Abbott
AXSYM® SYSTEM

HBsAg
List No. 9B01-20
66-8427/R1

HBsAg

Customer Service
United States: 1-877-4ABBOTT

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

CAUTION:
United States Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

ABBOTT LABORATORIES
Diagnostics Division
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NAME

AxSYM® HBsAg

INTENDED USE

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

WARNING: Not intended for use in screening blood, plasma, or tissue donors. The effectiveness of AxSYM HBsAg for use in screening blood, plasma, or tissue donors has not been established.

SUMMARY AND EXPLANATION OF THE TEST

Enzyme immunoassays for the detection of antigens were first described by Engvall and Perlmann[1-3] and VanWeemen and Schuurs[4] 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.[5-7] In the early 1980's, monoclonal anti-HBs based assays were developed for the detection of HBsAg.[8-13] AxSYM HBsAg is an enzyme immunoassay that uses microparticles coated with monoclonal anti-HBs for the detection of HBsAg.

HBsAg assays are routinely used to aid in the diagnosis of suspected HBV infection and to monitor the status of infected individuals, i.e., whether the patient's infection has resolved or the patient has become a chronic carrier of the virus.[14] For the diagnosis of acute or chronic hepatitis, HBsAg reactivity should be correlated with patient history and the presence of other hepatitis B serological markers. Prenatal testing has been recommended by the United States Centers for Disease Control so that newborns from HBV carrier mothers may obtain prophylactic treatment.[15,16]

Samples nonreactive by AxSYM HBsAg are considered negative for HBsAg and need not be tested further. A reactive sample must be retested by AxSYM HBsAg to determine whether it is repeatedly reactive. A sample found to be repeatedly reactive should be confirmed by the AxSYM HBsAg Confirmatory assay, a neutralization procedure utilizing human anti-HBs. If the sample is reactive and neutralized in the AxSYM HBsAg Confirmatory assay, the sample is considered confirmed positive for HBsAg. If the sample is reactive and not neutralized in the AxSYM HBsAg Confirmatory assay, the sample is considered repeat reactive and nonconfirming for HBsAg. It is recommended that confirmatory testing be performed prior to disclosure of the presence or absence of HBsAg.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM HBsAg is based on MEIA technology and utilizes the principle of direct binding of the HBsAg in the sample to anti-HBs coated microparticles and indirect detection of the HBsAg by biotinylated anti-HBs followed by anti-biotin:alkaline phosphatase conjugate. The AxSYM HBsAg reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

Sample and all AxSYM HBsAg reagents required for one test are pipetted by the Sampling Pipettor into various wells of a Reaction Vessel (RV).

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Pipettor.

PROCESSING CENTER

- Sample, Anti-HBs (Mouse, Monoclonal, IgM) Coated Microparticles, and Biotinylated Anti-HBs (Goat, IgG) are combined in one RV well.
- When HBsAg is present in the sample, it binds to the Anti-HBs (Mouse, Monoclonal, IgM) Coated Microparticles and Biotinylated Anti-HBs (Goat, IgG), forming an antibody-antigen-antibody complex in the reaction mixture.
- An aliquot of the reaction mixture is transferred to the Matrix Cell. The microparticles bind irreversibly to the glass fiber matrix.
- The Anti-biotin (Rabbit):Alkaline Phosphatase Conjugate is dispensed onto the Matrix Cell and binds to any microparticle-bound antibody-antigen-antibody complex.
- The Matrix Cell is washed with Matrix Cell Wash to remove materials not bound to the microparticles.
- The substrate, 4-Methylumbelliferyl Phosphate (MUP), is added to the Matrix Cell, and the fluorescent product formed is measured by the MEIA optical assembly.

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

For further information regarding MEIA technology, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS

REAGENT KIT, 100 TESTS

AxSYM HBsAg Reagent Pack (No. 9B01-20)*

- 1 Bottle (15.5 mL) Anti-biotin (Rabbit):Alkaline Phosphatase Conjugate in TRIS buffer with protein (0.497% bovine, 2.42% piscine) stabilizers. Minimum concentration: 0.05 µg/mL. Preservative: 0.1% Sodium Azide. (Reagent Bottle 1)
- 1 Bottle (5.1 mL) Anti-HBs (Mouse, Monoclonal, IgM) Coated Microparticles in sodium phosphate buffer with protein (1.0% bovine) stabilizer. Minimum concentration: 0.2% solids. Preservative: 0.1% Sodium Azide. (Reagent Bottle 2)
- 1 Bottle (17.5 mL) Biotinylated Anti-HBs (Goat, IgG, adsorbed with human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV) in TRIS buffer containing animal sera. Minimum concentration: 1.25 µg/mL. Preservatives: 0.1% Sodium Azide and Antimicrobial Agents. (Reagent Bottle 3)
- 1 Bottle (49.2 mL) Probe Wash Solution prepared in TRIS buffer. Preservative: Antimicrobial Agent. (Reagent Bottle 4)

Index Calibrator

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

- No. 9B01-66 includes the AxSYM HBsAg Reagent Pack and Index Calibrator (100 tests), Reaction Vessels (100 each), and Matrix Cells (100 each).

AxSYM HBsAg Controls (No. 9B01-10) (sold separately)

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

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

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

The AxSYM HBsAg Controls have the following ranges:

Control	Color	HBsAg Concentration (ng/mL)	Control Range (S/CO)
Negative	Natural	0.0	0.20 - 0.80
Positive	Blue[a]	0.6 - 1.0[b]	1.00 - 4.50

[a] Dye: Bromophenol Blue

[b] Concentration (ng/mL) is approximately 0.15 - 0.25 IU/mL.

OTHER REAGENTS (sold separately)

AxSYM Probe Cleaning Solution (No. 9A35-05)

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

Solution 1 (MUP) (No. 8A47-04)

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

Solution 3 (Matrix Cell Wash) (No. 8A81-04)

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

Solution 4 (Line Diluent) (No. 8A46)

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

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

SAFETY PRECAUTIONS

- **CAUTION:** This product contains human sourced infectious 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 or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced material be considered potentially infectious and handled with appropriate biosafety practices. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[17] Biosafety Level 2[18] or other appropriate biosafety practices[19,20] should be used for materials that contain or are suspected of containing infectious agents.

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- This product contains Sodium Azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

HANDLING PRECAUTIONS

- **Do not use Solution 1 (MUP) beyond the expiration date or a maximum of 14 days on board the AxSYM System. When loading new Solution 1 (MUP), it is important to immediately tighten the instrument cap for MUP to minimize exposure to air. Prolonged exposure of MUP to air may compromise performance.**
- Do not use AxSYM HBsAg Reagent Kit beyond the expiration date.
- Do not use AxSYM HBsAg Reagent Pack beyond a maximum of 112 cumulative hours on board the AxSYM System.
- Do not mix reagents from different Reagent Packs. Do not mix reagents and index calibrators from different lots.
- Avoid microbial contamination of specimens and reagents. Use of disposable pipettes or pipette tips is recommended.
- Avoid chemical contamination of reagents and equipment.
- Ensure that sufficient sample volume is present. If sample volume is insufficient, the AxSYM System will give an error code and no result will be reported. For a description of the system error codes, refer to the AxSYM System Operations Manual, Section 10.
- Use caution in handling patient specimens to prevent cross contamination. Transfer of any amount of an HBsAg reactive specimen may contaminate an adjacent nonreactive specimen and cause a falsely reactive result.

Refer to the AxSYM System Operations Manual, Sections 7 and 8, for a more detailed discussion of the safety and handling precautions during system operation.

STORAGE INSTRUCTIONS

Upon receipt, the AxSYM HBsAg Reagent Pack, Index Calibrator, and Controls must be stored at 2-8°C. They may be used immediately after removal from the refrigerator. Index Calibrator and Controls should be returned to 2-8°C storage immediately after use.

Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM HBsAg Reagent Pack may be on board the AxSYM System for a maximum of 112 cumulative hours; for example, 14 eight-hour shifts. After 112 hours, the Reagent Pack and associated Index Calibrator must be discarded. Refer to the AxSYM System Operations Manual, Sections 2 and 5, for further information on tracking onboard time.

Solution 1 (MUP) must be stored at 2-8°C (do not freeze). It may be used immediately after removal from the refrigerator. MUP may be on board the AxSYM System for a maximum of 14 days. After 14 days, it must be discarded.

The AxSYM Probe Cleaning Solution, Solution 3 (Matrix Cell Wash), and Solution 4 (Line Diluent) must be stored at 15-30°C.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When an AxSYM HBsAg Negative or Positive Control value is out of the expected range, it may indicate deterioration of the reagents or errors in technique. The test results of associated specimens are invalid and these specimens must be retested. Assay recalibration may be necessary. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

INSTRUMENT PROCEDURE

NOTE: AxSYM HBsAg must only be used with AxSYM System software version 3.60 or higher.

ASSAY FILE INSTALLATION

The AxSYM HBsAg/Confirmatory Assay Disk, List No. 2K14-01 or higher, contains 4 assay/ratio files: HBsAg_US, HBsAg_CF, NeutUS (Ratio), and NeutDiUS (Ratio). The HBsAg_US assay file must be installed on the AxSYM System from the assay disk prior to performing the AxSYM HBsAg assay. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM HBsAg ASSAY PARAMETERS

Assay parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. Selected assay parameters used for the AxSYM HBsAg assay are listed below.

	Assay Parameters
1	Long Assay Name (English): HBsAg_US
6	Abbrev Assay Name (English): HBsAg_US
11	Assay Number: 107
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Index Cal
45	Selected Result Concentration Units > S/CO
80	Interpretation Option to use > 1

NOTE: Parameters 43, 44, 45, and 80 cannot be edited.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma (collected in potassium EDTA, sodium citrate, sodium heparin, lithium heparin, or plasma separator tubes) may be used with the AxSYM HBsAg assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.
- The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify that the correct sample type is tested with the AxSYM HBsAg assay.
- Specimens from heparinized patients may be incompletely coagulated and inconsistent results could occur due to the presence of fibrin. To prevent partially coagulated specimens, draw the specimen prior to heparin therapy or into a plasma collection tube. Serum collection tubes should not be used with heparinized patients.
- This assay was designed for use with human serum or plasma from individual patient specimens. Pooled specimens must not be used.
- Gravity separation is not sufficient for specimen preparation. Specimens containing clots, red blood cells, or particulate matter may give inconsistent results and must be clarified by centrifugation prior to testing.
- **All patient specimens to be tested in Primary Tubes must be centrifuged to remove red blood cells or particulate matter. Follow the manufacturer's instructions for centrifugation.**
- **Specimens must be transferred to a centrifuge tube and centrifuged at a Relative Centrifugal Force (RCF) of 10,000 x g for 10 minutes if:**
  - **they still contain clots, red blood cells, or particulate matter after being centrifuged according to the collection tube manufacturer's instructions, or**
  - **they require repeat or confirmatory testing, or**
  - **they have been frozen and thawed.**

  Transfer the clarified specimen to an aliquot tube or a sample cup for testing.

  NOTE: AxSYM System Software Version 3.60 and higher offers an Auto Retest/Auto Dilution feature. Due to the centrifugation requirements discussed above, this feature must not be used with this assay.
- Centrifuged specimens with a lipid layer on top of the liquid must be transferred to an aliquot tube or a sample cup. Care must be taken to transfer only the clarified specimen and not the lipemic material.
  - The Clinical Laboratory Standards Institute (CLSI) provides the following recommendations for storing blood specimens[21].
    - Store samples at 22°C (72°F) for no longer than 8 hours.
    - If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C (36-46°F).
    - If the assay will not be completed within 48 hours, freeze at or below -20°C (-4°F).
  - Note: Per manufacturer's recommendations, plasma collected in heparin collection tubes should be stored at room temperature to minimize latent fibrin formation promoted by cold temperatures.[22]
- Specimens that are not tested within the specified time period listed above must be removed from the clot or red blood cells, and stored frozen (-20°C or colder).
- Multiple freeze-thaw cycles should be avoided. Frozen specimens collected in all recommended collection tubes may be subjected to up to 2 freeze/thaw cycles prior to being tested.* Specimens must be mixed **thoroughly** after thawing, by LOW speed vortexing or by gentle inversion, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.
  - * Note: Freeze/thaw cycles 1 and 2 for serum collected in red top glass collection tubes demonstrated up to 18% (95% confidence limit) negative bias on average in a low positive sample (1.2 S/CO target); however, the mean value remained reactive.
- Specimens may be shipped at -20°C or colder (dry ice) or 2-8°C (wet ice) and must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances. For shipment at 2-8°C (wet ice), do not exceed the storage limitations listed above. It is recommended to ship specimens off the clot or red blood cells.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter.
- Specimens with obvious microbial contamination should not be used.
- Do not use heat-inactivated specimens.
- Inspect all samples for bubbles. Remove bubbles prior to testing the sample. Refer to the AxSYM System Operations Manual, Section 7, for detailed instructions on removing bubbles.

- To minimize the effects of evaporation, all samples (patient specimens, controls, and calibrators) should be tested within 3 hours of being placed on board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of onboard sample storage constraints.

SAMPLE VOLUME

The sample volume required to perform a single AxSYM HBsAg test on the AxSYM System varies depending on the type of sample container used. For sample cups, a ROUTINE test and a STAT test each require 194 µL. For every additional AxSYM HBsAg test performed (ROUTINE or STAT) from the same sample container, an additional 144 µL of sample is required.

The sample cup minimum volume for both ROUTINE and STAT tests is calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is(are) ordered. The sample cup STAT minimum volume is printed on the Orderlist Report. When using Host Order Query, the Order screen information and Orderlist Report are not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query Option.

To obtain the recommended volume requirements for the AxSYM HBsAg Index Calibrator and Controls, hold the bottles **vertically** and dispense 15 drops of Index Calibrator or 5 drops (per replicate) of each control into each respective sample cup.

For sample volume requirements in primary or aliquot tubes, and calibrator/control volume requirements for multiple AxSYM HBsAg reagent lots, refer to the AxSYM System Operations Manual, Section 5.

AxSYM HBsAg PROCEDURE

MATERIALS PROVIDED

- No. 9B01-66 — AxSYM HBsAg Reagent Kit, containing:
  - AxSYM HBsAg Reagent Pack
  - AxSYM HBsAg Index Calibrator
  - 100 Reaction Vessels (RV)
  - 100 Matrix Cells

MATERIALS REQUIRED BUT NOT PROVIDED

- No. 9B01-10 — AxSYM HBsAg Controls
- No. 8A47-04 — Solution 1 (MUP)
- No. 8A81-04 — Solution 3 (Matrix Cell Wash)
- No. 8A46 — Solution 4 (Line Diluent)
- No. 9A35-05 — AxSYM Probe Cleaning Solution
- No. 8A76-01 — Sample Cups
- Pipettes or pipette tips (optional) to deliver the volumes specified on the Order screen

CAUTION:

- Mix the AxSYM HBsAg Index Calibrator and Controls by gentle inversion prior to use.
- When manually dispensing samples into sample cups, verify that dispensing equipment does not introduce cross contamination and delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

ASSAY PROCEDURE

CAUTION: The System status must be WARMING, PAUSED, READY, or STOPPED before adding or removing sample segments, Reagent Packs, or Reaction Vessels.

NOTE: The AxSYM System Auto Retest/Auto Dilution feature must **not** be used for this assay. Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert.

1. Check for sufficient onboard inventory of Matrix Cells and bulk solutions, and sample segment availability.
2. Check for sufficient waste collection capacity.
   CAUTION: Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in progress. If opened, all processing will stop. All tests will be terminated and must be repeated.
3. Order the AxSYM HBsAg Index Calibrator, AxSYM HBsAg Controls, and/or patient specimens as required. Assign or modify sample segment position (S/P) for each sample, as necessary. Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for calibration and control requirements.

   Index Calibration

   Perform AxSYM HBsAg calibration by testing 5 replicates of the Index Calibrator. Invert gently to mix and dispense at least 15 drops of the Index Calibrator into a sample cup. Do not simultaneously calibrate more than one AxSYM HBsAg reagent lot.

   Controls

   Perform quality control by testing the Negative and Positive Controls (one test each). Invert gently to mix and dispense at least 5* drops each of the Negative and Positive Controls into individual sample cups.

   * When more than one AxSYM HBsAg reagent lot is on board the AxSYM System, multiply the control volume by the number of lots.

   Patient Specimens

   Ensure that sufficient volume is present in the sample cups or tubes. The sample cup minimum volume is 194 µL for the first AxSYM HBsAg test plus 144 µL for each additional AxSYM HBsAg test. For volume requirements in Primary or Aliquot Tubes, refer to the AxSYM System Operations Manual, Section 5.

NOTE: The operator may obtain an Orderlist Report by accessing the Orderlist screen and pressing PRINT. The printout contains sample placement information and minimum STAT sample cup volume requirements for all tests ordered. When using the Host Order Query, the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5: Ordering Patient Samples, for a description of the Host Query Option.

4. Place sample segments containing the ordered samples into the Sample Carousel.
5. Open Reagent Bottle 4 containing the Probe Wash Solution. Place the AxSYM HBsAg Reagent Pack into the Reagent Pack Carousel.
   NOTE: The cap for Reagent Bottle 4 must be manually opened prior to running an AxSYM HBsAg assay. Upon completion of the run, close the Reagent Bottle 4 cap securely.
6. Ensure that RVs are present on the RV Carousel. Additional RVs may be added as needed.
7. Press RUN. All entries on the Orderlist screen are transferred to the Order Status screen for sample processing.
8. Review the results to determine whether retesting is required.
9. When testing is completed, close Reagent Bottle 4 and remove the samples and the AxSYM HBsAg Reagent Pack from the Sampling Center. Store reagent pack at 2-8°C.

NOTE: When using the onboard reagent tracking feature, the operator must perform a reagent pack scan after removing any pack from the system in order to maintain the validity of the reagent pack stability time.

Sections 5 and 6 of the AxSYM System Operations Manual contain detailed steps for performing assay calibration and sample testing procedures.

QUALITY CONTROL PROCEDURES

CALIBRATION

A minimum of five replicates of the AxSYM HBsAg Index Calibrator must be tested for an AxSYM HBsAg calibration. A single sample of both the Negative and Positive Controls must be tested as a means of evaluating the assay calibration. Once the AxSYM HBsAg calibration is accepted and stored, all subsequent samples may be tested without further calibration unless one or more of the following occur:

- A reagent pack with a new lot number is used.
- Either of the AxSYM HBsAg Control values is out of its specified range.
- The MEIA Optics Verification Update has been performed.

Refer to the AxSYM System Operations Manual, Section 6, for further information on:

- Setting up an assay calibration
- Determining when recalibration may be necessary
- Calibration verification

The operator must verify that the AxSYM HBsAg Control values are within the ranges specified in this package insert. Refer to the **REAGENTS**, CONTROLS section of this package insert for AxSYM HBsAg Control ranges. If a control value is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendix E, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

QUALITY CONTROL

The performance of the Abbott AxSYM HBsAg Controls has not been established with any other HBsAg assays.

The AxSYM HBsAg controls are in a serum matrix made from recalcified plasma. The user should provide alternate control material for plasma when necessary.

Good laboratory practice recommends the use of positive and negative controls to assure functionality of reagents and proper performance of the assay procedure. Positive control and negative control are intended to monitor for substantial reagent failure. Quality Control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. It is recommended that the user refer to CLSI document C24-A2, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions:* [Approved Guideline – Second Edition][23] or other published guidelines for general quality control recommendations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1202(c)[24].

The minimum control requirement for an AxSYM HBsAg assay is a single sample of each of the Negative and Positive Controls tested once every 24 hours, each day of use for each reagent lot. Controls may be placed in any position in the Sample Carousel.

Additional controls may be tested in conformance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control procedures.

The operator must verify that the AxSYM HBsAg Control values are within the ranges specified in this package insert. Refer to the **REAGENTS**, CONTROLS section of this package insert for AxSYM HBsAg Control ranges. If a control value is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Deterioration of the reagents or errors in technique may be indicated when an AxSYM HBsAg Positive or Negative Control value is out of the expected range. If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. Retest patient specimens before reporting results for this run. Recalibration may be indicated. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

FLUORESCENCE BACKGROUND ACCEPTANCE CRITERIA

Quality control with regard to the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64, Max Intercept-Max MUP intercept, each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the result is invalid. The test request will be moved to the Exceptions List where it will appear with the message "1064 Invalid test result, intercept too high" and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10, when this error message is obtained.

Refer to the AxSYM System Operations Manual, Section 2, for further information on this parameter.

RESULTS

CALCULATION

The AxSYM HBsAg assay protocol calculates the Cutoff Rate (CO) using the mean rate of five Index Calibrator replicates, multiplied by a factor of 2, and stores the result.

Cutoff Rate (CO) = Index Calibrator Mean Rate × 2

The AxSYM HBsAg assay protocol calculates a result based on the ratio of the sample rate (S) to the stored cutoff rate (CO) for each sample and control.

S/CO = Sample Rate (S) / Cutoff Rate (CO)

FLAGS

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Sections 1 and 2.

INTERPRETATION OF RESULTS

Initial AxSYM HBsAg Results

Initial Result (S/CO)	Instrument Interpretation	Retest Procedure
≥ 1.00	REACTIVE	Recentrifuge; Retest in Duplicate[a]
< 1.00	NONREACTIVE	No Retest Required

[a] Initially reactive specimens must first be recentrifuged according to directions in the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert and then retested in duplicate.

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

Final AxSYM HBsAg Results

AxSYM HBsAg			
Initial Interpretation	Retest Results	Final Result	Interpretation
Initially Reactive	One or both of the duplicate retests are reactive.	Repeatedly Reactive	Presumptive evidence of HBsAg; an AxSYM HBsAg Confirmatory test should be performed prior to disclosure of the presence of HBsAg.
	Both of the duplicate retests are nonreactive.	Negative	HBsAg not detected; does not exclude the possibility of exposure to or early acute infection with HBV.
Nonreactive	No Retest Required		

False reactive results may be obtained with any diagnostic test. Two types of false reactive results may occur with AxSYM HBsAg: nonrepeatable reactives and nonspecific reactives.

Nonrepeatable Reactives: Some samples that are initially reactive in the AxSYM HBsAg assay may not be repeatedly reactive upon retesting. The most common reasons for nonrepeatable reactives are:

- Particulate matter in the patient sample, particularly fibrin clots and cellular material.
- Contamination of nonreactive samples caused by transfer of a high titer antigen sample.[25]

Nonspecific Reactives: All highly sensitive immunoassay systems have a potential for nonspecific reactions. The specificity of a repeatedly reactive sample should be confirmed by the AxSYM HBsAg Confirmatory assay. A nonspecific reactive sample will be repeatedly reactive but will not confirm by neutralization. Therefore, it is recommended that this specific antibody neutralization procedure be performed before disclosing HBsAg status to the patient. For additional information on neutralization testing, refer to the AxSYM HBsAg Confirmatory assay package insert.

LIMITATIONS OF THE PROCEDURE

- **WARNING: Not intended for use in screening blood, plasma, or tissue donors.** The effectiveness of AxSYM HBsAg for use in screening blood, plasma, or tissue donors has not been established.
- Current methods for the detection of HBsAg may not detect all potentially infected individuals. A nonreactive test result does not exclude the possibility of exposure to or early acute infection with hepatitis B virus. Nonreactive test results in individuals with prior exposure to HBsAg may be due to antigen levels below the detection limit of this assay.
- For diagnostic purposes, HBsAg reactivity should be correlated with patient history and the presence of other hepatitis markers. Reactive results do not discriminate between acute or chronic HBV infections.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[26] Such specimens may exhibit either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.[27] These specimens should not be tested with the AxSYM HBsAg assay.
- Samples with HBsAg mutations within amino acids 121-124 may be nonreactive by AxSYM HBsAg. If acute or chronic HBV infection is suspected, and the AxSYM HBsAg result is nonreactive, it is recommended that other HBV serological markers be tested to confirm the HBsAg nonreactivity.
- No high dose hook effect was observed in the HBsAg assay up to approximately 3,800 IU/mL HBsAg. When the high dose hook effect was observed, samples containing up to approximately 3,800,000 IU/mL of HBsAg remained reactive, i.e., no qualitative change in assay results occurred.

5

EXPECTED VALUES

Due to geographic locations or demographics, assay results obtained in individual laboratories may vary from data presented.

Of the prospective subjects participating in the clinical investigation 45.93% (1,314/2,861) were from individuals at increased risk of HBV infection. All subjects were at risk of HBV infection due to lifestyle, behavior, occupation, or known exposure event but were asymptomatic and reported no current signs or symptoms of hepatitis. The population was 47.56% Caucasian, 36.30% African American, 12.71% Hispanic, 1.45% Asian, and 0.46% American Indian/Alaska Native with the remaining 1.52% represented by other ethnic groups. The population was 62.10% female and 37.90% male ranging in age from 18 to 75 years. AxSYM HBsAg was reactive in 1.67% of the individuals in this population. Table 1 is a summary of the percent of individuals at increased risk of HBV infection enrolled at each location and the percent of AxSYM HBsAg reactive results observed from each location. Table 2 is a summary of the percent AxSYM HBsAg reactive results by age range and gender.

Table 1
AxSYM HBsAg Reactive Results by Specimen Collection Site or Specimen Vendor for Individuals at Increased Risk of HBV Infection

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

Table 2
AxSYM HBsAg Results by Age Range and Gender for Individuals at Increased Risk of HBV Infection

		AxSYM HBsAg Result		
Age Range	Gender	+ Number of Specimens	- Number of Specimens	Total
10 to 19	Female	0	14	14
	Male	0	11	11
20 to 29	Female	1	183	184
	Male	0	97	97
30 to 39	Female	0	184	184
	Male	5	102	107
40 to 49	Female	5	246	251
	Male	3	156	159
50 to 59	Female	1	136	137
	Male	6	103	109
60 to 69	Female	0	35	35
	Male	1	11	12
70 to 79	Female	0	8	8
	Male	0	3	3
Unknown[a]	Female	0	3	3
	Total	**22 (1.67%)**	**1,292 (98.33%)**	**1,314**

[a] Age was not provided for three subjects.

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from data presented.

PRECISION

System Reproducibility

A five-day precision study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) documents EP15-A2[28]. Testing was conducted at three clinical testing sites using three AxSYM HBsAg reagent lots and three control lots per site. Testing included two precision runs per day (a minimum of two hours apart) for each reagent lot, on each of five days. Each precision run included four replicates of each of the three panel members and the AxSYM HBsAg Negative Control and Positive Control. Panel members were prepared by adding purified HBsAg to nonreactive human serum. The data are summarized in Table 3.

Table 3
AxSYM HBsAg System Reproducibility-Three Reagent Master Lots, Three Clinical Testing Sites

Sample	Total No. Reps	Grand Mean S/CO	Within-Run		Within-Day		Within-Laboratory Precision (Total)			Precision with Additional Component of Between-Lot		Precision with Additional Component of Between-Site		Precision with Additional Components of Site and Lot (Overall)	
			SD	%CV	SD	%CV	SD	%CV	CL	SD	%CV	SD	%CV	SD	%CV
Panel 1	360	0.76	0.039	5.2	0.041	5.4	0.043	5.6	6.0	0.065	8.5	0.065	8.5	0.065	8.5
Panel 2	360	1.24	0.047	3.8	0.052	4.2	0.054	4.3	4.7	0.094	7.6	0.090	7.2	0.094	7.6
Panel 3	360	3.55	0.197	5.5	0.207	5.8	0.213	6.0	6.4	0.342	9.6	0.317	8.9	0.342	9.6
NC	360	0.51	0.032	6.3	0.034	6.7	0.037	7.2	7.8	0.048	9.4	0.048	9.4	0.048	9.4
PC	360	2.50	0.089	3.6	0.100	4.0	0.105	4.2	4.5	0.211	8.4	0.196	7.8	0.211	8.4

NC = Negative Control, PC = Positive Control, Reps = Replicates, SD = Standard Deviation, CV = Coefficient of Variation, CL = Upper One-sided 95% Confidence Limit

Within Laboratory Precision

A 20-day precision study was conducted based on guidance from CLSI EP5-A2[29]. Testing was conducted at Abbott Laboratories using two AxSYM HBsAg reagent lots, one control lot, and two AxSYM instruments. Testing included two precision runs per day (a minimum of two hours apart) for each reagent lot, on each instrument, on each of 20 days. Each precision run included two replicates of each of the six HBsAg subtype *ad* or *ay* panel members and the AxSYM HBsAg Negative Control and Positive Control. Panel members were prepared by adding purified HBsAg to recalcified nonreactive human plasma. The data are summarized in Table 4.

Table 4
AxSYM HBsAg Within Laboratory Precision

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

CLINICAL PERFORMANCE

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

AxSYM HBsAg assay was further evaluated by testing a total of 117 acute and chronic subjects, which included prospectively-collected specimens from 6 individuals diagnosed with acute HBV infection, 15 seroconversion panel members classified as acute by four-marker HBV reference testing, 53 specimens prospectively-collected in Vietnam and classified as chronic by four-marker HBV reference testing and 43 specimens prospectively-collected in the U.S. from individuals classified as chronic defined by the presence of HBsAg for ≥ 6 months. (Table 8).

The HBV classification for each subject was determined by a serological assessment using an HBV reference marker pattern consisting of four FDA-approved reference assays for the detection of HBsAg, anti-HBc IgM, total anti-HBc, and anti-HBs. All reference assays used were from a single manufacturer. Testing of these specimens occurred at clinical testing sites located in Port Jefferson, NY (44.03%); Dallas, TX (12.88%); and Raritan, NJ (42.39%), and at Abbott Laboratories, IL (0.70%).

The specimens were assigned an HBV classification (Table 5), and the AxSYM HBsAg results were compared to the reference HBsAg confirmed results (Table 6). Agreement of the AxSYM HBsAg assay for the increased risk and signs and symptoms populations was assessed relative to the reference HBsAg confirmed results (Table 7). Agreement of the AxSYM HBsAg assay for the individuals with acute and chronic infection was assessed relative to the reference HBsAg confirmed results (Table 8).

Results of HBV Classification

Specimens were assigned an HBV classification using the positive (+) and negative (–) patterns for the four HBV reference markers. Table 5 is a summary of how these classifications were derived and the number of specimens in each classification. There were 16 unique HBV reference marker patterns observed in the AxSYM HBsAg clinical investigation.

Table 5
HBV Classification for Individuals at Increased Risk of HBV Infection, Individuals With Signs and Symptoms of Hepatitis Infection and Individuals with Acute and Chronic HBV Infection

HBV Reference Markers					HBV Classification
Number of Specimens	HBsAg	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
17	+	-	-	-	Early Acute
11	+	+	+	-	Acute
1	+	+	+	I	Chronic
3	+	-	+	+	Chronic
85	+	-	+	-	Chronic
2	+	-	-	+	Chronic
4	+	-	+	I	Chronic
43	Presence of HBsAg ≥ 6 months				Chronic
1	+	+	+	+	Late Acute/Recovering
4	-	+	+	+	Recovering Acute
3	-	+	+	I	Early Recovery
193	-	-	+	+	Immune Due to Natural Infection
31	-	-	+	I	Distantly Immune/Anti-HBs Unknown
107	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
508	-	-	-	+	Immune Due to HBV Vaccination
66	-	-	-	I	Unknown
1,056	-	-	-	-	Susceptible
2,135					Total

I = Indeterminate

Comparison of Results

Table 6 is a comparison of the AxSYM HBsAg results to the reference HBsAg assay results by HBV classification.

Table 6
Comparison of AxSYM HBsAg Results With Reference HBsAg Results by HBV Classification
Individuals at Increased Risk of HBV Infection, Individuals With Signs and Symptoms of Hepatitis Infection and Individuals with Acute and Chronic HBV Infection

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

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

[b] Includes retesting of initial reactives, with the exception of the 15 well-characterized seroconversion panel members.

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

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

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

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

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

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

Percent Agreement

Table 7 is a summary, for each HBV classification, of the percent agreement between AxSYM HBsAg and the reference HBsAg assay for Individuals at Increased Risk of HBV Infection and Individuals With Signs and Symptoms of Hepatitis Infection.

Table 7
Percent Agreement Between AxSYM HBsAg Results and Reference HBsAg Results Summarized by HBV Classification
Individuals at Increased Risk of HBV Infection and Individuals With Signs and Symptoms of Hepatitis Infection

HBV Classification	Positive Percent Agreement (%)	95% Confidence Interval (%)	Negative Percent Agreement (%)	95% Confidence Interval (%)
Early Acute	2/2 (100.00)	[15.81, 100.00]		
Acute	5/5 (100.00)	[47.82, 100.00]		
Chronic	41/42 (97.62)	[87.43, 99.54]		
Late Acute/Recovering	1/1 (100.00)	[2.50, 100.00]		
Recovering Acute			4/4 (100.00)	[39.76, 100.00]
Early Recovery			3/3 (100.00)	[29.24, 100.00]
Immune Due to Natural Infection			190/193 (98.45)	[95.52, 99.68]
Distantly Immune/Anti-HBs Unknown			31/31 (100.00)	[88.78, 100.00]
Distantly Immune/Anti-HBs Not Detected			104/107 (97.20)	[92.02, 99.42]
Immune Due to HBV Vaccination			507/508 (99.80)	[98.91, 100.00]
Unknown			66/66 (100.00)	[94.59, 100.00]
Susceptible			1,053/1,056 (99.72)	[99.17, 99.94]
Overall	49/50 (98.00)	[89.35, 99.95]	1,956/1,966 (99.49)	[99.07, 99.76]

Percent Agreement for Individuals With Acute or Chronic HBV Infection

Table 8 is a summary of the percent agreement between AxSYM HBsAg and the reference HBsAg assay for Individuals With Acute or Chronic HBV Infection.

Table 8
Percent Agreement Between AxSYM HBsAg and Reference HBsAg Results
Individuals With Acute or Chronic HBV Infection

Specimen Category	Positive Percent Agreement (%)	95% Confidence Interval (%)	Negative Percent Agreement (%)	95% Confidence Interval (%)
Individuals With Acute HBV Infection[a]	21/21 (100.00)	[83.89, 100.00]	N/A	N/A
Individuals With Chronic HBV Infection[b]	94/94 (100.00)	[96.15, 100.00]	1/2 (50.00)	[1.26, 98.74]

[a] Includes 6 diagnosed acute and 15 members of seroconversion panels with HBV acute classification by four-marker reference testing.

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

Clinical Performance in Pregnant Females

The performance of AxSYM HBsAg in detecting HBV infection in pregnant females was evaluated by testing prospectively-collected serum specimens from pregnant females at low risk or increased risk of HBV infection. A total of 741 specimens were obtained from specimen vendors. Table 9 is a demographic summary of the population.

Table 9
Demographic Summary of Pregnant Females at Low or Increased Risk of HBV Infection

	Low Risk Number of Specimens (%)	Increased Risk Number of Specimens (%)	Total Number of Specimens (%)
Trimester			
First	24 (4.38)	7 (3.63)	31 (4.18)
Second	261 (47.63)	74 (38.34)	335 (45.21)
Third	263 (47.99)	112 (58.03)	375 (50.61)
Ethnicity			
Caucasian	10 (1.82)	41 (21.24)	51 (6.88)
African American	52 (9.49)	24 (12.44)	76 (10.26)
Hispanic	468 (85.40)	120 (62.18)	588 (79.35)
Asian	16 (2.92)	0 (0.00)	16 (2.16)
American Indian/Alaska Native	0 (0.00)	2 (1.04)	2 (0.27)
Other	2 (0.36)	6 (3.11)	8 (1.08)
Age Range			
16 to 31	323 (58.94)	159 (82.38)	482 (65.05)
32 to 45	225 (41.06)	34 (17.62)	259 (34.95)
Total	**548 (73.95)**	**193 (26.05)**	**741 (100.00)**

Percent Agreement for Pregnant Females by Risk and Trimester

Tables 10 and 11 are a comparison of AxSYM HBsAg results and reference HBsAg results by trimester for low risk and increased risk pregnant females.

Table 10
Comparison of AxSYM HBsAg Results With Reference HBsAg Results by Trimester for Low Risk Pregnant Females

	First Trimester			Second Trimester			Third Trimester		
AxSYM HBsAg Result[a]	Reference HBsAg Result[b]			Reference HBsAg Result[b]			Reference HBsAg Result[b]		
	+	-	Total	+	-	Total	+	-	Total
+	0	0	0	1	0	1	0	0	0
-	0	24	24	0	260	260	0	263	263
Total	**0**	**24**	**24**	**1**	**260**	**261**	**0**	**263**	**263**

[a] Includes retesting of initial reactives.

[b] Includes retesting and confirmatory testing performed according to the package insert.

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Table 11
Comparison of AxSYM HBsAg Results with Reference HBsAg Results by Trimester for Increased Risk Pregnant Females

AxSYM HBsAg Result[a]	First Trimester			Second Trimester			Third Trimester		
	Reference HBsAg Result[b]			Reference HBsAg Result[b]			Reference HBsAg Result[b]		
	+	-	Total	+	-	Total	+	-	Total
+	0	0	0	0	0	0	1	0	1
-	0	7	7	0	74	74	0	111	111
Total	0	7	7	0	74	74	1	111	112

[a] Includes retesting of initial reactives.

[b] Includes retesting and confirmatory testing performed according to the package insert.

Overall Summary and Percent Agreement for Pregnant Females

Table 12 is a comparison of AxSYM HBsAg results and reference HBsAg results for 741 pregnant females at low risk or increased risk of HBV infection.

Table 12
Comparison of AxSYM HBsAg Results With Reference HBsAg Results in Pregnant Females

AxSYM HBsAg Result[a]	Reference HBsAg Result[b]		Total Number of Specimens (%)
	+ Number of Specimens (%)	- Number of Specimens (%)	
+	2 (100.00)	0 (0.00)	2 (0.27)
-	0 (0.00)	739 (100.00)	739 (99.73)
Total	2 (100.00)	739 (100.00)	741 (100.00)

[a] Includes retesting of initial reactives.

[b] Includes retesting and confirmatory testing performed according to the package insert.

Table 13 is the overall positive and negative percent agreement for pregnant females between AxSYM HBsAg and the reference HBsAg assay.

Table 13
Percent Agreement Between AxSYM HBsAg and Reference HBsAg in Pregnant Females

Specimen Category	Positive Percent Agreement (%)	95% Confidence Interval (%)	Negative Percent Agreement (%)	95% Confidence Interval (%)
Pregnant Females	2/2 (100.00)	[15.81, 100.00]	739/739 (100.00)	[99.50, 100.00]

Clinical Performance in a Pediatric Population

The performance of AxSYM HBsAg in a pediatric population was evaluated by testing specimens from a surplus pediatric population. A total of 100 specimens were collected in Fall River, MA by a specimen vendor. Table 14 is a demographic summary of the population by age range and gender with AxSYM HBsAg results.

Table 14
Demographic Summary With AxSYM HBsAg Results for the Pediatric Population

Age Range	Gender	AxSYM HBsAg Result		
		+ Number of Specimens	- Number of Specimens (%)	Total
> 2 to 12 Years	Female	0	21 (100.00)	21
	Male	0	29 (100.00)	29
> 12 to 18 Years	Female	0	36 (100.00)	36
	Male	0	14 (100.00)	14
	Total	0	100 (100.00)	100

Table 15 is the overall negative percent agreement for the pediatric population between AxSYM HBsAg and the reference HBsAg assay.

Table 15
Percent Agreement Between AxSYM HBsAg and Reference HBsAg for the Pediatric Population

Specimen Category	Negative Percent Agreement (%)	95% Confidence Interval (%)
Pediatric Specimens	100/100 (100.00)	[96.38, 100.00]

ANALYTICAL SPECIFICITY

A study was conducted to evaluate the potential for cross-reactivity in the AxSYM HBsAg assay when used to test specimens from individuals with medical conditions unrelated to HBV infection. A total of 229 specimens from 16 different categories were tested. The first 13 of 16 categories were antibody, antigen, or PCR positive. Two hundred twenty four specimens were nonreactive (97.8%) and 5 specimens were reactive (2.2%) by AxSYM HBsAg. One of the 5 reactive specimens was confirmed positive for HBsAg by AxSYM HBsAg Confirmatory. The data are summarized in Table 16.

Table 16
Cross-reactivity of AxSYM HBsAg in Specimens from Individuals with Medical Conditions Unrelated to HBV

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

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

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

Interference

At the concentrations listed below, bilirubin (unconjugated), hemoglobin, total protein, and triglycerides showed ≤ 0.1 S/CO interference in the AxSYM HBsAg assay for high negative samples (0.8 S/CO target), and ≤ 10% interference for low positive samples (1.2 S/CO target):

- Bilirubin ≤ 20 mg/dL
- Hemoglobin ≤ 500 mg/dL
- Total Protein ≤ 12 g/dL
- Triglycerides ≤ 3,000 mg/dL

In addition, high negative (0.8 S/CO target) and low positive (1.2 S/CO target) serum samples were spiked with bacteria (*Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*) and viral or parasitic antigens (cytomegalovirus, Epstein-Barr virus, herpes simplex virus, rubella, *Toxoplasma gondii*, and varicella-zoster virus). The bacteria were spiked to 10^{2-3}, 10^{3-4}, and 10^{5-6} colony-forming units per mL (CFU/mL). The viral or parasitic antigens were spiked to 1 µg/mL and 1 ng/mL. All samples were tested in replicates of 22. All replicates of the high negative samples (0.8 S/CO target) remained nonreactive and all replicates of the low positive samples (1.2 S/CO target) remained reactive.

Tube Type Matrix Comparison

The following tube types are acceptable for use with the AxSYM HBsAg assay:

- Glass: serum and serum separator
- Plastic: serum, serum separator, plasma separator, potassium EDTA, sodium citrate, sodium heparin and lithium heparin

On average, the lower one-sided 95% confidence interval of %bias for 25 low positive samples (1.2 S/CO target) was less than 11% compared to the control sample type (serum in glass).

Table 17
Sample Types (Serum and Plasma) Study of AxSYM HBsAg % Bias by Sample Type

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

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

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NEONATES

A study was performed to evaluate the performance of the AxSYM HBsAg when used to test neonatal serum (cord blood). Twenty cord blood specimens from babies born to apparently healthy women at low risk of exposure to HBV were obtained. Thirteen of the specimens were spiked with HBsAg to create low positive samples (1.2 S/CO target). The data are summarized in Table 18.

Table 18
Evaluation of Neonatal (Cord Blood) Specimens by AxSYM HBsAg/AxSYM HBsAg Confirmatory

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

HBsAg MUTANT DETECTION

The hepatitis B virus, unlike other DNA viruses, replicates through reverse transcription. The reverse transcription process lacks proofreading capability; therefore, HBV is subject to a mutation rate 10 times higher than the mutation rate of other DNA viruses.[30] Some of these mutations may cause changes in the antigenic structure of HBsAg, resulting in epitopes that are no longer recognized by anti-HBs. HBsAg mutants have been reported in a wide range of patient populations, including blood donors, vaccine recipients, renal dialysis patients, orthotopic liver transplant recipients, infants born to HBsAg positive mothers, and patients undergoing nucleoside analog treatment for HBV.[30-37] HBsAg mutations may result in a less favorable outcome in some patients[30,31,33] and false negative results in some HBsAg assays.[30-32]

The immunodominant "a" determinant portion of the HBsAg protein spans the region bound by amino acids 100–158.[38] This region includes at least two antigenic loops; the second loop (amino acids 139–147) binds a large proportion of anti-HBs in immune serum.[30,33] Immunological pressure by anti-HBs, whether induced by natural infection, vaccination, or therapeutic administration, may be a method by which HBsAg mutants are selected.[30-33] The most frequent and stable mutation reported is the glycine to arginine mutation at amino acid position 145 in the second loop of the "a" determinant.[32]

A panel of 27 recombinant HBsAg mutant samples and one wild type control sample were prepared as described by Coleman et al.[39] Twenty-five of the 27 samples spanned the "a" determinant region. Each sample was prepared to a concentration of approximately 1 ng/mL and tested by AxSYM HBsAg. Three of the 27 samples were nonreactive by AxSYM HBsAg; these three samples contained antigen with various mutations at amino acid position 123. All of the remaining 24 samples were reactive by AxSYM HBsAg. These reactive samples included seven samples with mutations surrounding the amino acid 123 site (amino acids 115-120 and 126-133) and ten samples with mutations in the second loop (amino acids 139-147). These results are consistent with those reported in the literature.[39,40] The data are summarized in Table 19.

Table 19
Detection of Recombinant HBsAg Mutants by AxSYM HBsAg/AxSYM HBsAg Confirmatory

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

HBsAg GENOTYPE DETECTION

The binding epitope for AxSYM HBsAg is conserved across all known genotypes of HBsAg.[38,41,42] This was verified by testing a commercially-available genotype panel containing genotypes A through G. All genotypes were detected by AxSYM HBsAg and AxSYM HBsAg Confirmatory. The data are summarized in Table 20.

Table 20
AxSYM HBsAg
HBsAg Genotype Detectability Study

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

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

ANALYTICAL SENSITIVITY

The sensitivity of AxSYM HBsAg was evaluated using a 16-member panel composed of eight HBsAg subtype *ad* members and eight HBsAg subtype *ay* members, and the AxSYM HBsAg Index Calibrator as a nonreactive sample. The panel and Index Calibrator were tested in replicates of five with each of three AxSYM HBsAg reagent lots for a total of 24 runs. The HBsAg concentration at the assay cutoff (sensitivity) was estimated using a linear regression analysis. The expected sensitivity of the AxSYM HBsAg assay is less than or equal to 0.6 ng/mL through expiration. The data are summarized in Table 21.

Table 21
Analytical Sensitivity (ng/mL) of AxSYM HBsAg

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

In addition, the sensitivity of AxSYM HBsAg was evaluated using serial dilutions of the World Health Organization (WHO) International HBsAg Standard. The dilutions ranged from 0.020 to 0.625 International Units (IU)/mL. Recalcified nonreactive human plasma was used as the diluent and represented the 0 IU/mL sample. The samples were tested in replicates of five with each of three AxSYM HBsAg reagent lots for a total of 23 runs. The HBsAg concentration at the assay cutoff (sensitivity) was estimated using a linear regression analysis. The data are summarized in Table 22.

Table 22
Analytical Sensitivity (IU/mL) of AxSYM HBsAg

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

SEROCONVERSION DETECTION

The ability of the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays to detect HBsAg was evaluated by testing 15 seroconversion panels obtained from three commercial vendors. The results were compared to the results of an FDA-licensed HBsAg assay (reference). HBsAg was detected by AxSYM HBsAg and confirmed positive by AxSYM HBsAg Confirmatory 3 to 7 days earlier than the reference HBsAg assay in five panels, and coincident with the reference HBsAg assay in ten panels. The data are summarized in Table 23.

Table 23
Seroconversion Detection by AxSYM HBsAg/AxSYM HBsAg Confirmatory

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

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June, 2006

Abbott
AXSYM® SYSTEM

HBsAg Confirmatory

Customer Service
United States: 1-877-4ABBOTT

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

CAUTION:
United States Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

ABBOTT LABORATORIES
Diagnostics Division
Abbott Park, IL 60064 USA

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NAME

AxSYM® HBsAg Confirmatory

INTENDED USE

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

WARNING: Not intended for use in screening blood, plasma, or tissue donors. The effectiveness of AxSYM HBsAg Confirmatory for use in screening blood, plasma, or tissue donors has not been established.

SUMMARY AND EXPLANATION OF THE TEST

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

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

AxSYM HBsAg Confirmatory differs from AxSYM HBsAg in that the sample is automatically treated with the AxSYM HBsAg Confirmatory Reagent A (anti-HBs [human]) or Reagent B (recalcified plasma [human], nonreactive for anti-HBs). If HBsAg is present in the sample, it will be bound by the antibody in Reagent A. The neutralized HBsAg is blocked from binding to the anti-HBs coated microparticles in the AxSYM HBsAg assay.

The assay principle involves two steps: treatment of the sample and HBsAg testing. The AxSYM HBsAg Confirmatory reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- All samples are tested undiluted and with an automated 1:500 dilution procedure. The dilution is performed by the AxSYM System with Dilution Reagent.
- Sample and all AxSYM HBsAg Confirmatory and AxSYM HBsAg reagents required for one test are pipetted by the Sampling Pipettor into various wells of a Reaction Vessel (RV).
- The sample and its 1:500 dilution are each pipetted into two RVs. For each sample and dilution, Reagent A is added to one RV and Reagent B is added to the other.

The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Pipettor.

PROCESSING CENTER

- When HBsAg is present in the sample, it is bound (neutralized) by the antibody in Reagent A.
- Anti-HBs (Mouse, Monoclonal, IgM) Coated Microparticles and Biotinylated Anti-HBs (Goat, IgG) solution are added to the reaction mixture.
- Any nonneutralized HBsAg in the sample simultaneously binds to the Anti-HBs (Mouse, Monoclonal, IgM) Coated Microparticles and Biotinylated Anti-HBs (Goat, IgG), forming an antibody-antigen-antibody complex in the reaction mixture.
- An aliquot of the reaction mixture is transferred to the Matrix Cell. The microparticles bind irreversibly to the glass fiber matrix.
- The Anti-biotin (Rabbit):Alkaline Phosphatase Conjugate is dispensed onto the Matrix Cell and binds with any microparticle-bound antibody-antigen-antibody complex.
- The Matrix Cell is washed with Matrix Cell Wash to remove materials not bound to the microparticles.
- The substrate, 4-Methylumbelliferyl Phosphate (MUP), is added to the Matrix Cell, and the fluorescent product formed is measured by the MEIA optical assembly.

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

For further information regarding MEIA technology, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS

CONFIRMATORY REAGENT KIT, 30 TESTS

AxSYM HBsAg Confirmatory Reagent Kit (No. 9B01-60)

- 1 Bottle (1 mL) Reagent A. Antibody to Hepatitis B Surface Antigen (Human) nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV. Preservative: 0.1% Sodium Azide. Dye: Violet (FD&C Red No. 33 and Acid Blue No. 9).
- 1 Bottle (1.5 mL) Reagent B. Recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs. Preservative: 0.1% Sodium Azide. Dye: Yellow (Acid Yellow No. 23).
- 1 Bottle (18 mL) Dilution Reagent. Recalcified human plasma nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs. Preservative: 0.1% Sodium Azide.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

SAFETY PRECAUTIONS

- **CAUTION:** This product contains human sourced infectious 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 or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced material be considered potentially infectious and handled with appropriate biosafety practices. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[1] Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

HANDLING PRECAUTIONS

- **Do not use Solution 1 (MUP) beyond the expiration date or a maximum of 14 days on board the AxSYM System. When loading new Solution 1 (MUP), it is important to immediately tighten the instrument cap for MUP to minimize exposure to air. Prolonged exposure of MUP to air may compromise performance.**
- Do not use AxSYM HBsAg Confirmatory Reagent Kit beyond the expiration date.
- Do not use AxSYM HBsAg Reagent Kit beyond the expiration date.
- Do not use AxSYM HBsAg Reagent Pack beyond a maximum of 112 cumulative hours on board the AxSYM System.
- Do not mix reagents from different Confirmatory Reagent Kits or Reagent Packs. Do not mix reagents and index calibrators from different lots.
- Avoid microbial contamination of specimens and reagents. Use of disposable pipettes or pipette tips is recommended.
- Avoid chemical contamination of reagents and equipment.
- Ensure that sufficient sample volume is present. If sample volume is insufficient, the AxSYM System will give an error code and no result will be reported. For a description of the system error codes, refer to the AxSYM System Operations Manual, Section 10.
- Use caution in handling patient specimens to prevent cross contamination. Transfer of any amount of an HBsAg reactive specimen may contaminate an adjacent nonreactive specimen and cause a falsely reactive result.

Refer to the AxSYM System Operations Manual, Sections 7 and 8, for a more detailed discussion of the safety and handling precautions during system operation.

STORAGE INSTRUCTIONS

Upon receipt, the AxSYM HBsAg Confirmatory Reagent Kit must be stored at 2-8°C. The AxSYM HBsAg Confirmatory Reagent Kit may be used immediately after removal from the refrigerator.

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Reagents are stable until the expiration date when stored and handled as directed.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When the S/CO for an AxSYM HBsAg Negative Control or Positive Control treated with Reagent B, or the % Neutralization for an AxSYM HBsAg Positive Control treated with Reagent B is out of the expected range, it may indicate deterioration of the reagents or errors in technique. The test results of associated specimens are invalid and these specimens must be retested. Assay recalibration may be necessary. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

INSTRUMENT PROCEDURE

NOTE: AxSYM HBsAg Confirmatory must only be used with AxSYM System software version 3.60 or higher.

ASSAY FILE INSTALLATION

The AxSYM HBsAg/Confirmatory Assay Disk, List No. 2K14-01 or higher, contains 4 assay/ratio files: HBsAg_US, HBsAg_CF, NeutUS (Ratio), and NeutDiUS (Ratio). The assay/ratio files must be installed on the AxSYM System from the assay disk prior to performing the AxSYM HBsAg Confirmatory assay. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM HBsAg CONFIRMATORY ASSAY PARAMETERS

Assay parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. Selected assay parameters used for the AxSYM HBsAg Confirmatory assay are listed below.

Assay Parameters	
1	Long Assay Name (English): HBsAg_CF
6	Abbrev Assay Name (English): HBsAg_CF
11	Assay Number: 113
43	Default Dilution Protocol > REAGENT B
44	Default Calibration Method > Index Cal
45	Selected Result Concentration Units > S/CO
80	Interpretation Option to use > 1

NOTE: Parameters 43, 44, 45, and 80 cannot be edited.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma (collected in potassium EDTA, sodium citrate, sodium heparin, lithium heparin, or plasma separator tubes) may be used with the AxSYM HBsAg Confirmatory assay. Follow the manufacturer's processing instructions for serum and plasma collection tubes.
- The AxSYM System does not provide the capability to verify sample type. It is the responsibility of the operator to verify that the correct sample type is tested with the AxSYM HBsAg Confirmatory assay.
- Specimens from heparinized patients may be incompletely coagulated and inconsistent results could occur due to the presence of fibrin. To prevent partially coagulated specimens, draw the specimen prior to heparin therapy or into a plasma collection tube. Serum collection tubes should not be used with heparinized patients.
- This assay was designed for use with human serum or plasma from individual patient specimens. Pooled specimens must not be used.
- Gravity separation is not sufficient for specimen preparation. Specimens containing clots, red blood cells, or particulate matter may give inconsistent results and must be clarified by centrifugation prior to testing.
- **All patient specimens must be transferred to a centrifuge tube and centrifuged at a Relative Centrifugal Force (RCF) of 10,000 x g for 10 minutes prior to confirmatory testing.**

  Transfer the clarified specimen to an aliquot tube or a sample cup for testing.

  NOTE: AxSYM System Software Version 3.60 and higher offers an Auto Retest/Auto Dilution feature. Due to the centrifugation requirements discussed above, this feature must not be used with this assay.
- Centrifuged specimens with a lipid layer on top of the liquid must be transferred to an aliquot tube or a sample cup. Care must be taken to transfer only the clarified specimen and not the lipemic material.
  - The Clinical Laboratory Standards Institute (CLSI) provides the following recommendations for storing blood specimens[5].
    - Store samples at 22°C (72°F) for no longer than 8 hours.
    - If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C (36-46°F).
    - If the assay will not be completed within 48 hours, freeze at or below -20°C (-4°F).
  - Note: Per manufacturer's recommendations, plasma collected in heparin collection tubes should be stored at room temperature to minimize latent fibrin formation promoted by cold temperatures.[6]
- Specimens that are not tested within the specified time period listed above must be removed from the clot or red blood cells, and stored frozen (-20°C or colder).
- Multiple freeze-thaw cycles should be avoided. Frozen specimens collected in all recommended collection tubes may be subjected to up to 2 freeze/thaw cycles prior to being tested.* Specimens must be mixed **thoroughly** after thawing, by LOW speed vortexing or by gentle inversion, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.
  - * Note: Freeze/thaw cycles 1 and 2 for serum collected in red top glass collection tubes demonstrated up to 18% (95% confidence limit) negative bias on average in a low positive sample (1.2 S/CO target); however, the mean value remained reactive.
- Specimens may be shipped at -20°C or colder (dry ice) or 2-8°C (wet ice) and must be packaged and labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances. For shipment at 2-8°C (wet ice), do not exceed the storage limitations listed above. It is recommended to ship specimens off the clot or red blood cells.
- For optimal results, specimens must be free of fibrin, red blood cells, or other particulate matter.
- Specimens with obvious microbial contamination should not be used.
- Do not use heat-inactivated specimens.
- Inspect all samples for bubbles. Remove bubbles prior to testing the sample. Refer to the AxSYM System Operations Manual, Section 7, for detailed instructions on removing bubbles.
- To minimize the effects of evaporation, all samples (patient specimens, controls, and calibrators) should be tested within 3 hours of being placed on board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of onboard sample storage constraints.

SAMPLE/REAGENT VOLUME

Refer to the ASSAY PROCEDURE section of this package insert for the minimum reagent and sample volumes required for calibration and/or testing patient specimens using AxSYM HBsAg Confirmatory.

AxSYM HBsAg CONFIRMATORY PROCEDURE

MATERIALS PROVIDED

- No. 9B01-60 AxSYM HBsAg Confirmatory Reagent Kit

MATERIALS REQUIRED BUT NOT PROVIDED

- No. 9B01-20 AxSYM HBsAg Reagent Kit
- No. 9B01-10 AxSYM HBsAg Controls
- No. 8A75 100 Reaction Vessels
- No. 8A73-02 100 Matrix Cells
- No. 8A47-04 Solution 1 (MUP)
- No. 8A81-04 Solution 3 (Matrix Cell Wash)
- No. 8A46 Solution 4 (Line Diluent)
- No. 9A35-05 AxSYM Probe Cleaning Solution
- No. 8A76-01 Sample Cups
- Pipettes or pipette tips (optional) to deliver the volumes specified on the Order screen

ADDITIONAL MATERIALS AVAILABLE

- No. 1L89-01 AxSYM HBsAg Confirmatory Templates A and B (Color-coded, plastic forms that can be placed on top of AxSYM Sample Segments as a guide for the proper positioning of reagents and samples.)

CAUTION:

- Mix the AxSYM HBsAg Index Calibrator and Controls by gentle inversion prior to use.
- When manually dispensing samples into sample cups (or aliquot tubes), verify that dispensing equipment does not introduce cross contamination and delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

ASSAY PROCEDURE

When testing samples with AxSYM HBsAg Confirmatory, the following points should be noted:

- **An active calibration for the AxSYM HBsAg lot in use must be in place prior to running the AxSYM HBsAg Confirmatory assay.**
- The AxSYM System Auto Retest/Auto Dilution feature must not be used for this assay. Refer to the **SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS** section of this package insert.
- A specific sample segment position (S/P) configuration is required when setting up a sample segment for either calibration or testing patient specimens. The AxSYM HBsAg Confirmatory Templates A (for calibration) and B (for testing patient specimens) are color-coded, plastic forms that can be placed on top of sample segments as a guide for proper positioning of reagents and samples. When using the AxSYM HBsAg Confirmatory Templates A and B, place the template on the selected sample segment. Follow the assigned S/P positions as indicated on the template. Visually verify reagent color against the template.

CAUTION: The System status must be WARMING, PAUSED, READY, or STOPPED before adding or removing sample segments, Reagent Packs, or Reaction Vessels.

CAUTION: The AxSYM HBsAg Confirmatory assay pipettes Reagent A, Reagent B, and Dilution Reagent directly from the Sample Carousel. When the Sampling Pipettor moves to the Sample Carousel to pipette these reagents, the Sampling Center Motion Detection Light illuminates at the same time the Sample Carousel begins to move. To avoid risk of physical injury, keep fingers away from the Sample Carousel when amber motion detection light is illuminated.

1. Check for sufficient onboard inventory of Matrix Cells and bulk solutions, and sample segment availability.
2. Check for sufficient waste collection capacity.
   CAUTION: Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in progress. If opened, all processing will stop. All tests will be terminated and must be repeated.
3. Centrifuge the specimens at an RCF of 10,000 x g for 10 minutes.
4. Pipette each reagent and sample into a sample cup (or aliquot tube) and place the sample cups (or aliquot tubes) into the appropriate sample segment positions (S/P).
   - For calibration, refer to Step 5 for the correct S/P positions, and reagent and sample volumes.
   - For testing patient specimens, refer to Step 6 for the correct S/P positions, and reagent and sample volumes.

   CAUTION: For the AxSYM System to correctly calculate the test results required for confirmation, the sample segment position (S/P) configuration described in Steps 5 and 6 must be followed.
5. Sample Segment Setup for Calibration:
   - Refer to the **QUALITY CONTROL PROCEDURES** section of this package insert for calibration requirements.
   - When calibration is required, AxSYM HBsAg Confirmatory reagents (Reagent A or Reagent B) are automatically ordered by the AxSYM System when HBsAg_CF is selected. These reagents must be manually pipetted into sample cups (or aliquot tubes) and placed in S/P positions 1 (Reagent A) and 2 (Reagent B) of the appropriate sample segment. Therefore, tests **must not** be assigned to S/P positions 1 or 2 when calibrating the assay. Refer to the following table for the correct S/P positions, and reagent and sample volumes.

Sample Segment Setup for Calibration Acceptance and Storage[a]

Sample Segment Position (S/P)	Reagent or Sample	Reagent Color	Minimum Volume Required
1	Reagent A	Violet	100 µL
2	Reagent B	Yellow	175 µL
3	Index Calibrator	Green	270 µL (8 drops)
4	Negative Control	Natural	160 µL (4 drops)
5	Positive Control	Blue	250 µL (7 drops)

[a] AxSYM HBsAg Confirmatory Template A may be used as a guide when setting up a sample segment for calibration.

- For calibration, the AxSYM HBsAg Index Calibrator is ordered using the Calibration Order Screen as follows:

  Index Calibrator (270 µL or 8 drops):

  Select F4-CAL. Select HBsAg_CF assay. **Assign S/P to position 3.** The AxSYM System automatically orders two replicates with REAGENT_B. Do not simultaneously calibrate more than one AxSYM HBsAg Reagent Pack lot.
- For calibration, the AxSYM HBsAg Negative and Positive Controls are ordered using the Patient Orderlist screen as follows:

  Negative Control (160 µL or 4 drops):

  Select F6-PATIENT. **Assign S/P to position 4.** Select HBsAg_CF assay. Order one REP of REAGENT_B.

  Positive Control (250 µL or 7 drops):

  Select F6-PATIENT. **Assign S/P to position 5.** Select HBsAg_CF assay. Select F4-DILS/REPS. Order one REP of REAGENT_B and one REP of REAGENT_A.

6. Sample Segment Setup for Testing Patient Specimens:
   - When testing patient specimens, AxSYM HBsAg Confirmatory reagents (Reagent A, Reagent B, or Dilution Reagent) are automatically ordered by the AxSYM System when HBsAg_CF is selected. The minimum volume required for these reagents increases depending on the number of patient specimens (one to five) being tested. These reagents must be manually pipetted into sample cups (or aliquot tubes) and placed in S/P positions 1 (Reagent A), 2 (Reagent B), and 3 (Dilution Reagent) of the appropriate sample segment. Therefore, tests **must not** be assigned to S/P positions 1-3 when patient specimens are being ordered. These S/P positions **must not** appear on the Orderlist Report printout. Refer to the following table for the correct S/P positions and reagent volumes.

Sample Segment Setup for Testing Patient Specimens – Reagent Volumes[a]

Sample Segment Position (S/P)	Reagent	Reagent Color	Minimum Volume Required (µL) per Number (One to Five) of Patient Specimens Being Tested				
			One	Two	Three	Four	Five
1	Reagent A	Violet	150	200	250	300	350
2	Reagent B	Yellow	175	225	275	325	375
3	Dilution Reagent	Natural	500	875	1250	1625	2000

[a] AxSYM HBsAg Confirmatory Template B may be used as a guide when setting up a sample segment for testing patient specimens.

- For Sample Segment Positions 4-10: Controls must be placed into S/P positions 4 (Negative Control) and 5 (Positive Control) of the sample segment. One to five patient specimens can be tested per sample segment, and must be placed sequentially into S/P positions 6-10 of the sample segment. For more than five patient specimens, additional sample segments with Reagent A, Reagent B, Dilution Reagent, Negative Control, and Positive Control must be set up. Refer to the following table for the correct S/P positions and sample volumes.

Sample Segment Setup for Testing Patient Specimens – Sample Volumes[a]

Sample Segment Position (S/P)	Sample	Reagent Color	Minimum Volume Required
4	Negative Control	Natural	160 µL (4 drops)
5	Positive Control	Blue	250 µL (7 drops)
6	Patient Specimen #1		275 µL
7	Patient Specimen #2		275 µL
8	Patient Specimen #3		275 µL
9	Patient Specimen #4		275 µL
10	Patient Specimen #5		275 µL

[a] AxSYM HBsAg Confirmatory Template B may be used as a guide when setting up a sample segment for testing patient specimens.

- For testing patient specimens, each specimen must be tested both undiluted and with the automated 1:500 dilution. All specimen dilutions are made with the AxSYM HBsAg Confirmatory Dilution Reagent.
- For testing patient specimens, the Negative and Positive Controls, and all patient specimens are ordered using the Patient Orderlist screen as follows:

  Negative Control (160 µL or 4 drops):

  Select F6-PATIENT. **Assign S/P to position 4.**

  Select HBsAg_CF assay. Order one REP of REAGENT_B.

  Positive Control (250 µL or 7 drops):

  Select F6-PATIENT. **Assign S/P to position 5.**

  Select HBsAg_CF assay. Select F4-DILS/REPS.

  Order one REP of REAGENT_B and one REP of REAGENT_A.

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Patient Specimens (275 µL):

Select F6-PATIENT. **Assign S/P to positions 6-10** as needed for up to five (5) patients. Select HBsAg_CF assay.

Select F4-DILS/REPS for each patient and order four tests:

one with REAGENT_B, one with REAGENT_A,

one with REAG_B_DIL, and one with REAG_A_DIL.

NOTE: The operator may obtain an Orderlist Report by accessing the Orderlist screen and pressing PRINT. The printout contains sample placement information and minimum STAT sample cup (or aliquot tube) volume requirements for all tests ordered. When using Host Order Query, the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5: Ordering Patient Samples, for a description of the Host Query Option.

7. Place sample segments containing the AxSYM HBsAg Confirmatory reagents and ordered samples into the Sample Carousel.
8. Open AxSYM HBsAg Reagent Bottle 4 containing the Probe Wash Solution. Place the AxSYM HBsAg Reagent Pack into the Reagent Pack Carousel.

NOTE: The cap for AxSYM HBsAg Reagent Bottle 4 must be manually opened prior to running an AxSYM HBsAg assay. Upon completion of the run, close the Reagent Bottle 4 cap securely.

9. Ensure that RVs are present on the RV Carousel. Additional RVs may be added as needed.
10. Press RUN. All entries on the Orderlist screen are automatically transferred to the Order Status screen for sample processing.
11. Review the results to determine whether retesting with a manual sample dilution is required.
12. When testing is completed, close Reagent Bottle 4 and remove the samples and the AxSYM HBsAg Reagent Pack from the Sampling Center. Store reagent pack at 2-8°C. Discard any AxSYM HBsAg Confirmatory reagents, Index Calibrator, Negative Control, or Positive Control remaining in the sample cups (or aliquot tubes).

NOTE: When using the onboard reagent tracking feature, the operator must perform a reagent pack scan after removing any pack from the system in order to maintain the validity of the reagent pack stability time.

Sections 5 and 6 of the AxSYM System Operations Manual contain detailed steps for performing assay calibration and sample testing procedures.

SPECIMEN DILUTION PROCEDURES

Manual Sample Dilution

If a sample is reactive but is not neutralized using the undiluted and the automated 1:500 dilution procedure, an additional dilution is required. Prepare a manual 1:50 dilution using a 20 µL sample plus 980 µL of the Dilution Reagent. This manually diluted sample is tested using the automated 1:500 dilution, resulting in a final dilution of 1:25,000.

To Order Patient Specimens Diluted 1:25,000

Select F6-PATIENT. Assign S/P to positions 6-10 as needed for up to five (5) patients. Select HBsAg_CF assay. Select F4-DILS/REPS for each patient and order two tests: one with REAG_B_DIL and one with REAG_A_DIL. (Edit REAGENT_A and REAGENT_B to 0.)

Refer to the AxSYM System Operations Manual, Section 5, for additional information on ordering specimen dilutions.

QUALITY CONTROL PROCEDURES

CALIBRATION

A minimum of two replicates of the AxSYM HBsAg Index Calibrator treated with Reagent B must be tested for an AxSYM HBsAg Confirmatory calibration.

One each of the following must be tested as a means of evaluating the assay calibration:

- AxSYM HBsAg Negative Control treated with Reagent B
- AxSYM HBsAg Positive Control treated with Reagent A
- AxSYM HBsAg Positive Control treated with Reagent B

Once an AxSYM HBsAg Confirmatory calibration is accepted and stored (ACTIVE), AND there is an active calibration with the AxSYM HBsAg Reagent Pack lot in use, all subsequent samples may be tested without further calibration unless one or more of the following occur:

- An AxSYM HBsAg Reagent Pack or an AxSYM HBsAg Confirmatory Reagent Kit with a new lot number is used.
- Either of the AxSYM HBsAg Control values is out of its specified range.
- The MEIA Optics Verification Update has been performed.

Refer to the AxSYM System Operations Manual, Section 6, for further information on:

- Setting up an assay calibration
- Determining when recalibration may be necessary
- Calibration verification

The operator must verify that the AxSYM HBsAg Control values are within the ranges specified in the **RESULTS**, INTERPRETATION OF RESULTS section of this package insert. If a control value is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendix E, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

QUALITY CONTROL

The AxSYM HBsAg controls are in a serum matrix made from recalcified plasma. The user should provide alternate control material for plasma when necessary.

Good laboratory practice recommends the use of positive and negative controls to assure functionality of reagents and proper performance of the assay procedure. Positive control and negative control are intended to monitor for substantial reagent failure. Quality Control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. It is recommended that the user refer to CLSI document C24-A2, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions:* [Approved Guideline - Second Edition][7] or other published guidelines for general quality control recommendations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1202(c)[8].

The minimum control requirement for each AxSYM HBsAg Confirmatory sample segment is a single sample of each of the following:

- AxSYM HBsAg Negative Control treated with Reagent B
- AxSYM HBsAg Positive Control treated with Reagent A
- AxSYM HBsAg Positive Control treated with Reagent B

Additional controls may be tested in conformance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control procedures.

The operator must verify that the AxSYM HBsAg Control values are within the ranges specified in the **RESULTS**, INTERPRETATION OF RESULTS section of this package insert. If a control value is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Deterioration of the reagents or errors in technique may be indicated when an AxSYM HBsAg Positive or Negative Control value is out of the expected range. If control results fall outside the stated range or outside your established acceptable range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. Retest patient specimens before reporting results for this run. Recalibration may be indicated. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

The AxSYM System has the capability to generate a Levey-Jennings plot of each assay's quality control performance. Refer to the AxSYM System Operations Manual, Section 5. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

FLUORESCENCE BACKGROUND ACCEPTANCE CRITERIA

Quality Control with regard to the MUP substrate blank is automatically determined by the instrument and checked under Assay Parameter 64, Max Intercept-Max MUP intercept, each time a test result is calculated. If the MUP intercept value is greater than the maximum allowable value, the result is invalid. The test request will be moved to the Exceptions List where it will appear with the message "1064 Invalid test result, intercept too high" and the calculated intercept value. Refer to the AxSYM System Operations Manual, Section 10, when this error message is obtained.

Refer to the AxSYM System Operations Manual, Section 2, for further information on this parameter.

RESULTS

CALCULATION

The AxSYM HBsAg Confirmatory assay protocol calculates the Cutoff Rate (CO) using the Index Calibrator treated with Reagent B mean rate (from the rate of the two Index Calibrator treated with Reagent B replicates), multiplied by a factor of 1.5, and stores the result.

Cutoff Rate (CO) = Index Calibrator Treated with Reagent B Mean Rate x 1.5

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There is one result reported (REAGENT_B) for the Negative Control being tested. There are three result reports for each sample* being tested. The three reported results are identified as REAGENT_A, REAGENT_B, and %NeutUS for undiluted samples and REAG_A_DIL, REAG_B_DIL, and %NeutDiUS for 1:500 diluted samples.

The AxSYM HBsAg Confirmatory assay protocol calculates a result (S/CO) and provides an interpretation for each sample* treated with Reagent A and Reagent B.

For the Reagent A report:

$$S/CO\ (A) = \frac{\text{Rate of Sample Treated with Reagent A}}{\text{Cutoff Rate}}$$

For the Reagent B report:

$$S/CO\ (B) = \frac{\text{Rate of Sample Treated with Reagent B}}{\text{Cutoff Rate}}$$

The AxSYM HBsAg Confirmatory assay calculates the percent neutralization (%NeutUS or %NeutDiUS) for the sample* using the results of the sample treated with Reagent A* [S/CO (A)] and the sample treated with Reagent B* [S/CO (B)] as follows:

%Neutralization =

$$\frac{S/CO\ (B) - S/CO\ (A)}{S/CO\ (B) - S/CO \text{ of Index Calibrator Treated with Reagent B}} \times 100$$

* Positive Control, undiluted patient specimens, and 1:500 or 1:25,000 diluted patient specimens.

FLAGS

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Sections 1 and 2.

INTERPRETATION OF RESULTS

Prior to the final interpretation of patient specimen results, the assay validity (as described below) must be verified by the operator.

For an AxSYM HBsAg Confirmatory assay to be valid, the following conditions must be met for each sample segment:

- The Calibration is "ACTIVE" for both AxSYM HBsAg and AxSYM HBsAg Confirmatory assays. (A Calibration report must be printed after each calibration.)
- The S/CO value of the Negative Control treated with Reagent B must be 0.30 to 0.95.
- The S/CO value of the Positive Control treated with Reagent B must be 1.00 to 4.50.
- The Positive Control %NeutUS must be greater than or equal to 50% and have an interpretation of POSITIVE.

A sample is **confirmed POSITIVE for HBsAg** by the AxSYM HBsAg Confirmatory assay when validity has been verified by the operator. In addition, POSITIVE must appear in the INTRP field of the %NeutUS or %NeutDiUS report for the sample. POSITIVE will appear in the INTRP field when the following occur:

- The INTRP field of the sample treated with Reagent B is REACTIVE (S/CO value is greater than or equal to 1.00),
- AND the percent neutralization value is greater than or equal to 50.

If POSITIVE does **not** appear as an interpretation in either of the percent neutralization reports, proceed with the appropriate interpretation as follows:

- When NEGATIVE appears in the INTRP field for an undiluted sample treated with Reagent B, the sample is **nonreactive for HBsAg** by AxSYM HBsAg Confirmatory.
- When REACTIVE appears in the INTRP field for an undiluted sample treated with Reagent B and the interpretation for the 1:500 diluted sample treated with Reagent B is NEGATIVE, the sample is **repeat reactive, nonconfirming for HBsAg** by AxSYM HBsAg Confirmatory.
- When REACTIVE appears in the INTRP field for both the undiluted and the 1:500 diluted sample treated with Reagent B and both percent neutralizations are less than 50, the **AxSYM HBsAg Confirmatory testing must be repeated at a sample dilution of 1:25,000** as described in the Manual Sample Dilution section.

The following table summarizes the possible AxSYM HBsAg Confirmatory interpretations based on the INTRP field of the Reagent B result reports for undiluted and 1:500 diluted samples and the INTRP field of the percent neutralization reports.

AxSYM HBsAg Confirmatory Interpretations[a]

	INTRP Fields for Undiluted Sample		INTRP Fields for 1:500 Diluted Sample		Final Interpretation
	REAGENT_B	%NeutUS	REAG_B_DIL	%NeutDiUS	
1	REACTIVE	POSITIVE	NEGATIVE	[blank][b]	CONFIRMED POSITIVE
2	REACTIVE	[blank][b]	REACTIVE	POSITIVE	CONFIRMED POSITIVE
3	REACTIVE	POSITIVE	REACTIVE	POSITIVE	CONFIRMED POSITIVE
4	NEGATIVE	[blank][b]	NEGATIVE	[blank][b]	NONREACTIVE for HBsAg
5	REACTIVE	[blank][b]	NEGATIVE	[blank][b]	REPEAT REACTIVE, Nonconfirming for HBsAg
6	REACTIVE	[blank][b]	REACTIVE	[blank][b]	Repeat confirmatory testing using a 1:25,000 sample dilution
7[c]	NA	NA	REACTIVE	POSITIVE	CONFIRMED POSITIVE
8[c]	NA	NA	NEGATIVE	[blank][b]	REPEAT REACTIVE, Nonconfirmingfor HBsAg

NA = Not Applicable

[a] No interpretation is made directly from the Reagent A [S/CO (A)] results; Reagent A results are used to calculate %Neutralization only.

[b] The INTRP Field of the report will be blank.

[c] Interpretations 7 and 8 are ONLY for those samples tested at a 1:25,000 dilution. A 1:50 manual dilution is made and the sample is tested at 1:500 only (final dilution = 1:25,000). The AxSYM assay report will note results as the 1:500 Diluted Sample.

LIMITATIONS OF THE PROCEDURE

- **WARNING: Not intended for use in screening blood, plasma, or tissue donors.** The effectiveness of AxSYM HBsAg Confirmatory for use in screening blood, plasma, or tissue donors has not been established.
- Although there is an association between the presence of HBsAg and infectivity, it is recognized that presently available methods for HBsAg confirmation may not detect all possible cases of hepatitis B viral infection.
- For diagnostic purposes, HBsAg reactivity should be correlated with patient history and the presence of other hepatitis markers. Reactive results do not discriminate between acute or chronic HBV infections.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[9] Such specimens may exhibit either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.[10] These specimens should not be tested with the AxSYM HBsAg assay.

EXPECTED VALUES

Not Applicable

SPECIFIC PERFORMANCE CHARACTERISTICS

Assay results obtained in individual laboratories may vary from data presented.

AxSYM HBsAg CONFIRMATORY PERFORMANCE

In a multi-site clinical evaluation, 178 specimens were tested by AxSYM HBsAg Confirmatory and an FDA-approved HBsAg confirmatory reference assay. As shown in Table 1, 166 specimens were confirmed positive and five specimens were negative for the presence of HBsAg by both methods.

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Table 1
Comparison of AxSYM HBsAg Confirmatory Results
to Reference HBsAg Confirmatory Results

	Reference HBsAg Confirmatory Result				
	+[a]		-		
	AxSYM HBsAg Confirmatory Result				
Specimen Category	+	-	+	-	Total
Individuals at Increased Risk of HBV Infection	18	1[b]	2[c]	2	23
Individuals With Signs and Symptoms of Hepatitis Infection	31	0	3[d]	3	37
Individuals With Acute HBV Infection	21	0	0	0	21
Individuals With Chronic HBV Infection	41	0	1[e]	0	42
Specimens From Vietnam	53	0	0	0	53
Pregnant Females	2	0	0	0	2
Total	166	1	6	5	178

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

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

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

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

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

Table 2 is a comparison of the AxSYM HBsAg Confirmatory results to the reference HBsAg confirmatory assay results by HBV classification.

Table 2
Comparison of AxSYM HBsAg Confirmatory Results
to Reference HBsAg Confirmatory Results
by HBV Classification

	Reference HBsAg Confirmatory Result				
	+[a]		-		
	AxSYM HBsAg Confirmatory Result				
HBV Classification	+	-	+	-	Total
Early Acute	17	0	0	0	17
Acute	11	0	0	0	11
Chronic[b]	135	1	1	0	137
Late Acute/Recovering	1	0	0	0	1
Immune Due to Natural Infection	0	0	0	3	3
Distantly Immune/Anti-HBs Not Detected	0	0	2	1	3
Immune Due to HBV Vaccination	0	0	1	0	1
Susceptible	0	0	2	1	3
Total	164	1	6	5	176[c]

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

b 42 of the chronic specimens were defined by the presence of HBsAg for ≥ 6 months.

c The total number of specimens is fewer than the total shown in Table 1 because two specimens from pregnant females were not tested for HBV classification determination.

Refer to AxSYM HBsAg Package Insert (List 9B01-20) Specific Performance Characteristics for cross-reactivity and interfering substances results, HBsAg Seroconversion Panels, HBsAg Mutant detection and HBsAg Genotyping results.

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Abbott
AXSYM® SYSTEM

In Vitro Test
List No. 9B01-10
66-8778/R1

HBsAg
Controls

ABBOTT LABORATORIES
Abbott Park, IL 60064 USA

Printed in USA

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Intended Use

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

- **Warnings and Precautions: For *In Vitro* Diagnostic Use.**

  CAUTION: This product contains human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should 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 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.
  - The Negative Control is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
  - The Positive Control is reactive for HBsAg, and nonreactive for HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs. Reactive plasma is heat-inactivated.

Warning: This product contains Sodium Azide; for a specific listing, refer to the **Materials Provided** section of this package insert. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

Materials Provided:

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

Reagent Preparation and Storage

- AxSYM HBsAg Controls are stable until the expiration date when stored and handled as directed. Do not use past the expiration date. **Store at 2-8°C**

Quality Control Procedure

Refer to the AxSYM HBsAg and AxSYM HBsAg Confirmatory assay package inserts and the AxSYM System Operations Manual for additional information.

AxSYM HBsAg Controls should be run every 24 hours.

Limitations

- The AxSYM HBsAg Controls must only be used with the AxSYM HBsAg assay (List No. 9B01-20) and the AxSYM HBsAg Confirmatory assay (List No. 9B01-60).
- Values have not been established for assays other than the AxSYM HBsAg and AxSYM HBsAg Confirmatory assays. If the user intends to use this control material with other assays it is their responsibility to establish the appropriate ranges.
- The AxSYM HBsAg controls are in a serum matrix made from recalcified plasma. The user should provide alternate control material for plasma when necessary.
- The controls are not calibrators and should not be used for assay calibration.

Expected Results

The AxSYM HBsAg Controls have the following ranges:

Control	Color	HBsAg Concentration (ng/mL)	Control Range (S/CO)
Negative	Natural	0.0	0.20 - 0.80
Positive	Blue[a]	0.6 - 1.0[b]	1.00 - 4.50

[a] Dye: Bromophenol Blue

[b] Concentration (ng/mL) is approximately 0.15 - 0.25 IU/mL.

AxSYM is a registered trademark of Abbott Laboratories.

Bibliography

1. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. Washington, DC: US Government Printing Office; May 1999.
3. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline - Third Edition*. CLSI Document M29-A3. Wayne, PA: CLSI, 2005.