Antibody to Hepatitis B Surface Antigen (Mouse Monoclonal IgM)

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

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.

Key to symbols used

- **LOT** Lot Number
- **REP** List Number
- **IVD** In Vitro Diagnostic Medical Device
- **Caution** Consult instructions for use
- **Store at 2-8°C**
- **Store at 15-30°C**
- **Expiration Date**
- **Authorized Representative in the European Community**
- **Manufacturer**
- **Activator Line Treatment**
- **Assay Kit Card**
- **Calibrators**
- **Contains: Azoide**
- **WARNING: SEVERE IRRITANT**

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

U.S. License No. 43
NAME AND INTENDED USE

The ABBOTT PRISM HBsAg assay is an in vitro chemiluminescent immunoassay (CLIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma specimens. The ABBOTT PRISM HBsAg (CLIA) 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.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B virus (HBV) is a small, partially double stranded, DNA virus and a member of the Hepadnaviridae family. The HBV genome contains four overlapping reading frames representing the core, polymerase, surface, and X genes. This virus is responsible for infecting approximately one third of the global population. Approximately 350 million individuals, worldwide, are chronic carriers of HBV. HBV is primarily transmitted through sexual, parenteral, and perinatal routes. Premature mortality from chronic liver disease occurs in 15-25% of the chronically infected HBV patients. HBsAg, hepatitis B surface antigen, is the first viral antigen to circulate in the infected individual. HBV, unlike other DNA viruses, replicates through reverse transcription. The reverse transcription process lacks proofreading capability; therefore, HBV mutation rate is 10 times higher than the mutation rate of other DNA viruses. Some of these mutations may cause changes in the antigenic structure of HBsAg, resulting in epitopes that are no longer recognized by anti-HBs. Sensitive immunoassays for the detection of HBsAg were first described in the early 1970s and were subsequently 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. In addition, 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.

BIOLICAL PRINCIPLES OF THE PROCEDURE

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

1. The sample (either plasma, serum, or control) is incubated in the reaction well of the reaction tray. During incubation, HBsAg present in the sample binds to the antibody on the Microparticles.
2. 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 beating. 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 filter with the Conjugate Wash.
3. The chemiluminescent signal is enhanced by addition of an alkaline hydrogen peroxide substrate. The resulting photons are counted.

WARRANTS AND PRECAUTIONS

The ABBOTT PRISM HBsAg assay meets FDA potency requirements.

NOTE: Each batch MUST end in a release control (ABBOTT PRISM Positive Control). The ABBOTT PRISM Positive Control (included in Kit (REF 3E60-10 or 3L27-01) must be used as the release control which has been configured to validate the system functionality and release sample results. Refer to the ABBOTT PRISM Run Control Kit package insert or the ABBOTT PRISM Positive Run Control Kit package insert for detailed handling and use instructions.

SAFETY PRECAUTIONS

CAUTION: This product contains human sourced and/or potentially infectious components. 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 materials must be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

1. Wear gloves when handling specimens or reagents.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in work areas where specimens or reagents are handled.
4. Clean and disinfect all spills of reagents or specimens using an appropriate disinfectant, such as 0.1% sodium hypochlorite, or other suitable disinfectants.
5. Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.
6. The human plasma used in the Conjugate is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
7. The human plasma used in the Negative Calibrator is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
8. The human plasma used in the Positive Calibrator is reactive for HBsAg and nonreactive for HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2, and anti-HCV.
Handling Precautions

- Do not use kits beyond the expiration date.
- Gently invert each component several times prior to loading the original container on the ABBOTT PRISM System to ensure a homogenous solution. Additional gentle inversion may be required to thoroughly resuspend microparticles. Avoid foaming.
- Gently invert calibrators in the calibrator pack several times prior to each use.
- Each component of the ABBOTT PRISM HbsAg Wash Kit should be at room temperature (15 - 30°C) and then mixed before loading onto the ABBOTT PRISM System.
- Do not mix reagents or calibrators from different bottles. Do not mix or interchange reagents from different ABBOTT PRISM HbsAg Assay Kits.
- Any lot of ABBOTT PRISM HbsAg Wash Kit can be used with any lot of ABBOTT PRISM HbsAg Assay Kit.
- Any lot of ABBOTT PRISM Activator Concentrate, ABBOTT PRISM Activator Diluent, and Control from ABBOTT PRISM Run Control Kit or ABBOTT PRISM Positive Run Control Kit may be used with any lot of any ABBOTT PRISM System.
- Treat Negative and Positive Calibrators and Controls as specimens.
- Avoid microbial and chemical contamination of samples, reagents and equipment. The use of disposable pipette tips is recommended for all preliminary sample transfer.
- Use accurately calibrated equipment.
- Do not freeze reagents.
- Failure to adhere to instructions in the ABBOTT PRISM Operations Manual or package insert may result in erroneous test 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, Sections 7 and 8.

Preparation of Activator Solution

Activator solution must be prepared using equal parts of ABBOTT PRISM Activator Concentrate and ABBOTT PRISM Activator Diluent. The activator solution expires 24 hours from preparation. The ABBOTT PRISM Activator Concentrate may be used immediately after removing from the refrigerator. The volume of activator solution required for multiple tests is calculated by the user.

Twenty nonreactive and 20 low-level reactive specimens showed no qualitative performance differences when subjected to 6 freeze-thaw cycles. However, some specimens that have undergone multiple freeze-thaw cycles and 18 low-level reactive specimens were spiked with elevated levels of bilirubin (≤ 0.4% v/v), triglycerides (≤ 3000 mg/dL), or protein (≤ 12 g/dL). However, specimens that contain greater concentrations of these potentially interfering substances have not been tested. The impact of greater concentrations of these potentially interfering substances on the ABBOTT PRISM HBsAg assay is unknown.

Performance has not been established using umbilical cord blood, or body fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid. These specimens should not be tested using the ABBOTT PRISM HbsAg assay.

Specimens collected by phlebotomy, that have not been frozen, do not require centrifugation. All other specimens (including previously frozen phlebotomy specimens) must be centrifuged as follows:

- Spin 10 minutes at 1000 x g.
- Carefully remove the supernatant.
- Store unopened vials containing tested specimen material at 4°C until testing.
Non-frozen specimens (excluding non-frozen plasmapheresis specimens) must be centrifuged such that g-minutes is between 30,000 and 75,000. A refrigerated or non-refrigerated centrifuge is acceptable for use. The acceptable time and force ranges that meet this criterion are listed in Table I.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>RCF (x g)</th>
<th>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>

Convert rpm to RCF as follows: $\text{RCF} = 1.12 \times \frac{\text{rpm}}{1000}$

Convert RCF to rpm as follows: $\text{rpm} = \frac{\text{RCF}}{1.12}$

**RCF** - The relative centrifugal force generated during centrifugation.

**rpm** - The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).

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

$f_{\text{max}}$ - Radius of the rotor in millimeters. The radius measured is dependent 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 the fixed angle rotor, $f_{\text{max}}$ is the measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor or rotor adapter. For the swinging bucket rotor, $f_{\text{max}}$ is a measure of the distance from the rotor axis (center) to the bottom of the specimen tube in the rotor adapter or bucket at full extension.

**NOTE:** If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used, then the radius ($f_{\text{max}}$) should be manually measured in millimeters and the RCF calculated.

**g-minutes** - The unit of measure for the product of RCF $(x ~ g)$ and centrifugation time (minutes).

Previously frozen specimens must be centrifuged such that the g-minutes is between 180,000 and 300,000. A refrigerated or non-refrigerated centrifuge is acceptable for use. The acceptable time and force ranges that meet this criterion are listed in Table II.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>RCF (x g)</th>
<th>g-minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>12,000</td>
<td>180,000</td>
</tr>
<tr>
<td>20</td>
<td>9,000 - 12,000</td>
<td>180,000 - 240,000</td>
</tr>
<tr>
<td>25</td>
<td>7,500 - 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 re-centrifuged from 30,000 to 75,000 g-minutes as defined for non-frozen specimens.

**NOTE:** Specimens retested within 24 hours of initial centrifugation do not need to be re-centrifuged.

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

Centrifuged cadaveric SERUM specimens tested with ABBOTT PRISM HBsAg may be filtered using the instructions indicated below. If testing includes ABBOTT PRISM HIV O Plus, then the following instructions must be performed.

**NOTE:** Failure to adhere to the following instructions may result in erroneous or inconsistent test results for ABBOTT PRISM HIV O Plus.

Filteration of Centrifuged Cadaveric SERUM Specimens

Weard personal protective equipment, including eyewear.

After centrifugation, filter each cadaveric specimen through a Millipore GV Filter as follows:

1. Label an empty tube with the specimen identification number matching the original tube.
2. Remove the plunger from a sterile 10 cc syringe.

**NOTE:** Do not use a syringe smaller than 10 cc because excess pressure may build up, potentially causing damage to the filter unit or personal injury.

3. Remove the sterile filter from the package.
4. Securely screw the syringe to the filter.

**NOTE:** Do not touch the tip of the filter to avoid possible contamination.
5. Pour a minimum of 1 mL of the centrifuged cadaveric serum into the syringe.

**NOTE:** Additional volume may be required based on the number of ABBOTT PRISM assays performed. Refer to the Specimen Volume section of this package insert.
6. While holding the filter syringe unit over the tube, insert the plunger and slowly apply pressure to deliver the filtered cadaveric serum.

**NOTE:** A clogged filter will resist pressure and no additional sample volume will pass through.

7. If necessary, replace the clogged filter as follows:
   a. Remove the sterile filter from the package.
   b. Carefully invert the syringe to a filter-side-up position with the syringe plunger intact to prevent sample leakage. Gently remove the clogged filter and dispose of it in a potentially infectious waste container.
   c. Securely screw the syringe to the filter.
   d. Slowly apply pressure on the plunger to deliver the filtered cadaveric serum into the tube.
   e. Repeat this step as needed to successfully complete the filtration process.

**NOTE:** Filtered cadaveric specimens that are not tested within 24 hours of initial centrifugation must be re-centrifuged, but do not need to be re-filtered.

**Specimen Volume**

The specimen volume required to perform a single assay on the ABBOTT PRISM System varies according to the number and type of assays, and the different specimen containers. The ABBOTT PRISM HBsAg assay requires 100 µL sample dispense. For ABBOTT PRISM Sample Cups, the minimum specimen volume required for one ABBOTT PRISM HBsAg assay is 400 µL. For either primary or aliquot tubes, all additional assay volume requirements, refer to the ABBOTT PRISM Operations Manual, Section 5.

**PROCEDURE**

**Materials Provided**

- **REF:** 6D19-68 ABBOTT PRISM HBsAg Assay Kit
- **Materials Required but not Provided**
  - ABBOTT PRISM HBsAg Wash Kit
  - ABBOTT PRISM HBsAg Activator Concentrate
  - ABBOTT PRISM HBsAg Activator Diluent
  - ABBOTT PRISM Reaction Trays
  - ABBOTT PRISM Pipette Tips
  - ABBOTT PRISM Accessory Kit
  - ABBOTT PRISM Run Control Kit
  - ABBOTT PRISM Positive Run Control Kit
  - ABBOTT PRISM Run Control Adapters
  - Protective Disposable Gloves
  - Disinfectant
  - Purified Water-rinsed or Clean Disposable Measuring Equipment

**Additional Materials Available**

- **REF:** 7B36-01 ABBOTT PRISM SAMPLE CUPS
- **REF:** 1A75-10 or 3L27-10 ABBOTT PRISM ACTIVATOR LINE TREATMENT
- **REF:** 7A03-01 or 3L00-01 ABBOTT PRISM PRIME/PURGE ACCESSORIES
- **REF:** 7A03-30 or 3L00-30 ABBOTT PRISM PURGE CONCENTRATE
- **REF:** 7A03-31 ABBOTT PRISM LINE CLEANER

For Cadaveric Specimens Only

- **REF:** 2P41-01 Millipore GV Filters
- **10 cc Sterile Syringes**

**ABBOTT PRISM HBsAg ASSAY PROCEDURE**

Key procedures that require operator interaction for testing samples are listed below. For detailed information concerning batch time, maximum batch size, reagent handling and loading, and associated procedural steps, refer to the ABBOTT PRISM Operations Manual, Sections 2, 5, and 7.

1. Enter a Plan Work Load (refer to the ABBOTT PRISM Operations Manual, Section 5).
2. Replace reagents as needed (refer to the ABBOTT PRISM Operations Manual, Sections 5 and 7).

**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. Gently invert calibrators in the calibrator pack several times prior to each use. Each component of the ABBOTT PRISM HBsAg Wash Kit should be at room temperature (15 - 30°C) and then mixed before loading onto the ABBOTT PRISM System.
Verify that all tubing label symbols match the symbols on each reagent label. (Refer to the symbol key in the REAGENTS section of this package insert, and the ambient reagent bay and refrigerator diagrams provided with the ABBOTT PRISM System).

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

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

Prepare activator solution (Refer to the Preparation of Activator Solution section of this package insert) and load onto the ABBOTT PRISM System.

Verify that an adequate number of ABBOTT PRISM Reaction Trays are in the Tray Loader.

Verify that an adequate number of ABBOTT PRISM Pipette Tips are in the Pipette Tip Racks.

Perform the prime procedure (Refer to the ABBOTT PRISM Operations Manual, Section 5).

Initiate sample processing. Gently insert calibrators in the calibrator pack (several times). Open the bottles in the calibrator pack and place in the calibrator rack. Load the calibrator rack and sample racks, including the run controls. (Refer to the QUALITY CONTROL PROCEDURES, Controls, Control Handling Procedure, in this package insert.)

After the calibrators have been automatically pipetted, remove the calibrator rack. Close the calibrator bottles and return them to 2 - 8°C storage.

Each specimen is initially tested once, unless the operator overrides this automatic function of the ABBOTT PRISM System.

Sample racks may be removed after the samples have been pipetted.

NOTE: No operator interaction is required for the following steps, which are automatically carried out by the ABBOTT PRISM System: reaction tray transport, calibrator/sample/release control pipetting, incubation, reagent dispense, sample reading, data reduction, run validity and result determination.

After specimen processing 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. The ABBOTT PRISM HBsAg assay is a two-step ChLIA procedure.

QUALITY CONTROL PROCEDURES

Calibration

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

Controls

1. The ABBOTT PRISM Positive Control MUST be included as the last sample in each batch as a release control. The operator is prompted to include this control as the last sample in every batch, and the ABBOTT PRISM Positive Control is then automatically tested as a single specimen. This control must meet specifications defined in the ABBOTT PRISM HBsAg Control Kit package insert or the ABBOTT PRISM Positive Run Control Kit package insert in order to validate the system function and release sample results. If this control does not meet specifications defined in the ABBOTT PRISM HBsAg Control Kit package insert or the ABBOTT PRISM Positive Run Control Kit package insert, refer to the ABBOTT PRISM Operations Manual, Section 10, for additional information.

2. Invalid controls may be run at the operator’s discretion (refer to the ABBOTT PRISM Operations Manual, Section 3).

Invalid controls: Additional controls may be run anywhere within a batch as an invalid control. Specifications may be assigned to invalidating controls. If an invalid control fails to meet assigned specifications, sample processing is shutdown and no sample results are calculated or provided by the instrument. When an invalid control meets assigned specifications, sample processing continues and a valid release control (ABBOTT PRISM Positive Control) result is required to release data.

Non-validating controls: Additional controls may be run anywhere within a batch as a non-validating control. Specifications may be assigned to non-validating controls. A valid release control (ABBOTT PRISM Positive Control) result is required to release data. If the user-assigned specifications for the non-validating control(s) are not met and the release control specifications are met, there will be no effect on sample processing. In this case, reactive sample results must not be considered invalid.

3. Control Handling Procedure
   a. Place run control adapters into the sample rack. The adapters can be placed in any rack position except 1, 2, 27, or 28.
   b. Place each run control bottle into an adapter in the sample rack such that when the bottle flip-top cap is opened, it can be snapped into an open position within the adapter.
   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.

Refer to the ABBOTT PRISM Operations Manual, Section 3, for additional information on calibrators, assay controls and run controls.

ASSAY PARAMETER SPECIFICATIONS

The ABBOTT PRISM HBsAg 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:

\[
\text{Cutoff Value} = \frac{\text{Mean Negative Calibrator (NC) Net Counts} \times (0.19 \times \text{Mean Positive Calibrator (PC) Net Counts})}{\text{Mean Positive Calibrator (PC) Net Counts}}
\]

Example:

\[
\begin{align*}
\text{Mean NC Net Counts} &= 100 \\
\text{Mean PC Net Counts} &= 1,000 \\
\text{Cutoff Value} &= \frac{100 \times (0.19 \times 1,000)}{1,000} = 190
\end{align*}
\]

The ABBOTT PRISM System calculates the ABBOTT PRISM HBsAg assay S/CO for each sample and controls using the following formula:

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

Example:

\[
\begin{align*}
\text{Sample Net Counts} &= 580 \\
\text{Cutoff Value} &= 290 \\
\text{S/CO} &= \frac{580 \times 290}{580 + 290} = 0.99
\end{align*}
\]

Interpretation of Results

A positive ABBOTT PRISM HBsAg assay specimen with Net Counts less than the cutoff value are nonreactive and need not be tested further. Nonreactive specimens are considered negative for HBsAg by the criteria of ABBOTT PRISM HBsAg:

- Specimens with Net Counts greater than or equal to the cutoff value are considered initially reactive by the criteria of the ABBOTT PRISM HBsAg assay. All specimens (excluding non-frozen plasmapheresis specimens) that are reactive on initial testing must be centrifuged prior to retesting according to the table in the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert. Initially reactive specimens must be retested in duplicate using the ABBOTT PRISM HBsAg Assay Kit.

NOTE: Specimens restested within 24 hours of initial centrifugation do not require recentrifugation.

- If the sample Net Counts for both retests are less than the cutoff value, the specimen is nonreactive. Nonreactive specimens are considered negative for HBsAg by the criteria of ABBOTT PRISM HBsAg.

- If the sample Net Counts for either duplicate retest are greater than or equal to the cutoff value, the specimen is considered repeatedly reactive. Repeatedly reactive specimens must be tested by the ABBOTT PRISM HBsAg Confirmatory assay, a licensed neutralizing confirmatory test. Only the specimens which are confirmed by specific neutralization with anti-HBs are considered positive for HBsAg.

- Individuals who are repeatedly reactive may be referred for medical evaluation which may include additional testing.

Although the association of infectivity of donated blood or plasma and the presence of HBsAg is strong, it is recognized that presently available methods for HBsAg detection are not sensitive enough to detect all potentially infectious units of blood, plasma or possible cases of HBV infection. A nonreactive test result does not exclude infection.

Reading Results

Some S/CO values may be flagged with “<” or “>” symbols. For more information on sample reports, refer to the ABBOTT PRISM Operations Manual, Section 5: Operating Instructions, Reports. The ABBOTT PRISM System reports sample results in Net Counts and S/CO. Net Counts are used by the ABBOTT PRISM System to interpret results. The S/CO value is provided in reports to show reactivity relative to the cutoff value. In the ABBOTT PRISM HBsAg assay, specimens with S/CO values of less than 1.00 are considered nonreactive. Specimens with an S/CO value of greater than or equal to 1.00 are considered reactive.

System Errors

For a description of the error codes that appear on ABBOTT PRISM System reports, refer to the ABBOTT PRISM Operations Manual, Section 10.
LIMITATIONS OF THE PROCEDURE

- This assay was designed and validated for use with individual human serum and plasma specimens. This assay has not been validated for use with pooled specimens.
- Do not use specimens collected in heparin. Use of heparin as an anticoagulant may cause a reduction in Sample Net Counts and in S/CO for ABBOTT PRISM HCV; therefore, heparin is not recommended for any ABBOTT PRISM assay.
- Serum from heparinized patients may be incompletely coagulated. Emorros or inconsistent test results may occur due to the presence of fibrin. To prevent this phenomenon, draw specimen prior to heparin therapy.
- False-reactive test results can be expected with any test kit. False-reactive test results have been observed due to nonspecific interactions. Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS section of this package insert for assay performance characteristics.
- Some specimens that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results.
- Previously frozen specimens must be centrifuged prior to the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert prior to running the assay.
- An increased occurrence of drain time errors may be observed for cadaveric specimens.
- Do not use cadaveric plasma specimens.
- Performance has not been established using umbilical cord blood, or body fluids such as urine, saliva, semen, amniotic fluid, cerebrospinal fluid, or pleural fluid. These specimens should not be tested using the ABBOTT PRISM HBsAg assay.
- Do not use heat-inactivated specimens.
- Do not use specimens with obvious microbial contamination, gross lipemia or gross hemolysis.

SPECIFIC PERFORMANCE CHARACTERISTICS

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 on each subchannel. The intra-assay variability contains intra-assay variability.

TWO replicates were invalid due to instrument detection of sample drain time error.

One replicate was invalid due to instrument detection of sample dispense error.

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-EV 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).

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% (9/13,911) 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.

Some specimens that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results.

An increased occurrence of drain time errors may be observed for cadaveric specimens.

The intra-assay variability contains intra-assay variability.

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

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

TABLE II

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABBOTT PRISM HBsAg Assay Reproducibility</strong></td>
</tr>
<tr>
<td><strong>Panel Number</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>440</td>
</tr>
</tbody>
</table>

**Negative Control**

| 439 | 0.26 | 0.03 | 14.6 | 0.04 | 15.6 |

**Positive Control**

| 440 | 2.63 | 0.13 | 5.2 | 0.20 | 7.8 |

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

**TABLE III**

<table>
<thead>
<tr>
<th>TABLE III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABBOTT PRISM HBsAg Assay Reproducibility</strong></td>
</tr>
<tr>
<td><strong>Panel Number</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

**Calibrator**

| **Number of Replicates** | **S/CO Mean** | **%CV SD** | **%CV Inter-assay SD** |
| 660 | 89 | 9.6 | 10.8 | 13.1 | 14.7 |

**Positive**

| 1299 | 73.3 | 5.6 | 73.3 | 5.6 |

Cutoff Value = Mean Negative Calibrator Net Counts + (0.19 X Mean Positive Calibrator Net Counts)
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%.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number Tested</th>
<th>Number Reactively Positive (% of Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preselected</td>
<td>514</td>
<td>514 (100.00)</td>
</tr>
<tr>
<td>HBsAg Positive Acute HBV</td>
<td>98</td>
<td>98 (100.00)</td>
</tr>
<tr>
<td>HBsAg Positive Chronic HBV</td>
<td>101</td>
<td>101 (100.00)</td>
</tr>
<tr>
<td>HBsAg Positive Recovered</td>
<td>47</td>
<td>47 (0.00)</td>
</tr>
<tr>
<td>Risk for HBV Infection</td>
<td>1,212</td>
<td>767 (63.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>754 (63.31)</td>
</tr>
</tbody>
</table>

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, 21 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.
f 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.
g Specimen was not tested due to insufficient specimen volume.

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 panel was tested as described in the ASSAY REPRODUCIBILITY section of this package insert. The detection of HBsAg ad and ay subtypes is presented in Tables VI and VII, respectively.

<table>
<thead>
<tr>
<th>HBsAg Concentration (ng/mL)</th>
<th>Mean S/CO Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.917</td>
<td>6.98</td>
<td>+</td>
</tr>
<tr>
<td>0.525</td>
<td>4.06</td>
<td>+</td>
</tr>
<tr>
<td>0.124</td>
<td>1.39</td>
<td>+</td>
</tr>
<tr>
<td>0.000</td>
<td>0.34</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HBsAg Concentration (ng/mL)</th>
<th>Mean S/CO Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.002</td>
<td>8.86</td>
<td>+</td>
</tr>
<tr>
<td>0.485</td>
<td>4.62</td>
<td>+</td>
</tr>
<tr>
<td>0.131</td>
<td>1.37</td>
<td>+</td>
</tr>
<tr>
<td>0.000</td>
<td>0.34</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

PERFORMANCE CHARACTERISTICS OF CADAVERIC SERUM TESTING

Reproducibility

Inter-assay reproducibility of PRISM HBsAg 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 lots of 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.

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 lots of PRISM HBsAg. The mean sample to cutoff (S/CO) ratio for the 162 nonreactive postmortem replicates (51 specimens with three reagent lots; see Table VIII) was 0.37, and the mean S/CO for 162 normal donor replicates (54 specimens with three reagent lots) was 0.24. Results are presented in Table VIII.

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of Specimens</th>
<th>No. of Replicates</th>
<th>Mean S/CO</th>
<th>Nonreactive</th>
<th>Initial Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmortem</td>
<td>51</td>
<td>137</td>
<td>0.37</td>
<td>136</td>
<td>(99.27%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.73%)</td>
<td></td>
</tr>
<tr>
<td>Normal Donor</td>
<td>54</td>
<td>162</td>
<td>0.24</td>
<td>162</td>
<td>(100.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

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 tested due to insufficient specimen volume.

Assuming the specimen with the initial reactive result would have a reactive result upon retest, the 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 16.1 hours after death.

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 lots of PRISM HBsAg. The mean sample to cutoff (S/CO) for the 142 postmortem replicates (51 specimens, with three reagent lots; see Table IX, footnote a) was 2.05, and the mean S/CO for 162 normal donor replicates (54 specimens, with three reagent lots) was 2.07. Results are presented in Table IX.

<table>
<thead>
<tr>
<th>Population</th>
<th>No. of Specimens</th>
<th>No. of Replicates</th>
<th>Mean S/CO</th>
<th>Nonreactive</th>
<th>Initial Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmortem</td>
<td>51</td>
<td>142</td>
<td>2.05</td>
<td>0</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0%)</td>
<td>(100.0%)</td>
</tr>
<tr>
<td>Normal Donor</td>
<td>54</td>
<td>162</td>
<td>2.07</td>
<td>0</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0%)</td>
<td>(100.0%)</td>
</tr>
</tbody>
</table>

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

The 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.
BIBLIOGRAPHY


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