr>
<tr>
<td>i</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot Number</td>
</tr>
<tr>
<td></td>
<td>Expiration Date</td>
</tr>
<tr>
<td>MASTER LOT</td>
<td>Master Lot</td>
</tr>
<tr>
<td>CALIBRATORS</td>
<td>Calibrators</td>
</tr>
<tr>
<td>PIPETTE TIPS</td>
<td>Pipette Tips</td>
</tr>
<tr>
<td>LINE CLEANER</td>
<td>Line Cleaner</td>
</tr>
<tr>
<td>ASSAY KIT CARD</td>
<td>Assay Kit Card</td>
</tr>
<tr>
<td>REACTION TRAYS</td>
<td>Reaction Trays</td>
</tr>
<tr>
<td>REAGENT COMPONENTS</td>
<td>Reagent Components</td>
</tr>
<tr>
<td>RUN CONTROL ADAPTERS</td>
<td>Run Control Adapters</td>
</tr>
</tbody>
</table>

See REAGENTS section for a full explanation of symbols used in reagent component naming.
NAME AND 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 or plasma. The ABBOTT PRISM HBsAg ChLIA is intended as a screen for donated blood to prevent transmission of hepatitis B virus (HBV) to recipients of blood and blood components and as an aid in the diagnosis of ongoing or previous hepatitis B viral infection.

SUMMARY AND EXPLANATION OF THE TEST
Sensitive immunoassays for the detection of HBsAg were first described in 1971. In 1976 and 1977, solid phase “sandwich” enzyme immunoassays were developed in which HBsAg was captured on a solid phase coated with polyclonal antibodies against HBsAg (anti-HBs) and then detected with anti-HBs conjugated to an enzyme. In recent years, monoclonal anti-HBs assays have been developed for the detection of HBsAg. Enzyme immunoassays have been used to screen blood and blood products for the presence of HBsAg to prevent transmission of HBV infection to recipients of blood or blood products. The assays for HBsAg are routinely used to diagnose suspected HBV infection and to monitor the status of infected individuals, i.e., whether the patient has resolved infection or has become a chronic carrier of the virus. The Centers for Disease Control and Prevention have recommended the prenatal screening of all pregnant women so that newborns from HBV carrier mothers may obtain prophylactic treatment. Prenatal transmission of HBV infection from mother to neonate is a major mode of transmission in an HBV endemic population. As with all immunoassays, the ABBOTT PRISM HBsAg assay may yield non-specific reactivity due to other causes, particularly when testing low prevalence populations.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE
The ABBOTT PRISM HBsAg assay is a two-step sandwich ChLIA that detects HBsAg in human serum or plasma. The reactions occur in the following sequence:

- The first reaction is the Tracer Wash, the Microparticles are captured by the matrix, while the remaining mixture flows through to the absorbent blotter.
- The Acidinium 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 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. For further information regarding ChLIA technology, refer to the ABBOTT PRISM Operations Manual. Section 3. 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 an ABBOTT PRISM calibration performed in the same batch. 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. Specimens which are not reactive by the ABBOTT PRISM HBsAg assay are considered nonreactive for HBsAg. These specimens need not be tested further. Specimens which are initially reactive should be centrifuged according to the table in the Specimen Collection and Preparation for Analysis section and tested in duplicate.

REAGENTS
NOTE: Each specific component description noted below is accompanied by a unique symbol. These symbols appear on both the component labels and on corresponding instrument tubing identifier labels. They are meant to facilitate identification and installation of reagent bottles within the ambient reagent bay and refrigerator.

ABBOTT PRISM HBsAg Assay Kit (3A47-48)

- **MICROPARTICLES** 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. Preservative: Sodium Azide. (Symbol: )
- **CONJUGATE** 1 bottle (328 mL) Antibody to Hepatitis B Surface Antigen (Goat Polyclonal):Acridinium Conjugate in Phosphate Buffered Saline with Calf Serum and Recalified, Heat-Inactivated Human Plasma. Minimum Concentration: 0.025 µg/mL. Preservative: Sodium Azide. (Symbol: )
- **CAL** 3 bottles (10.4 mL each) Negative Calibrator (Human). Recalified Plasma Nonreactive for HBsAg, HIV RNA or HIV-1 Ag, Anti-HBs, Anti-HCV, and Anti-HIV-1/HIV-2. Preservative: Sodium Azide. (Symbol: NC)
- **POS** 3 bottles (10.4 mL each) Positive Calibrator (Human). Recalified, Heat-Inactivated Plasma Reactive for HBsAg, Nonreactive for Anti-HBs, Anti-HCV, and Anti-HIV-1/HIV-2. HBsAg Concentration: 0.25 - 0.65 ng/mL (0.03 - 0.07 PEI Units/mL). Preservative: Sodium Azide. (Symbol: PC)

ABBOTT PRISM HBsAg Wash Kit (3A47-38)

- **TRANSFER WASH** 1 bottle (3393 mL) Transfer Wash. Phosphate Buffered Saline. Preservative: Sodium Azide. (Symbol: )
- **CONJUGATE WASH** 1 bottle (2811 mL) Conjugate Wash. Borate Buffered Saline. Preservative: Sodium Azide. (Symbol: )

Other Reagents Required
ABBOTT PRISM Activator Concentrate (1A75-02 or 3L27-02)
- **ACTIVATOR CONCENTRATE** 4 bottles (900 mL each) Activator Concentrate. 0.4% Hydrogen Peroxide/0.06% Diethylenetriamine-pentaacetic Acid.

ABBOTT PRISM Activator Diluent (1A75-01 or 3L27-01)
- **ACTIVATOR DILUENT** 4 bottles (900 mL each) Activator Diluent. 0.3 N Sodium Hydroxide.

Other Reagents Available
ABBOTT PRISM Run Control Kit (5E22-10)
- **CONTROL** 2 Bottles (10 mL each) Positive Control (Human). Purified anti-HBc IgG (Concentration: 0.9 - 2.6 PEI Units/mL) and recalified, heat-inactivated plasma reactive for HBsAg (HBsAg Concentration: 0.10 - 0.40 ng/mL [0.01 - 0.05 PEI Units/mL]), anti-HCV, anti-HIV-1, and anti-HTLV-I. Preservative: Sodium Azide. (Symbol: POS)

Refer to NOTE listed at the end of this section.

- **SUP CONTROL** 1 Bottle (10 mL) Supplemental Positive Control (Human). Recalified, heat-inactivated plasma reactive for anti-HIV-2 and anti-HTLV-II, nonreactive for HBsAg and anti-HCV. Preservative: Sodium Azide. (Symbol: SUP)

ABBOTT PRISM Run Control Kit (SE22-11)
- **CONTROL** 2 Bottles (10 mL each) Negative Control (Human). Recalified plasma nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HBc, anti-HBs, anti-HCV, anti-HIV-1/HIV-2, and anti-HTLV-I/II. Preservative: Sodium Azide. (Symbol: NEG)

ABBOTT PRISM Positive Run Control Kit (2K24-10)
- **CONTROL** 12 Bottles (10 mL each) Positive Control (Human). Purified anti-HBc IgG (Concentration: 0.9 - 2.6 PEI Units/mL) and recalified, heat-inactivated plasma reactive for HBsAg (HBsAg Concentration: 0.10 - 0.40 ng/mL [0.01 - 0.05 PEI Units/mL]), anti-HCV, anti-HIV-1, and anti-HTLV-I. Preservative: Sodium Azide. (Symbol: POS)

Refer to NOTE listed at the end of this section.

- **SUP CONTROL** 1 Bottle (10 mL) Supplemental Positive Control (Human). Recalified, heat-inactivated plasma reactive for anti-HIV-2 and anti-HTLV-II, nonreactive for HBsAg, HIV RNA or HIV-1 Ag, and anti-HCV. Preservative: Sodium Azide. (Symbol: SUP)

ABBOTT PRISM Negative Run Control Kit (2K24-10)
- **CONTROL** 2 Bottles (10 mL each) Negative Control (Human). Recalified plasma nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HBc, anti-HBs, anti-HCV, anti-HIV-1/HIV-2, and anti-HTLV-I/II. Preservative: Sodium Azide. (Symbol: NEG)

* Tween is a registered trademark of ICI Americas.

* Concentration standardized against the reference standard of the Paul Ehrlich Institute (PEI), Langen, Germany.
ABBOTT PRISM Positive Run Control Kit (2K24-11)
• [CONTROL A] 6 Bottles (10 mL each) Positive Control (Human). Purified anti-HBc IgG Concentration: 0.9 - 2.6 PEI Units/mL) and recalcified, heat-inactivated plasma reactive for HBsAg (HBsAg Concentration: 0.10 - 0.40 ng/mL [0.01 - 0.05 PEI Units/mL]), anti-HCV, anti-HIV-1, and anti-HTLV-I. Preservative: Sodium Azide. (Symbol: POS)
† Refer to NOTE listed at the end of this section.

ABBOTT PRISM Run Control Kit (4B48-10)
• [CONTROL A] 1 Bottle (20 mL) Positive Control (Human). Purified anti-HBc IgG Concentration: 1.5 - 3.5 PEI Units/mL) and recalcified, heat-inactivated plasma reactive for HBsAg (HBsAg Concentration: 0.10 - 0.40 ng/mL [0.01 - 0.05 PEI Units/mL]) and reactive for anti-HCV, anti-HIV-1, and anti-HTLV-I. Preservative: Sodium Azide. (Symbol: POS)
† Refer to NOTE listed at the end of this section.
• [SUP CONTROL] 1 Bottle (12 mL) Supplemental Positive Control (Human). Recalcified, heat-inactivated plasma reactive for anti-HIV-2 and anti-HTLV-II nonreactive for HBsAg and anti-HCV. Preservative: Sodium Azide. (Symbol: SUP)
• [CONTROL] 1 Bottle (20 mL) Negative Control (Human). Recalcified, heat-inactivated plasma nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-Hbc, anti-Hbs, anti-HCV, anti-HIV-1/HIV-2, and anti-HTLV-I/HTLV-II. Preservative: Sodium Azide. (Symbol: NEG)

ABBOTT PRISM Positive Control Kit (4B48-11)
• [CONTROL A] 3 Bottles (20 mL each) Positive Control (Human). Purified anti-HBc IgG Concentration: 1.5 - 3.5 PEI Units/mL) and recalcified, heat-inactivated plasma reactive for HBsAg (HBsAg Concentration: 0.10 - 0.40 ng/mL [0.01 - 0.05 PEI Units/mL]) and reactive for anti-HCV, anti-HIV-1, and anti-HTLV-I. Preservative: Sodium Azide. (Symbol: POS)
† Refer to NOTE listed at the end of this section.
† NOTE: Each batch MUST end in a release control. An HBsAg release control is any control reactive for HBsAg which has been configured to validate system function and release sample results. The configuration criteria are defined in the RUN CONTROLS file of the PRISM Resource Management software. Any customer specified control reactive for HBsAg may be used.

ABBOTT PRISM HBsAg Confirmatory Assay Kit (6D16-11)
• [REAGENT A] 1 Bottle (2 mL) Reagent A. Antibody to Hepatitis B Surface Antigen (Human) and recalcified human plasma nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. Minimum concentration: 0.01 mg/mL. Contains Red Dye D&C. Preservative: Sodium Azide. (Symbol: RGT A)
• [REAGENT B] 1 Bottle (2 mL) Reagent B. Recalified human plasma nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/ HIV-2. Contains bromocresol blue. Preservative: Sodium Azide. (Symbol: RGT B)
• [REAGENT C] 1 Bottle (4 mL) Reagent C. Specimen treatment reagent with 20 mM citrate buffer. (Symbol: RGT C)
• [DILUENT] 1 Bottle (18 mL) Diluent. Recalified human plasma nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. Preservative: Sodium Azide. (Symbol: DIL)
• [SAMPLE CUPS] 1 Package (100 units) ABBOTT PRISM Sample Cups
• [CONFIRMATORY BAR CODE] 1 Package (10 count) ABBOTT PRISM HBsAg Confirmatory Bar Code Labels

WARNINGS AND PRECAUTIONS

I) For In Vitro Diagnostic use.

CAUTION: This product contains human sourced and/or potentially infectious components. Some components sourced from human blood have been tested and found to be reactive for HBsAg, by approved tests. Refer to the Reagents section of this package insert. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced material must be considered potentially infectious. It is recommended that all samples and kit reagents be handled in accordance with Biosafety Level 2 practices as described in the CDC NIH publication, Biosafety in Microbiological and Biomedical Laboratories or other equivalent guidelines.

Safety Precautions
• Do not pipette by mouth.
• Do not smoke, eat, drink, apply cosmetics, or handle contact lenses in areas in which samples or reagents are handled.
• Wear disposable gloves when handling samples and reagents.
• Clean and disinfect all spills of samples and reagents using a tuberculocidal disinfectant, such as 0.5% sodium hypochlorite.
• Decontaminate and dispose of all samples, reagents and other potentially contaminated materials in accordance with applicable regulations. Generally accepted procedures for the treatment of potentially infectious solid waste include incineration or autoclaving. Due to variations among autoclaves and in waste configuration, each user must verify the effectiveness of the decontamination cycle using biological indicators.
• The ABBOTT PRISM Line Cleaner containing 2% Tetraethylammonium Hydroxide (TEAH) may cause mild eye irritation. If this solution comes in contact with eyes, rinse immediately with water (for additional information, refer to the ABBOTT PRISM Operations Manual, Section 8).
• All components of this product contain Sodium Azide. For a specific listing, refer to the Reagents section of this package insert. Sodium azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode upon percussion, such as hammering. To prevent formation of lead or copper azide, flush drains thoroughly with water after disposing of solutions containing sodium azide. To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health recommends the following: (1) siphon liquid from trap using a rubber or plastic hose, (2) fill with 10% sodium hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.
• The components containing Sodium Azide are classified per the applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate risk (R) and safety (S) phrases.

R22 Harmful if swallowed.
R32 Contact with acid liberates very toxic gas.
R35 This material and its container must be disposed of in a safe way.
S36 Wear suitable protective clothing.
S46 If swallowed, seek medical advice immediately and show this container or label.
• The ABBOTT PRISM Activator Diluent contains Sodium Hydroxide and is classified per the applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate risk (R) and safety (S) phrases.

R36 Irritating to eyes.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35 This material and its container must be disposed of in a safe way.
S46 If swallowed, seek medical advice immediately and show this container or label.

For product not classified as dangerous per European Directive 1999/45/EC - Safety data sheet available for professional user on request.

Handling Precautions
• Do not use kits beyond the expiration date.
• Gently invert each component several times prior to loading on the ABBOTT PRISM System to ensure a homogenous solution. Avoid foaming. Each component of the ABBOTT PRISM HBsAg Wash Kit should be at room temperature (15-30°C) before mixing.
• Do not mix reagents from different lots of the same assay kit lots.
• Any lot of ABBOTT PRISM HBsAg Wash Kit can be used with any lot of ABBOTT PRISM HBsAg Assay Kit.
• Avoid microbial and chemical contamination of samples, reagents, and equipment. The use of disposable pipette tips is recommended for any preliminary sample transfer.
• Do not freeze reagents.
• Failure to adhere to instructions in the ABBOTT PRISM Operations Manual or Package Insert may result in erroneous results.
• Use caution when handling samples, reagent bottles, and reagent caps to prevent cross contamination.

Additional safety and handling precautions and limitations for the assay kit, calibrators, specimens, controls, and other reagents are described in the ABBOTT PRISM Operations Manual, Section 7.
PREPARATION OF ACTIVATOR SOLUTION

Activator Solution is prepared daily by mixing equal parts of Activator Concentrate and Activator Diluent. The volume of Activator Solution required for multiple tests is calculated by the ABBOTT PRISM software. Refer to the ABBOTT PRISM Operations Manual, Section 5, for additional information. Use clean pipettes and/or metal-free containers (such as plasticware or acid-washed and distilled or deionized water-rinsed glassware) to measure. Prepare in the bottle provided in the Accessory Kit. Cover the bottle opening securely with the cap provided and invert gently five to ten times to mix. Load the Activator Solution on the PRISM System. Refer to the ABBOTT PRISM Operations Manual, Section 5, under Prepare and Load Activator Solution, for additional information.

NOTE: The Activator Solution must be used within 24 hours of preparation.

STORAGE INSTRUCTIONS

-2°C - 8°C
+2°C - +30°C
+15°C

- Store the ABBOTT PRISM HBsAg Assay Kit, ABBOTT PRISM Run Control Kit, ABBOTT PRISM Positive Run Control Kit, and ABBOTT PRISM Activator Concentrate at 2-8°C.
- Store the ABBOTT PRISM HBsAg Wash Kit and ABBOTT PRISM Activator Diluent at room temperature (15-30°C).
- Store ABBOTT PRISM Pipette Tips and ABBOTT PRISM Reaction Trays in their original package until use.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The ABBOTT PRISM System will not continue to process samples when calibrator values do not meet specifications. This may indicate either deterioration or contamination of reagents, or instrument failure. Refer to the ABBOTT PRISM Operations Manual, Section 10, for additional information.

INSTRUMENT PROCEDURE

- Refer to the ABBOTT PRISM Operations Manual for a detailed description of Instrument Procedures.
- Solutions required for instrument cleaning and maintenance are described in detail in the ABBOTT PRISM Operations Manual, Sections 5 and 9.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ABBOTT PRISM Operations Manual, Section 9.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Either serum (including serum collected in serum separator tubes) or plasma collected in EDTA, Sodium Heparin, Potassium Oxalate, Sodium Citrate, ACD, CP2D, CPD, or CPDA-1 anticoagulants may be used with the ABBOTT PRISM HBsAg assay.
- This assay was designed and validated for use with human serum or plasma from individual donor specimens. Pooled specimens must not be used.
- Heat-inactivated specimens should be avoided.
- Gravity separation is not sufficient for specimen preparation. Specimens collected by plasmapheresis which have not been frozen do not require centrifugation. All other specimens (including previously frozen plasmapheresis specimens) must be centrifuged.

Non-frozen specimens (excluding non-frozen plasmapheresis specimens) must be centrifuged such that g-minutes (the product of relative centrifugal force [RCF] and centrifugation time [minutes]) is between 30,000 and 75,000. The following chart lists acceptable time and force ranges that meet this criteria.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>RCF (x g)</th>
<th>RCF x Time (g-minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3,000</td>
<td>30,000</td>
</tr>
<tr>
<td>15</td>
<td>2,000 - 3,000</td>
<td>30,000 - 45,000</td>
</tr>
<tr>
<td>20</td>
<td>1,500 - 3,000</td>
<td>30,000 - 60,000</td>
</tr>
<tr>
<td>25</td>
<td>1,300 - 3,000</td>
<td>32,500 - 75,000</td>
</tr>
</tbody>
</table>

Conver RCF to rpm as follows: RCF = \(1.12 \times r_{\text{max}}\) (rpm/1000)=

Conver RCF to rpm as follows: \(rpm = \frac{RCF}{1.12 \times r_{\text{max}}}\)

RCF - The relative centrifugal force generated during centrifugation.
rpm - The rotation per minute of the rotor on which the specimen are being spun (usually the digital readout on the centrifuge will indicate rpm).

Time - The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.

\(r_{\text{max}}\) - Radius of the rotor in millimeters. The radius measured is dependant on whether the rotor is a fixed angle rotor or a swinging bucket rotor. This value is typically provided with the rotor, by the manufacturer.

* For fixed angle, \(r_{\text{max}}\) is a measure of the distance from the rotor axis (center) to the bottom of the tube cavity.
* For the swinging bucket, \(r_{\text{max}}\) is a measure of the distance from the rotor axis (center) to the bottom of the tube bucket while it is extended during rotation.

Previous frozen specimens must be centrifuged such that g-minutes (the product of relative centrifugal force [RCF] and centrifugation time [minutes]) is between 180,000 and 300,000. The following chart lists acceptable time and force ranges that meet this criteria.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>RCF (x g)</th>
<th>RCF x Time (g-minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>2,000</td>
<td>160,000</td>
</tr>
<tr>
<td>20</td>
<td>9,000 - 12,000</td>
<td>180,000 - 240,000</td>
</tr>
<tr>
<td>25</td>
<td>7,200 - 12,000</td>
<td>180,000 - 300,000</td>
</tr>
</tbody>
</table>

ANY specimen (excluding non-frozen plasmapheresis) not tested within 24 hours of initial centrifugation, must be recentrifuged from 30,000 to 75,000 (g-minutes) as defined for non-frozen specimens.

NOTE: If re-testing a specimen within 24 hours of initial centrifugation, the specimen is not required to be re-centrifuged.

FAILURE TO FOLLOW THE SPECIFIED CENTRIFUGATION PROCEDURE MAY GIVE ERRONEOUS OR INCONSISTENT RESULTS.

- Failure to follow the specified centrifugation procedure on specimens tested with the ABBOTT PRISM HBsAg assay may cause a reduction in Sample Net Counts and in S/CO (Sample Net Counts/Cutoff).
- Specimens may be stored at 2-8°C for up to fourteen days. If storage periods greater than fourteen days are anticipated, the serum or plasma should be removed from the clot or red blood cells to avoid hemolysis. Store the serum or plasma frozen (-10°C or colder).
- Previously frozen specimens must be mixed thoroughly after thawing and centrifuged according to the table in this section.
- Although 10 nonreactive and 10 low-level reactive specimens showed no qualitative performance differences when subjected to 6 freeze-thaw cycles, multiple freeze-thaw cycles should be avoided.

NOTE: Some specimens, nonreactive for HBsAg, that have been subjected to frozen storage have exhibited non-specific reactivity in the ABBOTT PRISM HBsAg assay.

- Clear, non-hemolized specimens should be used when possible. Specimens containing particulate matter may give erroneous or inconsistent test results.
- No qualitative performance differences were observed when nonreactive and low-level reactive specimens were spiked with elevated levels of bilirubin (< 20 mg/dL), hemoglobin (< 500 mg/dL), or lipids (< 3,000 mg/dL).
- When shipped, specimens must be packaged and labeled in compliance with applicable regulations covering the transport of clinical specimens and etiologic agents. Specimens may be shipped under ambient conditions, refrigerated on ice (2-8°C), or frozen on dry ice (-10°C or colder). Prior to freezing, the specimen should be removed from the clot or red cells.
- Performance has not been established for cadaver specimens, umbilical cord blood, or body fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid.

Specimen Volume

The specimen volume required to perform a single assay on the ABBOTT PRISM System varies according to the different specimen containers. For ABBOTT PRISM Sample Cups, the minimum specimen volume required for one assay is 400 µL. The ABBOTT PRISM HBsAg Assay requires 100 µL sample dispense. For volume requirements for each additional assay performed from the same specimen container and for volume requirements in primary or aliquot tubes, refer to the ABBOTT PRISM Operations Manual, Section 5.
ABBOTT PRISM HBsAg PROCEDURE

Materials Provided
- 3A47-48 ABBOTT PRISM HBsAg Assay Kit
- 3A47-39 ABBOTT PRISM HBsAg Wash Kit

Materials Required but not Provided
- 1A75-02 or 3L27-02 ABBOTT PRISM ACTIVATOR CONCENTRATE
- 1A75-01 or 3L27-01 ABBOTT PRISM ACTIVATOR DILUENT
- 5A07-01 ABBOTT PRISM REACTION TRAYS
- 5A07-10 ABBOTT PRISM PIPETTE TIPS
- 6A36-60 ABBOTT PRISM Accessory Kit

Additional Materials Available
- 1A75-10 or 3L27-10 ABBOTT PRISM ACTIVATOR LINE TREATMENT
- 2K24-10 ABBOTT PRISM Run Control Kit
- 2K24-11 ABBOTT PRISM Positive Run Control Kit
- 4B48-10 ABBOTT PRISM Run Control Kit
- 4B48-11 ABBOTT PRISM Positive Control Kit
- 5E22-10 ABBOTT PRISM Run Control Kit
- 5E22-11 ABBOTT PRISM Positive Run Control Kit
- 6A36-31 ABBOTT PRISM RUN CONTROL ADAPTERS
- 6D16-48 ABBOTT PRISM HBsAg Confirmatory Kit
- 7A03-01 or 3L00-01 ABBOTT PRISM PRIME/PURGE ACCESSORIES
- 7A03-30 or 3L00-30 ABBOTT PRISM PURGE CONCENTRATE
- 7B36-11 ABBOTT PRISM LINE CLEANER
- 7B36-01 ABBOTT PRISM SAMPLE CUPS

ABBOTT PRISM ASSAY PROCEDURE
For detailed information concerning batch time and maximum batch size, refer to the ABBOTT PRISM Operations Manual, Section 2.

STEP 1:
- Enter a Plan Workload (refer to ABBOTT PRISM Operations Manual, Section 5).
- Place reagents as needed.

NOTE: Gently invert each component several times prior to loading on the ABBOTT PRISM System to ensure a homogenous solution. Additional gentle inversion may be required to thoroughly resuspend microparticles. Avoid foaming. Each component of the ABBOTT PRISM HBsAg Wash Kit should be at room temperature (15-30°C) before mixing.

Verify that all tubing label symbols match the symbols on each reagent kit. (Refer to the symbol key in the section of this package insert and load into the ABBOTT PRISM System.)

Verify that all tubing is securely fastened to the corresponding wash and reagent bottles.

STEP 2:
- Inspect the waste containers. Empty and clean as defined in the ABBOTT PRISM Operations Manual, Section 9, if necessary.

STEP 3:
- Prepare Activator Solution (refer to the Preparation of Activator Solution section of this package insert) and load into the ABBOTT PRISM System.

STEP 4:
- Verify adequate number of Reaction Trays are in the Tray Loader.

STEP 5:
- Verify adequate number of Pipette Tips are in the Pipette Tip Racks.

STEP 6:
- Perform the prime procedure (refer to the ABBOTT PRISM Operations Manual, Section 5).

STEP 7:
- Initiate sample processing. Open the bottles in the Calibrator Pack and place in the Calibrator Rack. Load the Calibrator Rack and Sample Racks, including the Controls (refer to the Control Handling Procedure under Controls).

STEP 8:
- After the calibrators have been pipetted, remove Calibrator Rack. Close the Calibrator bottles and return to 2-8°C storage.

STEP 9:
- Each sample is initially tested once. Sample Racks may be removed after the samples have been pipetted.

STEP 10:
- Any specimen (excluding non-frozen plasmapheresis) that is initially reactive must be centrifuged according to the table in the Specimen Collection and Preparation for Analysis section and retested in duplicate (refer to the ABBOTT PRISM Operations Manual, Section 5).

NOTE: Specimens retested within 24 hours of initial centrifugation do not require refugation.

STEP 11:
- After the run is complete, perform the purge procedure (refer to the ABBOTT PRISM Operations Manual, Section 5).

Refer to the ABBOTT PRISM Operations Manual, Section 3, for a detailed description of ChLIA procedures.

QUALITY CONTROL PROCEDURES

Calibration
The ABBOTT PRISM HBsAg Negative and Positive Calibrators are tested in triplicate automatically at the beginning of each batch. The ABBOTT PRISM System will not release results when Calibrator values do not meet specifications. This may indicate either deterioration or contamination of reagents, or instrument failure.

Controls
1. A release control MUST be included as the last sample in each batch. A control such as the ABBOTT PRISM Positive Control or any customer-specified control reactive for HBsAg may be used. This control must test reactive in order to validate system function and to release results. If this control does not test reactive, refer to the ABBOTT PRISM Operations Manual, Section 10.

Sites using a release control other than the ABBOTT PRISM Positive Control must validate its performance on the ABBOTT PRISM System.

2. ABBOTT PRISM Run Control Handling Procedure
   a. Place each Run Control bottle into an adapter such that when the flip-top cap is opened, it can be snapped into an open position within the adapter.
   b. Place each Run Control within an adapter onto the Sample Rack. The controls can be placed in any rack position except 1, 2, 27, or 28.
   c. As mentioned above, place an ABBOTT PRISM Positive Control after the last sample tested in the batch. The controls can be placed in any rack position except 1, 2, 27, or 28.

3. Customer-Specified Control Handling Procedure
   a. Determine the volume of controls required. The control volume required to perform a single assay on the ABBOTT PRISM System varies according to the different specimen containers. For ABBOTT PRISM Sample Cups, the minimum control volume required for one assay is 600 µL (400 µL + 200 µL Sample Cup dead volume). For every additional assay performed from the same control container, an additional 200 µL is required. For volume requirements in primary or aliquot tubes, refer to the ABBOTT PRISM Operations Manual, Section 5.
   b. Refer to the ABBOTT PRISM Operations Manual, Section 5: Operating Instructions, Subsection: Sample Processing.
   c. Additional controls may be run at the operator’s discretion. Validity specifications may be assigned such that if these controls fail, no results are reported for that assay batch.

ASSAY PARAMETER SPECIFICATIONS
The PRISM assay parameter specifications have been factory set. These parameters cannot be printed, displayed, or edited.

RESULTS
Calculation of Cutoff and S/CO Values
The ABBOTT PRISM System calculates the ABBOTT PRISM HBsAg assay Cutoff Value using the following formula:

\[
\text{Cutoff} = \text{Mean Negative Calibrator Net Counts} + (0.2 \times \text{Mean Positive Calibrator Net Counts})
\]

Example: If the Mean NCC = 100, and the Mean PCC = 1,000
100 + (0.2 x 1,000) = 300
Cutoff = 300

* The Positive Calibrator and Negative Calibrator Net Counts are calculated using the two lowest replicates. An Instrument Code (22-211) will be displayed in place of the count value for the third replicate. Each of the two remaining replicates of the Positive Calibrator and the Negative Calibrator used to calculate the cutoff must meet all specifications.
The ABBOTT PRISM System calculates the ABBOTT PRISM HBsAg assay S/CO using the following formula:

\[ \text{S/CO} = \frac{\text{Sample Net Counts}}{\text{Cutoff}} \]

Example: If the Sample Net Counts = 600, and the Cutoff = 300

\[ \text{S/CO} = \frac{600}{300} = 2.00 \]

**INTERPRETATION OF RESULTS**

In the ABBOTT PRISM HBsAg assay, specimens with Net Counts less than the cutoff are considered nonreactive and need not be further tested. Specimens with Net Counts greater than or equal to the cutoff are considered initially reactive. All specimens that are reactive on initial testing must be centrifuged according to the table in the Specimen Collection and Preparation for Analysis section of this package insert and retested in duplicate.

**NOTE:** If re-testing a specimen within 24 hours of the initial centrifugation, the specimen is not required to be re-centrifuged. If repeat testing shows the Net Counts for both retests to be less than the cutoff, the sample is considered nonreactive. If either duplicate retest Net Count is greater than or equal to the cutoff, the specimen is repeatedly reactive. Repeatedly reactive specimens should be tested by a neutralizing confirmatory test. Only the specimens which are confirmed by neutralization with anti-HBs are considered positive for HBsAg.

ABBOTT PRISM reports display sample results in Net Counts and/or S/CO. Net Counts are used by ABBOTT PRISM to interpret results. The S/CO value is provided in reports to show relative reactivity to the cutoff. In the ABBOTT PRISM HBsAg assay, specimens with S/CO values of less than or equal to 1.00 are considered nonreactive. Specimens with an S/CO value of greater than or equal to 1.00 are considered reactive. Repeatedly reactive specimens should be tested by a neutralizing confirmatory test. Only the specimens which are confirmed by neutralization with anti-HBs are considered positive for HBsAg.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**Precision**

Assay reproducibility was determined by assaying a 28-member panel consisting of four replicates of the following: 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). The panel was tested in five runs over five days with each of three master lots at a total of three sites. The intra- and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) were analyzed with a variance components analysis, using a nested analysis of variance model.22 (Table I).

Mean S/CO is defined as the mean Sample Net Counts (NET) divided by the calculated cutoff.

**LIMITATIONS OF THE PROCEDURE**

- Testing of previously frozen samples with the ABBOTT PRISM HBsAg assay may cause an increase in non-specific reactivity. The assay was designed and validated for use with human serum or plasma. A total of 6,894 serum and plasma specimens from volunteer blood donors and plasmapheresis donors was collected from four blood centers (Table II). A total of 6,894 serum and plasma specimens from volunteer blood donors and plasmapheresis donors was collected from four blood centers (Table II).

**SPECIFIC PERFORMANCE CHARACTERISTICS**

**Precision**

Assay reproducibility was determined by assaying a 28-member panel consisting of four replicates of the following: 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). The panel was tested in five runs over five days with each of three master lots at a total of three sites. The intra- and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) were analyzed with a variance components analysis, using a nested analysis of variance model.22 (Table I).

Mean S/CO is defined as the mean Sample Net Counts (NET) divided by the calculated cutoff.

<table>
<thead>
<tr>
<th>Panel Member (ng/mL)</th>
<th>Number of Replicates</th>
<th>Mean S/CO</th>
<th>Intra-assay SD</th>
<th>Inter-assay SD</th>
<th>Inter-assay %CV</th>
<th>Inter-assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (ad 1.27)</td>
<td>120</td>
<td>9.06</td>
<td>0.45</td>
<td>4.9</td>
<td>0.46</td>
<td>5.1</td>
</tr>
<tr>
<td>2 (ad 0.71)</td>
<td>120</td>
<td>5.45</td>
<td>0.29</td>
<td>5.2</td>
<td>0.30</td>
<td>5.5</td>
</tr>
<tr>
<td>3 (ad 0.36)</td>
<td>118</td>
<td>2.84</td>
<td>0.15</td>
<td>5.3</td>
<td>0.15</td>
<td>5.3</td>
</tr>
<tr>
<td>4 (ay 0.98)</td>
<td>120</td>
<td>9.86</td>
<td>0.42</td>
<td>4.3</td>
<td>0.46</td>
<td>4.7</td>
</tr>
<tr>
<td>5 (ay 0.56)</td>
<td>119</td>
<td>5.73</td>
<td>0.24</td>
<td>4.2</td>
<td>0.28</td>
<td>4.8</td>
</tr>
<tr>
<td>6 (ay 0.34)</td>
<td>120</td>
<td>3.38</td>
<td>0.17</td>
<td>5.0</td>
<td>0.17</td>
<td>5.1</td>
</tr>
<tr>
<td>7 (0.00)</td>
<td>120</td>
<td>0.32</td>
<td>0.03</td>
<td>9.6</td>
<td>0.04</td>
<td>11.0</td>
</tr>
</tbody>
</table>

**Specificity**

The specificity of the ABBOTT PRISM HBsAg assay was estimated assuming a zero prevalence of HBsAg in volunteer blood donors and plasmapheresis donors. A total of 6,894 serum and plasma specimens from volunteer blood donors and plasmapheresis donors was collected from four blood centers (Table II). Of the nine repeatedly reactive specimens, six were excluded as confirmed positive by a neutralization assay. Therefore, of the 6,888 donations presumed seronegative for HBsAg, ABBOTT PRISM HBsAg has an estimated specificity of 99.96% (6,888/6,888). Specimens from individuals with medical conditions unrelated to HBV infection or containing potentially interfering substances and specimens from random hospital patients were tested with the ABBOTT PRISM HBsAg assay (Table II).

<table>
<thead>
<tr>
<th>Group/Type</th>
<th>Number of Specimens Tested</th>
<th>ABBOTT PRISM HBsAg</th>
<th>Number of ABBOTT PRISM HBsAg Repeatedly Reactive that were Confirmed Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volun-teer Serum Plasma</td>
<td>3,087 3,301</td>
<td>4 (0.13) 7 (0.21)</td>
<td>2 (0.06) 6 (0.18)</td>
</tr>
<tr>
<td>Blood Donors Plasmapheresis Donors</td>
<td>506</td>
<td>2 (0.40)</td>
<td>1 (0.20)</td>
</tr>
<tr>
<td>TOTAL DONORS</td>
<td>6,894</td>
<td>13 (0.19)</td>
<td>9 (0.13)</td>
</tr>
<tr>
<td>Random Hospital Patients</td>
<td>202</td>
<td>4 (1.98)</td>
<td>4 (1.98)</td>
</tr>
<tr>
<td>Medical Conditions Unrelated to HBV Infection and Potentially Interfering Substancesb</td>
<td>450</td>
<td>47 (10.44)</td>
<td>35 (7.78)</td>
</tr>
</tbody>
</table>

**SYSTEM ERRORS**

For a description of the error codes that appear in the ABBOTT PRISM Report, refer to the ABBOTT PRISM Operations Manual, Section 10.

**LIMITATIONS OF THE PROCEDURE**

- Testing of previously frozen samples with the ABBOTT PRISM HBsAg assay may cause an increase in non-specific reactivity. The assay was designed and validated for use with human serum or plasma. A total of 6,894 serum and plasma specimens from volunteer blood donors and plasmapheresis donors was collected from four blood centers (Table II).

Of the nine repeatedly reactive specimens, six were excluded as confirmed positive by a neutralization assay. Therefore, of the 6,888 donations presumed seronegative for HBsAg, ABBOTT PRISM HBsAg has an estimated specificity of 99.96% (6,888/6,888). Specimens from individuals with medical conditions unrelated to HBV infection or containing potentially interfering substances and specimens from random hospital patients were tested with the ABBOTT PRISM HBsAg assay (Table II).

**Table II**

<table>
<thead>
<tr>
<th>Group/Type</th>
<th>Number of Specimens Tested</th>
<th>ABBOTT PRISM HBsAg</th>
<th>Number of ABBOTT PRISM HBsAg Repeatedly Reactive that were Confirmed Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volunteer Serum Plasma</td>
<td>3,087 3,301</td>
<td>4 (0.13) 7 (0.21)</td>
<td>2 (0.06) 6 (0.18)</td>
</tr>
<tr>
<td>Blood Donors Plasmapheresis Donors</td>
<td>506</td>
<td>2 (0.40)</td>
<td>1 (0.20)</td>
</tr>
<tr>
<td>TOTAL DONORS</td>
<td>6,894</td>
<td>13 (0.19)</td>
<td>9 (0.13)</td>
</tr>
<tr>
<td>Random Hospital Patients</td>
<td>202</td>
<td>4 (1.98)</td>
<td>4 (1.98)</td>
</tr>
<tr>
<td>Medical Conditions Unrelated to HBV Infection and Potentially Interfering Substancesb</td>
<td>450</td>
<td>47 (10.44)</td>
<td>35 (7.78)</td>
</tr>
</tbody>
</table>
a A confirmed positive result in these studies was defined as neutralization by ABBOTT HBsAg CONFIRMATORY ASSAY, Procedure B.

b "Medical Conditions Unrelated to HBV Infection and Potentially Interfering Substances" included the following categories of previously frozen specimens: ANA Ab Positive, Rheumatoid Factor Positive, SLE, CMV Ab Positive, EBV Ab Positive, HAV Ab Positive, HCV Ab Positive, HIV 1-Ab Positive, HSV Ab Positive, Rubella Ab Positive, Non-Viral Liver Disease, Multiple Myeloma, Syphilis Positive, Toxoplasma Ab Positive, Fungal Infections, Animal Handlers, Elevated IgG, Elevated Bilirubin, Elevated Triglycerides, Elevated Cholesterol, Elevated Hemoglobin, Goat/Mouse Ab Positive, Multiparous Females, Multiple Transfusion Recipients, Vaccine Recipients, and Obstetric Patients.

c Frozen specimens that did not confirm positive include: ANA Ab Positive (1), Rheumatoid Factor Positive (2), CMV Ab Positive (1), HCV Ab Positive (3), HSV Ab Positive (2), Rubella Ab Positive (5), Fungal Infections (4), Elevated Bilirubin (2), Elevated Triglycerides (1), Obstetric Patients (5), HIV-1 Ab Positive (1), and Goat/Mouse Ab Positive (1).

Detectability
Specimens obtained from patients with acute, chronic, and recovered HBV infections; preselected HBsAg positive specimens; and populations at increased risk of HBV infection were tested with the ABBOTT PRISM HBsAg assay. The ABBOTT PRISM HBsAg assay detected all of the 437 confirmed positive specimens (100.00%) (Table III).

Table III
Reactivity of the ABBOTT PRISM HBsAg Assay in Selected Populations with HBV Infection, or at Increased Risk of HBV Infection

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Specimens Tested</th>
<th>Number Reactively Repeatedly</th>
<th>Number Con-PRISM HBsAg (%)</th>
<th>Number Con-confirmed Positive (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selected Populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>15</td>
<td>15 (100.00)</td>
<td>15 (100.00)</td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>15</td>
<td>15 (100.00)</td>
<td>15 (100.00)</td>
<td></td>
</tr>
<tr>
<td>Infection Recovered</td>
<td>14</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td></td>
</tr>
<tr>
<td>Preselected HBsAg</td>
<td>402</td>
<td>402 (100.00)</td>
<td>402 (100.00)</td>
<td></td>
</tr>
<tr>
<td>Positive Populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at Increased Risk of HBV Infectionb</td>
<td>163</td>
<td>9 (5.52)</td>
<td>5</td>
<td>5 (100.00)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>609</td>
<td>441 (72.41)</td>
<td>437 (100.00)</td>
<td></td>
</tr>
</tbody>
</table>

a A confirmed positive result in these studies was defined as neutralization by ABBOTT HBsAg CONFIRMATORY ASSAY, Procedure B.

b "Selected Populations at Increased Risk of HBV Infection" included the following categories: U.S. IV Drug Users, Hemodialysis Patients, Homosexual Males, and Hemophiliacs.

Sensitivity
The sensitivity of ABBOTT PRISM HBsAg was evaluated using a seven-member proficiency panel of purified HBsAg (ad and ay) prepared at Abbott Laboratories. The panel was tested over five days with each of three master lots at a total of three sites. Results are summarized in Tables IV, V, and VI.

Table IV
Detection of Purified HBsAg/ad

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Mean S/CO Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.27</td>
<td>9.06 +</td>
<td></td>
</tr>
<tr>
<td>0.71</td>
<td>5.45 +</td>
<td></td>
</tr>
<tr>
<td>0.36</td>
<td>2.84 +</td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>0.32 -</td>
<td></td>
</tr>
</tbody>
</table>

Table V
Detection of Purified HBsAg/ay

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Mean S/CO Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.98</td>
<td>9.86 +</td>
<td></td>
</tr>
<tr>
<td>0.56</td>
<td>5.73 +</td>
<td></td>
</tr>
<tr>
<td>0.34</td>
<td>3.38 +</td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>0.32 -</td>
<td></td>
</tr>
</tbody>
</table>

Table VI
Sensitivity Calculated by Linear Regression Analysis

<table>
<thead>
<tr>
<th>HBsAg Subtype</th>
<th>Sensitivity (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ad</td>
<td>0.10*</td>
</tr>
<tr>
<td>ay</td>
<td>0.08*</td>
</tr>
</tbody>
</table>

* In studies performed at Abbott Laboratories using the HBsAg ad/ay reference serum from the Paul Ehrlich Institute (PEI), the ABBOTT PRISM HBsAg assay calculated sensitivity was <0.1 U/ml.

BIBLIOGRAPHY
18. CDC: Guidelines for the prevention of transmission of Human Immunodeficiency Virus and Hepatitis B Virus to health-care and public safety workers. MMWR 1989,38 (S-6); 16S.

ABBOTT PRISM is a registered trademark of Abbott Laboratories.

For additional product information, please contact your local customer service organization.