

ABBOTT PRISM®
HBsAg Confirmatory
List No. 6E51
34-4213/R7



Antibody to Hepatitis B Surface Antigen (Human)



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NAME AND INTENDED USE

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.

SUMMARY AND EXPLANATION OF THE TEST

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 Plasma (Human), 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.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ABBOTT PRISM HBsAg Confirmatory assay uses the ABBOTT PRISM HBsAg assay reagents in addition to the reagents described below. For information on the ABBOTT PRISM HBsAg assay, refer to the ABBOTT PRISM HBsAg assay package insert.

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. For further information regarding ChLIA technology, refer to the ABBOTT PRISM Operations Manual, Section 3.

REAGENTS

NOTE: Each Confirmatory Reagent and Diluent description that follows is accompanied by a unique symbol. These symbols appear on the bottle labels.

ABBOTT PRISM HBsAg Confirmatory Kit, 10 Tests (No. 6E51-68)

NOTE: Do not mix reagents from different bottles. Do not mix or interchange reagents from different ABBOTT PRISM HBsAg Confirmatory Assay Kits.

- 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)
- 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)
- 1 Bottle (4 mL) Reagent C. Specimen treatment reagent with 20 mM citrate buffer. (Symbol: RGT C)
- 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)
- 1 Package (100 units) ABBOTT PRISM Sample Cups.
- 1 Package (10 count) ABBOTT PRISM HBsAg Confirmatory Bar Code Labels.

Other Reagents Required

ABBOTT PRISM Run Control Kit (No. 3E60-10)

NOTE: The ABBOTT PRISM Negative and Positive Controls must be included on each ABBOTT PRISM HBsAg Confirmatory Sample Rack. Refer to the ABBOTT PRISM Run Control Kit package insert for detailed handling and use instructions.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

The performance characteristics of this product have not been established for the laboratory diagnosis of HBV infection.

Safety Precautions

CAUTION: This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, HIV-1 Ag or HIV-1 NAT, anti-HCV, and anti-HIV-1/HIV-2, by FDA licensed 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 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,¹ Biosafety Level 2² or other appropriate biosafety practices^{3,4} should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in work areas where specimens or reagents are handled.
- Clean and disinfect all spills of specimens or reagents using an appropriate disinfectant, such as 0.1% sodium hypochlorite, or other suitable disinfectants.^{4,5,6}

- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local, state and federal regulations.^{7,8}
- Some 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 drain 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 acids liberates very toxic gas.
- S35 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.

Handling Precautions

- **Use extreme caution when performing off-line dilutions to prevent cross-contamination of samples.**
- Do not use kits beyond the expiration date.
- Do not mix reagents from different bottles. Do not mix or interchange reagents from different ABBOTT PRISM HBsAg Confirmatory Assay Kits.
- Treat Negative and Positive Controls as specimens.
- Avoid microbial and chemical contamination of samples, reagents and equipment. The use of disposable pipette tips is recommended for any 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.

Storage Instructions

Store the ABBOTT PRISM HBsAg Confirmatory kit at 2 - 8°C.

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 ABBOTT PRISM HBsAg reagents, or instrument failure. The ABBOTT PRISM HBsAg Confirmatory assay utilizes the ABBOTT PRISM Run Controls on each confirmatory rack to verify acceptable performance of the confirmatory reagents. When the treated controls do not meet specifications, the ABBOTT PRISM System will continue to process samples, but results for that Confirmatory Sample Rack will not be released. This may indicate either deterioration or contamination of ABBOTT PRISM HBsAg Confirmatory reagents, or instrument failure. Refer to the ABBOTT PRISM Operations Manual, Section 10, for additional information.

INSTRUMENT PROCEDURE

- ABBOTT PRISM software version 3.11 or higher must be used to perform the assay.
- Refer to the ABBOTT PRISM Operations Manual for a detailed description of Instrument Procedures.
- Refer to the ABBOTT PRISM Operations Manual, Section 7, for limitations associated with test management.

- 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

- Serum (including serum collected in serum separator tubes), plasma collected in EDTA, potassium oxalate, sodium citrate, ACD-A, ACD-B, CP2D, CPD, or CPDA-1 anticoagulants, or plasma collected from segmented tubing may be used with the ABBOTT PRISM HBsAg Confirmatory assay. Follow the manufacturer's processing instructions for serum and plasma collection tubes.

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.

- 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 heat-inactivated specimens.
- Do not use specimens with obvious microbial contamination.
- When shipped, specimens must be packaged and labeled in compliance with applicable regulations covering the transport of clinical specimens and infectious substances. Specimens may be shipped at 30°C or colder for a period not to exceed 7 days. Prior to freezing, the serum or plasma should be removed from the clot or red blood cells.
- Failure to follow the specified centrifugation procedure on specimens tested with the ABBOTT PRISM HBsAg Confirmatory assay may cause a reduction in Sample Net Counts and in S/CO.
- Specimens may be stored for up to 14 days at 2 - 8°C. If storage periods greater than 14 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 (-20°C or colder).
- Previously frozen specimens must be mixed gently and thoroughly after thawing and centrifuged according to Table II in this section.
- Some specimens that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may give erroneous or inconsistent test results.
- Clear, non-hemolyzed specimens should be used when possible. Specimens containing visible particulate matter may give erroneous or inconsistent test results.
- 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 Confirmatory assay.
- Specimens collected by plasmapheresis, that have not been frozen, do not require centrifugation. All other specimens (including previously frozen plasmapheresis specimens) must be centrifuged as follows:

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 I

Centrifugation Time (minutes)	RCF (x g)	g-minutes
10	3,000	30,000
15	2,000 - 3,000	30,000 - 45,000
20	1,500 - 3,000	30,000 - 60,000
25	1,300 - 3,000	32,500 - 75,000

Convert rpm to RCF as follows: $RCF = 1.12 \times r_{max} (rpm/1000)^2$

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

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 should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.
r_{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, r_{max} is a measure of the distance from the rotor axis (center) to the bottom of the tube cavity. For the swinging bucket rotor, r_{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.
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 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 II

Centrifugation Time (minutes)	RCF (x g)	g-minutes
15	12,000	180,000
20	9,000 - 12,000	180,000 - 240,000
25	7,200 - 12,000	180,000 - 300,000

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

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

Specimen Volume

The specimen volume required to perform the ABBOTT PRISM HBsAg Confirmatory assay (undiluted and diluted specimen) is 810 μ L.

PROCEDURE

Materials Provided

- No. 6E51-68 ABBOTT PRISM HBsAg Confirmatory Kit

NOTE: A kitcard is not provided with the ABBOTT PRISM HBsAg Confirmatory Assay Kit. Each laboratory is responsible for ensuring appropriate reagent documentation.

Materials Required but not Provided

- No. 6D19-68 ABBOTT PRISM HBsAg Assay Kit
- No. 6D19-58 ABBOTT PRISM HBsAg Wash Kit
- No. 1A75-02 ABBOTT PRISM Activator Concentrate
- No. 1A75-01 ABBOTT PRISM Activator Diluent
- No. 5A07-01 ABBOTT PRISM Reaction Trays
- No. 5A07-10 ABBOTT PRISM Pipette Tips
- No. 6A36-60 ABBOTT PRISM Accessory Kit
- No. 3E60-10 ABBOTT PRISM Run Control Kit
- No. 6A36-32 ABBOTT PRISM HBsAg Confirmatory Preparation Rack
- No. 6A36-33 ABBOTT PRISM HBsAg Confirmatory Template
- No. 6A36-31 ABBOTT PRISM Run Control Adapters
- Protective Disposable Gloves
- Disinfectant

For hazard information, refer to the **WARNINGS AND PRECAUTIONS** section in the package insert of each product and to the ABBOTT PRISM Operations Manual, Section 8.

ABBOTT PRISM HBsAg CONFIRMATORY ASSAY PROCEDURE

The following steps describe the off-line dilution and neutralization procedure for the ABBOTT PRISM HBsAg Confirmatory assay. The ABBOTT PRISM HBsAg Confirmatory Sample Rack can process a maximum of four specimens in addition to the treated Negative and Positive Controls. To process more than four specimens, additional Confirmatory Sample Racks must be prepared. The ABBOTT PRISM Negative and Positive Controls must be included on each Confirmatory Sample Rack. The ABBOTT PRISM HBsAg Confirmatory Preparation Rack is used as an aid to organize the off-line dilution and neutralization procedure, and allow for correct reagent dispensing (refer to the ABBOTT PRISM Operations Manual Section 5).

1. Sample Cup Labeling

Refer to Bar Code label ID in the following table. Four sample cups are required for each specimen. Label sample cups for the first specimen to be placed in the rack with bar codes xxxxx-1 through xxxxx-4. For additional specimens, continue labeling four sample cups per specimen using the bar code labels in numerical order as they are packaged in the ABBOTT PRISM HBsAg Confirmatory Kit. Four sample cups are required for the controls. Label these sample cups AC1 through AC4.

NOTE: xxxxx corresponds to the bar coded numbers 00001 through 00020 found on the bar code labels in the ABBOTT PRISM HBsAg Confirmatory Kit. Each specimen within a Confirmatory Sample Rack should have a unique bar code number.

2. Off-line Dilution

CAUTION: Use extreme caution when performing off-line dilutions to prevent cross contamination of samples. Change pipette tip after each dilution or reagent addition.

For each specimen to be tested, perform a 1:500 dilution (using ABBOTT PRISM HBsAg Confirmatory Diluent) as follows:

- 1:25 dilution (10 μ L specimen + 240 μ L Diluent);
- 1:20 dilution of the resultant solution (50 μ L dilution "a" + 950 μ L Diluent)

NOTE: Retain the original bar coded tube from the ABBOTT PRISM HBsAg repeatedly reactive specimen for use as the pilot tube on the Confirmatory Sample Rack.

3. Neutralization Procedure

Refer to the following table. (Change pipette tip after each reagent addition.)

- Pipette 400 μ L of the undiluted specimen into the sample cups labeled xxxxx-1 and xxxxx-2.
- Pipette 400 μ L of the diluted specimen into the sample cups labeled xxxxx-3 and xxxxx-4.
- If additional specimens are tested, repeat steps a and b using another set of bar code labeled sample cups.
- Pipette 400 μ L of the Negative Control into the sample cups labeled AC1 and AC2.
- Pipette 400 μ L of the Positive Control into the sample cups labeled AC3 and AC4.
- Add the indicated amounts of Reagent C, Reagent A, and Reagent B.

Sample Cup	1	2	3	4	5	6	7	8
Bar Code Label ID	xxxxx-1	xxxxx-2	xxxxx-3	xxxxx-4	AC1	AC2	AC3	AC4
Confirmatory Rack Positions	R-1 or S-1 or T-1 or U-1	R-2 or S-2 or T-2 or U-2	R-3 or S-3 or T-3 or U-3	R-4 or S-4 or T-4 or U-4	AC1	AC2	AC3	AC4
Sample Type	Undiluted Specimen	Undiluted Specimen	Diluted Specimen	Diluted Specimen	ABBOTT PRISM Negative Control	ABBOTT PRISM Negative Control	ABBOTT PRISM Positive Control	ABBOTT PRISM Positive Control
Amount of Sample	400 μ L	400 μ L	400 μ L	400 μ L	400 μ L	400 μ L	400 μ L	400 μ L
Amount of Reagent C	50 μ L	50 μ L	50 μ L	50 μ L	50 μ L	50 μ L	50 μ L	50 μ L
Amount of Reagent A	50 μ L	0	50 μ L	0	50 μ L	0	50 μ L	0
Amount of Reagent B	0	50 μ L	0	50 μ L	0	50 μ L	0	50 μ L
Final Color	RED	BLUE	RED	BLUE	RED	BLUE	RED	BLUE

4. Mix by gently tapping the sample cups and incubate at 15 - 30°C for 1 to 8 hours.

5. Confirmatory Sample Rack Loading
 - a. Install the ABBOTT PRISM HBsAg Confirmatory Template onto a standard ABBOTT PRISM Sample Rack. Confirmatory sample positions are labeled on the Confirmatory Template. Refer to the ABBOTT PRISM Operations Manual, Section 5, for Sample Rack Layout.
 - b. For the first specimen, load the pilot tube in the rack position labeled PILOT R.

NOTE: The Sample Pilot tube is only used to obtain the donor ID for the specimen. No sample will be dispensed from this tube.
 - c. Load treated samples labeled xxxxx-1, xxxxx-2, xxxxx-3, and xxxxx-4 into rack positions labeled R-1 through R-4, respectively.
 - d. Load second specimen, if necessary, in rack positions labeled Pilot S and S-1 through S-4.
 - e. Load the third specimen, if necessary, into rack positions labeled Pilot T and T-1 through T-4.
 - f. Load the fourth specimen, if necessary, into rack positions labeled Pilot U and U-1 through U-4.
 - g. Load the treated Negative Control labeled AC1 and AC2 into rack positions AC1 and AC2, respectively.
 - h. Load the treated Positive Control labeled AC3 and AC4 into rack positions AC3 and AC4, respectively.
6. For each Confirmatory Sample Rack, use the following table to determine the number of samples and treated Run Controls to be included in Resource Management, Plan Work Load Menu.

Number of Confirmatory Samples to be tested	1	2	3	4
Number of Samples in Plan Work Load	4	8	12	16
Number of treated Run Controls in Plan Work Load	4	4	4	4
Total Samples	8	12	16	20

7. Refer to the ABBOTT PRISM Operations Manual, Section 5, for instructions on how to load Confirmatory Sample Racks onto the ABBOTT PRISM. Once the Confirmatory Sample Rack is scheduled, the samples are processed by the ABBOTT PRISM HBsAg assay.

Refer to the ABBOTT PRISM HBsAg package insert for detailed use instructions.

NOTE: Confirmatory Sample Racks may be loaded onto the ABBOTT PRISM together with other sample racks as part of an ABBOTT PRISM HBsAg assay batch.

QUALITY CONTROL PROCEDURES

Calibration

The ABBOTT PRISM HBsAg Negative and Positive Calibrators are automatically tested in triplicate at the beginning of each ABBOTT PRISM HBsAg 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 and Negative Controls with Reagent A and with Reagent B are tested on each Confirmatory Sample Rack as a means of validating ABBOTT PRISM HBsAg Confirmatory results. These controls must meet specifications listed in the **ASSAY PARAMETER SPECIFICATIONS** section of this package insert. For each Confirmatory Sample Rack, if these criteria are not met, the Confirmatory Sample Rack is invalid and results will not be generated. This may indicate deterioration or contamination of reagents, or instrument failure.

NOTE: The ABBOTT PRISM HBsAg Positive and Negative Controls with Reagent A and with Reagent B are **NOT** release controls.

2. The ABBOTT PRISM Positive Control **MUST** be included as the last sample in the ABBOTT PRISM HBsAg assay batch as a release control. The ABBOTT PRISM Positive Control **MUST** be loaded after the Confirmatory Sample Rack onto a different sample rack in order to release HBsAg Confirmatory results. This control must meet specifications defined in the ABBOTT PRISM Run Control Kit package insert in order to validate the system functionality and release sample results. If this control does not meet specifications defined in the ABBOTT PRISM Run Control Kit package insert, refer to the ABBOTT PRISM Operations Manual, Section 10, for additional information.

Refer to the **QUALITY CONTROL PROCEDURES, Controls** section of the ABBOTT PRISM HBsAg package insert for more detailed information on controls and control handling procedures.

ASSAY PARAMETER SPECIFICATIONS

The ABBOTT PRISM HBsAg Confirmatory assay parameter specifications have been factory set. These parameters cannot be printed, displayed, or edited. Validity of ABBOTT PRISM HBsAg Confirmatory reagent performance is established by evaluating the following:

Assay Parameter	Specification
Negative Control with Reagent A Net Counts	< Cutoff
Negative Control with Reagent B Net Counts	> Cutoff
Positive Control with Reagent B Net Counts	≥ Cutoff
Positive Control % Neutralization	≥ 75%

RESULTS

Calculation of Cutoff and S/CO Values

The ABBOTT PRISM System calculates the ABBOTT PRISM HBsAg Confirmatory assay cutoff value with the ABBOTT PRISM HBsAg Positive and Negative Calibrators using the following formula:

$$\text{Cutoff Value} = \text{Mean Negative Calibrator (NC) Net Counts} + (0.1 \times \text{Mean Positive Calibrator [PC] Net Counts})$$

Example: Mean NC Net Counts = 100
 Mean PC Net Counts = 1,000
 $100 + (0.1 \times 1,000) = 200$
 Cutoff Value = 200

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

$$\text{S/CO} = \text{Sample Net Counts} \div \text{Cutoff Value}$$

Example: Sample Net Counts = 600
 Cutoff Value = 200
 $600 \div 200 = 3.00$
 S/CO = 3.00

The ABBOTT PRISM System calculates the ABBOTT PRISM HBsAg Confirmatory assay % neutralization for each sample and control using the following formula:

$$\% \text{ neutralization} = \frac{\text{Sample with Reagent B Net Counts} - \text{Sample with Reagent A Net Counts}}{\text{Sample with Reagent B Net Counts} - \text{Negative Control with Reagent B Net Counts}} \times 100\%$$

Example: Sample with Reagent B Net Counts = 500
 Sample with Reagent A Net Counts = 100
 Negative Control with Reagent B Net Counts = 80
 $\frac{500 - 100}{500 - 80} \times 100\% = \frac{400}{420} \times 100\% = 95.24\%$

Interpretation of Results

A specimen is confirmed positive for HBsAg if the specimen with Reagent B net counts are greater than or equal to the cutoff value and the calculated percent neutralization is greater than or equal to 50%. Refer to the following table for additional interpretation of ABBOTT PRISM HBsAg Confirmatory test results.

INTERPRETATION OF RESULTS

Undiluted Specimen		Diluted Specimen		INTERPRETATION
Specimen Net Counts with Reagent B	% Neutralization	Specimen Net Counts with Reagent B	% Neutralization	
< Cutoff	n/a	< Cutoff	n/a	NEGATIVE
< Cutoff	n/a	≥ Cutoff	any value	NO RESULT-REPEAT TEST
< Cutoff	n/a	Instrument Error	n/a	NO RESULT-REPEAT TEST
Instrument Error	n/a	< Cutoff	n/a	NO RESULT-REPEAT TEST
Instrument Error	n/a	≥ Cutoff	≥ 50%	CONFIRMED POSITIVE FOR HBsAg
Instrument Error	n/a	≥ Cutoff	< 50%	ADDITIONAL DILUTION REQUIRED*
Instrument Error	n/a	Instrument Error	n/a	NO RESULT-REPEAT TEST
≥ Cutoff	≥ 50%	≥ Cutoff	any value	CONFIRMED POSITIVE FOR HBsAg
≥ Cutoff	≥ 50%	< Cutoff	n/a	CONFIRMED POSITIVE FOR HBsAg
≥ Cutoff	≥ 50%	Instrument Error	n/a	CONFIRMED POSITIVE FOR HBsAg
≥ Cutoff	< 50%	≥ Cutoff	≥ 50%	CONFIRMED POSITIVE FOR HBsAg
≥ Cutoff	< 50%	≥ Cutoff	< 50%	ADDITIONAL DILUTION REQUIRED*
≥ Cutoff	< 50%	< Cutoff	n/a	REPEAT REACTIVE, NON-CONFIRMING
≥ Cutoff	< 50%	Instrument Error	n/a	NO RESULT-REPEAT TEST

*NOTE: If an additional dilution is required, a dilution greater than 1:500 should be prepared (*i.e.*, 1:1000, 1:5000). Run the 1:500 dilution in the ABBOTT PRISM HBsAg Confirmatory assay as the UNDILUTED specimen and the additional dilution as the DILUTED specimen.

- 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 negative (non-confirming) ABBOTT PRISM HBsAg Confirmatory test result does not exclude infection.

Reading Results

Some S/CO and % neutralization 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 Confirmatory 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. Erroneous 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.
- 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 per Table II in the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert prior to running the assay.
- 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 Confirmatory 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 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 analysis⁹ for a mixed model¹⁰ (Table III).

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

Panel Member or Control	Number of Replicates ^a	Mean S/CO ^b	Intra-assay		Inter-assay ^c	
			SD	%CV	SD	%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		Inter-assay ^c	
			SD	%CV	SD	%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

- Several panel member replicates were not obtained due to instrument detection of three control aspiration or dispense errors and two invalid control results.
- Mean S/CO = mean of all replicate S/CO values for each panel member or control tested.
- Inter-assay variability contains intra-assay variability.
- %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.

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 IV).

TABLE IV
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.

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