



AUSAB
List No. 3C74
66-6609/R1

AUSAB®

Antibody to Hepatitis B Surface Antigen (Anti-HBs)

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.

NAME

AxSYM® AUSAB®

INTENDED USE

AxSYM AUSAB is a microparticle enzyme immunoassay (MEIA) intended for the quantitative determination of antibody to hepatitis B surface antigen (anti-HBs) in adult and pediatric serum (including serum collected in serum separator tubes) or plasma (collected in sodium heparin tubes). The assay is used for the quantitative measurement of antibody response to hepatitis B virus (HBV) vaccination for the determination of HBV immune status, and for the diagnosis of HBV disease associated with HBV infection when used in conjunction with other laboratory results and clinical information.

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

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients. The user is responsible for establishing their own assay performance characteristics in these populations.

SUMMARY AND EXPLANATION OF THE TEST

Anti-HBs assays are often used to test individuals pre- and post-immunization or following exposure to HBV. The presence of anti-HBs has been shown to be important in protection against HBV infection.¹ Numerous studies have demonstrated the effectiveness of the hepatitis B vaccine in stimulating the immune system to produce anti-HBs and in preventing HBV infection.²⁻⁴

Individuals at risk for HBV (e.g., healthcare workers, sex partners of persons with HBV infection, or immunocompromised individuals) may be tested for anti-HBs. These individuals are screened to determine the need for vaccination, to establish an individual's initial level of anti-HBs after vaccination, or to determine whether revaccination is needed. Some individuals may be prescreened or evaluated after an exposure incident (e.g., needle stick) to determine the appropriateness of receiving hepatitis B immune globulin (H-BIG®, Nabi Biopharmaceuticals) and a subsequent vaccination series.⁵ Detection of anti-HBs in an asymptomatic individual may indicate previous infection with HBV and/or vaccination.

A protective anti-HBs response has been defined as serum levels greater than or equal to 10 mIU/mL.⁶ An anti-HBs level less than 10 mIU/mL in a patient is considered nonprotective for HBV infection unless a prior anti-HBs test was greater than or equal to 10 mIU/mL or a past clinical history of HBV infection is known.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM AUSAB is based on MEIA technology and utilizes the principle of indirect binding between anti-HBs in the sample and hepatitis B surface (Recombinant, produced in murine L cells) antigen (rHBsAg, subtypes *ad* and *ay*) coated on the microparticles and biotinylated rHBsAg. The AxSYM AUSAB reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM AUSAB reagents required for one test are pipetted by the Sampling Pipettor into various wells of a Reaction Vessel (RV). Sample and Hepatitis B Surface Antigen (Recombinant, produced in murine L cells) (Subtypes *ad* and *ay*) Coated Microparticles are combined in one RV well.
- When anti-HBs is present in the sample, it binds to the coated microparticles forming an antigen-antibody complex in the reaction mixture.
- The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Pipettor.

PROCESSING CENTER

- An aliquot of the reaction mixture is transferred to the Matrix Cell. The microparticles bind irreversibly to the glass fiber matrix.
- Hepatitis B Surface Antigen (Recombinant, produced in murine L cells) (Subtypes *ad* and *ay*):Biotin Conjugate is dispensed onto the Matrix Cell forming an antigen-antibody-antigen complex.
- The Anti-biotin (Rabbit):Alkaline Phosphatase Conjugate is dispensed onto the Matrix Cell and binds to any microparticle-bound antigen-antibody-antigen 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 concentration of anti-HBs in the sample is determined using a previously generated AxSYM AUSAB calibration curve.

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

REAGENTS

REAGENT KIT, 100 TESTS*

AxSYM AUSAB Reagent Pack (3C74-20)

- 1 Bottle (13.6 mL) Anti-biotin (Rabbit):Alkaline Phosphatase Conjugate in TRIS buffer with protein (0.5% bovine, 2.9% piscine) stabilizers. Minimum concentration: 0.1 µg/mL. Preservative: 0.1% Sodium Azide. (Reagent Bottle 1)

- 1 Bottle (5.3 mL) Hepatitis B Surface Antigen (Recombinant, produced in murine L cells) (Subtypes *ad* and *ay*) Coated Microparticles in TRIS buffer with protein (0.3% bovine) stabilizer. Minimum concentration: 0.125% solids. Preservative: 0.08% Sodium Azide. (Reagent Bottle 2)
 - 1 Bottle (13.5 mL) Hepatitis B Surface Antigen (Recombinant, produced in murine L cells) (Subtypes *ad* and *ay*):Biotin Conjugate in TRIS buffer with animal sera (50% bovine, 1% rabbit) and recalcified anti-HBs nonreactive human plasma. Minimum concentration: 1.25 µg/mL. Preservative: 0.1% Sodium Azide. (Reagent Bottle 3)
 - 1 Bottle (31.4 mL) Specimen Diluent is recalcified anti-HBs nonreactive human plasma. Preservative: 0.1% Sodium Azide. (Reagent Bottle 4)
- * 3C74-66 includes the AxSYM AUSAB Reagent Pack (100 tests), Reaction Vessels (100 each), and Matrix Cells (100 each).

AxSYM AUSAB Specimen Diluent (3C74-50) (sold separately)

1 Bottle (100 mL) AxSYM AUSAB Specimen Diluent is anti-HBs nonreactive human plasma. Preservative: 0.1% Sodium Azide.

AxSYM AUSAB Standard Calibrators (3C74-01) (sold separately)

6 Bottles (4 mL each) of AxSYM AUSAB Standard Calibrators:

- Standard Calibrator A is recalcified anti-HBs nonreactive human plasma. Preservative: 0.1% Sodium Azide.
- Standard Calibrators B through F are recalcified anti-HBs reactive human plasma. Preservative: 0.1% Sodium Azide.

The AxSYM AUSAB Standard Calibrators have the following concentrations:

Standard Calibrator	Anti-HBs Concentration ^a (mIU/mL)
A	0
B	10
C	50
D	100
E	500
F	1000

^a Concentration traceable to the World Health Organization (WHO) International Reference Preparation for antibody to HBsAg.

AxSYM AUSAB Controls (3C74-10) (sold separately)

3 Bottles (8 mL each) of AxSYM AUSAB Controls:

- The Negative Control is recalcified anti-HBs nonreactive human plasma. Preservative: 0.1% Sodium Azide.
- The Positive Controls are recalcified anti-HBs reactive human plasma. Preservative: 0.1% Sodium Azide.

The AxSYM AUSAB Controls have the following concentrations and ranges:

Control	Color	Anti-HBs Concentration (mIU/mL)	Control Range (mIU/mL)
Negative	Natural	0	0 - 2
Positive 1	Green ^b	15 ^a	10 - 20
Positive 2	Blue ^c	80 ^a	60 - 100

^a Concentration traceable to the World Health Organization (WHO) International Reference Preparation for antibody to HBsAg.

^b Dye: Acid Blue No. 9 and Yellow No. 23

^c Dye: Acid Blue No. 9

OTHER REAGENTS (sold separately)

AxSYM Probe Cleaning Solution (9A35-05)

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

Solution 1 (MUP) (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) (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) (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 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 material must be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens,⁷ Biosafety Level 2⁸ or other appropriate biosafety practices^{9,10} should be used for materials that contain or are suspected of containing infectious agents.

- The AxSYM AUSAB Conjugate is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- The AxSYM AUSAB Specimen Diluent is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- The AxSYM AUSAB Standard Calibrator A is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- The AxSYM AUSAB Standard Calibrators B through F are reactive for anti-HBs and nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.
- The AxSYM AUSAB Negative Control is nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, anti-HCV, and anti-HBs.
- The AxSYM AUSAB Positive Controls are reactive for anti-HBs and nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.
- 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

- **AxSYM AUSAB reagents are susceptible to bubbles/foaming and require inspection and removal of bubbles before loading. Refer to the AxSYM System Operations Manual, Section 9.**
- **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 AUSAB Reagent Pack beyond the expiration date.
- Do not use AxSYM AUSAB Reagent Pack beyond a maximum of 112 cumulative hours on board the AxSYM System.
- Do not mix reagents from different Reagent Packs.
- 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 anti-HBs reactive specimen may contaminate an adjacent nonreactive specimen and cause a falsely reactive result.
- Use accurately calibrated equipment.

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 AUSAB Reagent Pack, Standard Calibrators, Controls, and Specimen Diluent must be stored at 2-8°C. They may be used immediately after removal from the refrigerator. Calibrators 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 AUSAB 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 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 AUSAB Negative Control, Positive Control 1, or Positive Control 2 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 AUSAB must only be used with AxSYM System software version 3.60 or higher.

ASSAY FILE INSTALLATION

The AxSYM AUSAB assay file must be installed on the AxSYM System from the AxSYM AUSAB Assay Disk, List No. 3C89-01 or higher, prior to performing the AxSYM AUSAB assay. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM AUSAB 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 AUSAB assay are listed below.

Assay Parameters	
1	Long Assay Name (English): AUSAB_US
6	Abbrev Assay Name (English): AUSAB_US
11	Assay Number: 120
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > mIU/mL
80	Interpretation Option to use > 1

NOTE: Although allowed, Parameters 43, 44, 45, and 80 should not be edited.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma (collected in sodium heparin tubes) may be used with the AxSYM AUSAB assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.
 - * Note: Plasma collected in tubes containing potassium EDTA, lithium heparin, sodium citrate, or in plasma separator tubes is not recommended for use in the AxSYM AUSAB assay.
- 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 AUSAB assay.
- This assay was designed for use with human serum or plasma from individual patient specimens. Pooled specimens must not be used.
- 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.
- 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 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.¹¹
 - 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.¹²

- Specimens that are not tested within the specified time period listed must be removed from the clot or red blood cells, and stored frozen (-20°C or colder).
- Specimens must not be repeatedly frozen and thawed. Frozen specimens may be thawed only once prior to testing. 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.
- 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. 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.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
- 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, it is recommended that all samples (patient specimens, controls, and calibrators) 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 AUSAB 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 210 µL. For every additional AxSYM AUSAB test performed (ROUTINE or STAT) from the same sample container, an additional 160 µL of sample is required.

If the dilution protocol is selected, 150 µL of sample is required unless the test is performed STAT. The minimum sample volume for a STAT AxSYM AUSAB dilution is 94 µL. For every additional AxSYM AUSAB dilution performed from the same container, an additional 44 µL of sample is required. Refer to the AxSYM System Operations Manual, Section 5.

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 AUSAB Standard Calibrators and Controls, hold the bottles **vertically** and dispense 11 drops of each calibrator or 9 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 AUSAB reagent lots, refer to the AxSYM System Operations Manual, Section 5.

AxSYM AUSAB PROCEDURE

MATERIALS PROVIDED

- 3C74-66 AxSYM AUSAB Reagent Pack, containing:
AxSYM AUSAB Reagent Pack
100 Reaction Vessels (RV)
100 Matrix Cells

MATERIALS REQUIRED BUT NOT PROVIDED

- 3C74-50 AxSYM AUSAB Specimen Diluent
- 3C74-01 AxSYM AUSAB Standard Calibrators
- 3C74-10 AxSYM AUSAB Controls
- 8A47-04 Solution 1 (MUP)
- 8A81-04 Solution 3 (Matrix Cell Wash)
- 8A46 Solution 4 (Line Diluent)
- 9A35-05 AxSYM Probe Cleaning Solution
- 8A76-01 Sample Cups
- Pipettes or pipette tips (optional) to deliver the volumes specified on the Order screen
- Cotton-tipped applicators

CAUTION:

- Mix the AxSYM AUSAB Standard Calibrators 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 AUSAB Standard Calibrators, AxSYM AUSAB Controls, and/or patient specimens as required. Assign or modify the 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.

Calibration

Invert gently to mix and dispense 11 drops of the Standard Calibrators into individual sample cups. Do not simultaneously calibrate more than one AxSYM AUSAB reagent lot.

Controls

Perform quality control by testing the Negative and Positive Controls (one test each). Invert gently to mix and dispense 9* drops each of Negative Control, Positive Control 1, and Positive Control 2 into individual sample cups.

- * Separate control replicates must be run for each AxSYM AUSAB reagent lot that is on board the AxSYM System. When more than one AxSYM AUSAB reagent lot is on board the AxSYM System, multiply the control volumes 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 210 µL for the first AxSYM AUSAB test plus 160 µL for each additional AxSYM AUSAB 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 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 Specimen Diluent. Remove any droplets of Specimen Diluent on the inside of the cap with a cotton-tipped applicator. Failure to do so may result in contamination of the Reagent Pack Actuator. Place the AxSYM AUSAB Reagent Pack into the Reagent Pack Carousel.
- NOTE:** The cap for Reagent Bottle 4 must be manually opened prior to running an AxSYM AUSAB 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 or dilution of the sample is required. Refer to the **SPECIMEN DILUTION PROCEDURES** section of this package insert for more detailed information on the dilution of samples with concentrations greater than 1,000.0 mIU/mL.
9. When testing is completed, close Reagent Bottle 4 and remove the samples and the AxSYM AUSAB 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.

SPECIMEN DILUTION PROCEDURES

Automated Dilution Protocol

Patient specimens with anti-HBs concentrations reported as greater than 1,000.0 mIU/mL (High Range Undiluted, Assay Parameter 92) are flagged with the code "> 1,000.0 mIU/mL". If quantitation of these specimens is desired, perform the Automated Dilution Protocol.

The Automated Dilution Protocol is provided to assist in quantitating test results greater than 1,000.0 mIU/mL and up to 25,000.0 mIU/mL. The AxSYM System performs a 1:25 dilution of the specimen using one Reaction Vessel. The AxSYM System automatically calculates the concentration of the diluted specimen and reports the result.

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

Manual Dilution Protocol

Patient specimens with anti-HBs concentrations reported as greater than 25,000.0 mIU/mL (> 25,000.0 mIU/mL) by the Automated Dilution Protocol may be diluted using a manual dilution of 1:25. Add 10 µL of the patient specimen to 240 µL of the AxSYM AUSAB Specimen Diluent.

Repeat the Automated Dilution Protocol using this manually diluted specimen. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

Final Specimen Concentration = Printed (Reported)

Concentration × Manual Dilution Factor (25)

QUALITY CONTROL PROCEDURES

CALIBRATION

To perform an AxSYM AUSAB calibration, test Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of controls (Negative Control, Positive Control 1, and Positive Control 2) must be tested as a means of evaluating the assay calibration.

Once the AxSYM AUSAB 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.
- Any of the AxSYM AUSAB 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 AUSAB Control values are within the ranges specified in this package insert. Refer to the **REAGENTS** section of this package insert for AxSYM AUSAB 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 AUSAB Controls has not been established with any other anti-HBs assays.

The AxSYM AUSAB Controls are in a serum matrix made from recalified 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-A2¹³, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions*: [Approved Guideline - Second Edition] or other published guidelines for general quality control recommendations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1202(c)¹⁴.

The minimum control requirement for an AxSYM AUSAB assay is a single sample of each of the controls (Negative Control, Positive Control 1, and Positive Control 2) 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 assay control values are within the ranges specified in the package insert. Refer to the **REAGENTS** section of this package insert for AxSYM AUSAB 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 AUSAB 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

AxSYM AUSAB utilizes a Four-Parameter Logistic Curve fit (4PLC Analysis) to generate a Standard Calibration curve. Refer to the AxSYM System Operations Manual, Appendix E, for further information.

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 AUSAB Results

Initial Result (mIU/mL)	Instrument Interpretation	Retest Procedure
> 12.0	REACTIVE	No Retest Required
8.0 to 12.0	GRAYZONE	Recentrifuge; Retest in Duplicate ^a
< 8.0	NONREACTIVE	No Retest Required

^a Gray zone 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.

Final AxSYM AUSAB Results

AxSYM AUSAB			Interpretation
Initial Interpretation	Retest Results	Final Result	
REACTIVE	No Retest Required		> 12 mIU/mL anti-HBs.
GRAYZONE	Both of the duplicate retests are reactive.	Reactive	Reactive for anti-HBs and individual is considered immune to HBV infection.
	One or both of the duplicate retests are repeatedly gray zone.	Gray Zone	Gray zone for anti-HBs and the immune status of the individual should be further assessed by considering other factors such as clinical status, follow-up testing, associated risk factors, and the use of additional diagnostic information.
	One retest is reactive and the other nonreactive.		
NONREACTIVE	Both of the duplicate retests are nonreactive.	Nonreactive	< 8 mIU/mL anti-HBs. Nonreactive for anti-HBs and individual is considered not immune to HBV infection.
	No Retest Required		

Refer to the SPECIMEN DILUTION PROCEDURES section of this package insert for calculation of diluted sample results.

- For diagnostic purposes, anti-HBs reactivity may be correlated with patient history and the presence of other hepatitis markers.
- Immunosuppressed or immunocompromised individuals may not produce anti-HBs above the limit of detection of the AxSYM AUSAB assay.

Individuals that have received blood component therapy (e.g., whole blood, plasma, immune globulin administration) during the previous 3 to 6 months may have a reactive anti-HBs result due to passive transfer of anti-HBs.¹⁵

LIMITATIONS OF THE PROCEDURE

- Quantitative anti-HBs values obtained using the AxSYM AUSAB assay may not be used interchangeably with values obtained with other manufacturers' assays.
- Plasma specimens collected in tubes containing potassium EDTA, lithium heparin, sodium citrate anticoagulants or in plasma separator tubes are not recommended for use in the AxSYM AUSAB assay.
- False reactive results caused by nonspecific reactions may be obtained with this or any diagnostic test. The most common reason for nonspecific reactions is particulate matter in the patient sample, particularly fibrin clots and cellular material.

- This assay does not differentiate between vaccine-induced immune response and an immune response induced by HBV infection. To determine if the anti-HBs response is due to vaccine or HBV infection, a total anti-HBc assay may be performed.
- High dose hook (prozone) effect: Specimens with anti-HBs concentrations greater than 130,000 mIU/mL may read lower than the Standard Calibrator F when tested undiluted with AxSYM AUSAB.

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, 57.97% (1,313/2,265) 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 47.60% Caucasian, 36.25% African American, 12.72% Hispanic, 1.45% Asian, 0.46% American Indian/Alaska Native, with the remaining 1.52% represented by other ethnic groups. The population was 62.15% female and 37.85% male ranging in age from 18 to 75 years. AxSYM AUSAB was reactive in 38.31% 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 AUSAB reactive results observed from each location. Table 2 is a summary of the percent AxSYM AUSAB reactive results by age range and gender.

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

Specimen Collection Site/ Specimen Vendor Location	Percent of Individuals at Increased Risk of HBV Infection Enrolled at Each Location	Percent of AxSYM AUSAB Reactive Results Observed From Each Location
Site 1, Galveston, TX	56.51 (742/1,313)	34.23 (254/742)
Site 2, Dallas, TX	4.49 (59/1,313)	25.42 (15/59)
Site 3, Miami, FL	3.96 (52/1,313)	63.46 (33/52)
Site 4, St. Petersburg, FL	4.27 (56/1,313)	25.00 (14/56)
Site 5, Chicago, IL	0.61 (8/1,313)	25.00 (2/8)
Site 6, Denver, CO	2.74 (36/1,313)	63.89 (23/36)
Specimen Vendor Location:		
Colton, CA	5.79 (76/1,313)	43.42 (33/76)
Plymouth, MA	7.54 (99/1,313)	24.24 (24/99)
High Point, NC	14.09 (185/1,313)	56.76 (105/185)

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

Age Range	Gender	AxSYM AUSAB Result			Total
		+ Number of Specimens	GZ Number of Specimens	- Number of Specimens	
10 to 19	Female	10	0	4	14
	Male	7	0	4	11
20 to 29	Female	82	2	100	184
	Male	33	1	63	97
30 to 39	Female	77	5	102	184
	Male	31	0	76	107
40 to 49	Female	103	5	143	251
	Male	47	1	111	159
50 to 59	Female	61	5	71	137
	Male	24	3	81	108
60 to 69	Female	19	2	14	35
	Male	3	0	9	12
70 to 79	Female	3	0	5	8
	Male	2	0	1	3
Unknown ^a	Female	1	0	2	3
Total		503 (38.31%)	24 (1.83%)	786 (59.86%)	1,313

GZ = Gray Zone

^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 CLSI document EP15-A2¹⁶. Testing was conducted at three clinical testing sites using three AxSYM AUSAB reagent, standard calibrator, and 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 two panel members and the AxSYM AUSAB Negative Control, Positive Control 1, and Positive Control 2. Panel members were prepared by adding recalcified human plasma reactive for anti-HBs to nonreactive human serum. The data are summarized in Table 3.

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

Sample	Total No. Reps	Target Conc. (mIU/mL)	Grand Mean Conc. (mIU/mL)	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	8	8.5	0.52	6.1	0.58	6.8	0.62	7.2	7.8	0.75	8.8	0.82	9.6	0.85	9.9
Panel 2	360	12	13.5	0.76	5.7	0.80	5.9	0.87	6.5	7.0	1.14	8.5	1.15	8.5	1.25	9.3
NC	360	0	0.1	0.05	NA	0.07	NA	0.07	NA	NA	0.20	NA	0.08	NA	0.20	NA
PC 1	360	15	16.4	1.04	6.3	1.16	7.1	1.28	7.8	8.5	1.37	8.3	1.46	8.9	1.46	8.9
PC 2	360	80	84.4	4.85	5.7	5.07	6.0	5.97	7.1	7.7	6.45	7.6	7.01	8.3	7.01	8.3

NA = Not Applicable, NC = Negative Control, PC = Positive Control, Repls = 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-A2¹⁷. Testing was conducted at Abbott Laboratories using two AxSYM AUSAB reagent pack and standard calibrator 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 panel members and the AxSYM AUSAB Negative Control, Positive Control 1, and Positive Control 2. Panel members were prepared by adding recalcified human plasma reactive for anti-HBs to nonreactive human serum. The data are summarized in Table 4.

**Table 4
AxSYM AUSAB Within-Laboratory Precision**

Sample	Total No. Repls	Target Conc. (mIU/mL)	Grand Mean Conc. (mIU/mL)	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 1	320	8	7.8	0.53	6.7	0.53	6.7	0.59	7.5	8.1	0.61	7.8	0.62	7.9
Panel 2	320	12	12.5	0.70	5.6	0.77	6.2	0.83	6.7	7.2	0.87	7.0	0.86	6.9
Panel 3	320	50	47.7	2.69	5.6	2.69	5.6	3.02	6.3	6.8	3.05	6.4	3.02	6.3
Panel 4	320	100	89.8	4.57	5.1	4.78	5.3	5.30	5.9	6.4	5.53	6.2	5.53	6.2
Panel 5	320	500	401.7	28.07	7.0	28.07	7.0	30.57	7.6	8.2	42.49	10.6	37.47	9.3
Panel 6	320	800	610.9	54.10	8.9	55.77	9.1	64.65	10.6	11.4	91.02	14.9	80.56	13.2
NC	320	0	0.2	0.06	NA	0.07	NA	0.08	NA	NA	0.11	NA	0.08	NA
PC 1	320	15	15.9	0.98	6.2	1.07	6.7	1.13	7.1	7.6	1.17	7.4	1.13	7.1
PC 2	320	80	82.1	4.26	5.2	4.86	5.9	5.05	6.2	6.6	5.27	6.4	5.27	6.4

NA = Not Applicable, NC = Negative Control, PC = Positive Control, Repls = Replicates, SD = Standard Deviation, CV = Coefficient of Variation, CL = Upper One-sided 95% Confidence Limit

CLINICAL PERFORMANCE

A multi-site study was conducted to evaluate the clinical performance of AxSYM AUSAB with serum specimens from 2,014 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 (39.32%); Dallas, TX (5.81%); Miami, FL (4.42%); St. Petersburg, FL (4.17%); Chicago, IL (8.19%); and Denver, CO (6.11%), or were obtained from a specimen vendor at the following three locations: Colton, CA (5.86%); Plymouth, MA (16.93%); and High Point, NC (9.19%). The population was Caucasian (52.88%), African American (28.55%), Hispanic (14.65%), Asian (1.99%), and American Indian/Alaska Native (0.45%), with the remaining 1.49% represented by other ethnic groups. The population was 52.53% female and 47.47% male and ranged in age from 18 to 83 years. 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 (39.32%); Dallas, TX (40.17%); and Raritan, NJ (20.51%).

The specimens were assigned an HBV classification (Table 5), and the AxSYM AUSAB results were compared to the reference anti-HBs results (Table 6). Agreement of the AxSYM AUSAB assay was assessed relative to the reference anti-HBs results (Table 7).

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 AUSAB clinical investigation.

**Table 5
HBV Classification for Individuals at Increased Risk of HBV Infection and
Individuals With Signs and Symptoms of Hepatitis Infection**

Number of Specimens	HBV Reference Markers				HBV Classification
	HBsAg	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
2	+	-	-	-	Early Acute
5	+	+	+	-	Acute
1	+	+	+		Chronic
2	+	-	+	+	Chronic
35	+	-	+	-	Chronic
1	+	-	-	+	Chronic
2	+	-	+		Chronic
1	+	+	+	+	Late Acute/Recovering
4	-	+	+	+	Recovering Acute
3	-	+	+		Early Recovery
193	-	-	+	+	Immune Due to Natural Infection
31	-	-	+		Distantly Immune/Anti-HBs Unknown
107	-	-	+	-	Distantly Immune/Anti-HBs Not Detected
507	-	-	-	+	Immune Due to HBV Vaccination
66	-	-	-		Unknown
1,054	-	-	-	-	Susceptible
2,014					Total

| = Indeterminate

Comparison of Results

Table 6 is a comparison of the AxSYM AUSAB results to the reference anti-HBs assay results by HBV classification.

**Table 6
Comparison of AxSYM AUSAB Results With Reference Anti-HBs Results by HBV Classification**

HBV Classification	Reference Anti-HBs Result ^a									Total
	+						-			
	+	GZ	-	+	GZ	-	+	GZ	-	
Early Acute	0	0	0	0	0	0	0	0	2	2
Acute	0	0	0	0	0	0	0	0	5	5
Chronic	2	0	1	2	0	1	0	0	35	41
Late Acute/Recovering	0	0	1	0	0	0	0	0	0	1
Recovering Acute	4	0	0	0	0	0	0	0	0	4
Early Recovery	0	0	0	0	0	3	0	0	0	3
Immune Due to Natural Infection	180	4	9	0	0	0	0	0	0	193
Distantly Immune/Anti-HBs Unknown	0	0	0	9	5	17	0	0	0	31
Distantly Immune/Anti-HBs Not Detected	0	0	0	0	0	0	3	0	104	107
Immune Due to HBV Vaccination	503	2	2	0	0	0	0	0	0	507
Unknown	0	0	0	23	24	19	0	0	0	66
Susceptible	0	0	0	0	0	0	2	3	1,049	1,054
Total	689	6	13	34	29	40	5	3	1,195	2,014

| = Indeterminate; GZ = Gray Zone

^a Includes retesting and dilution performed according to the package insert as required.

^b Includes retesting and dilution as required.

Percent Agreement

Table 7 is a summary, for each HBV classification, of the percent agreement between AxSYM AUSAB and the reference anti-HBs assay. Samples that were indeterminate by the reference anti-HBs assay and reactive or nonreactive by AxSYM AUSAB were considered discordant for the percent agreement calculation.

Table 7
Percent Agreement Between AxSYM AUSAB Results and Reference Anti-HBs Results Summarized by HBV Classification

HBV Classification	Positive Percent Agreement (%)	95% Confidence Interval (%)	Negative Percent Agreement (%)	95% Confidence Interval (%)
Early Acute	NA	NA	2/2 (100.00)	[15.81, 100.00]
Acute	NA	NA	5/5 (100.00)	[47.82, 100.00]
Chronic	2/4 (50.00)	[6.76, 93.24]	35/37 (94.59)	[81.81, 99.34]
Late Acute/Recovering	0/1 (0.00)	[0.00, 97.50]	NA	NA
Recovering Acute	4/4 (100.00)	[39.76, 100.00]	NA	NA
Early Recovery	0/3 (0.00)	[0.00, 70.76]	NA	NA
Immune Due to Natural Infection	180/193 (93.26)	[88.76, 96.37]	NA	NA
Distantly Immune/Anti-HBs Unknown	0/17 (0.00)	[0.00, 19.51]	0/9 (0.00)	[0.00, 33.63]
Distantly Immune/Anti-HBs Not Detected	NA	NA	104/107 (97.20)	[92.02, 99.42]
Immune Due to HBV Vaccination	503/507 (99.21)	[97.99, 99.78]	NA	NA
Unknown	0/19 (0.00)	[0.00, 17.65]	0/23 (0.00)	[0.00, 14.82]
Susceptible	NA	NA	1,049/1,054 (99.53)	[98.90, 99.85]
Overall	689/748 (92.11)	[89.94, 93.94]	1,195/1,237 (96.60)	[95.44, 97.54]

NA = Not Applicable

Percent Agreement for Hepatitis B Vaccine Recipients

AxSYM AUSAB performance was further evaluated by testing 211 prospectively-collected serum specimens from hepatitis B vaccine recipients. Each specimen was tested using a reference total anti-HBc assay and found to be negative. Individuals received a full course of injections (three) of one of the following vaccines (See Table 8):

Table 8
Hepatitis B Vaccine Recipients

Vaccine Manufacturer and Trade Name	Number of Recipients	Percent of Recipients (%)
GlaxoSmithKline Engerix-B®	106	50.24
GlaxoSmithKline Twinrix®	1	0.47
Merck & Co. RECOMBIVAX HB®	49	23.22
Merck & Co. HEPTAVAX-B™	9	4.27
Merck & Co. Trade Name Unknown	8	3.79
Sanofi Pasteur MSD	12	5.69
Other ^a	5	2.37
Unknown	21	9.95
Total	211	100.00

^a Other includes different combinations of manufacturer and trade name for the three doses.

Positive and negative percent agreement between the AxSYM AUSAB assay and the reference anti-HBs assay were calculated. Positive percent agreement was 92.31% (144/156) with a 95% confidence interval of 86.95% to 95.96%. Negative percent agreement was 88.00% (44/50) with a 95% confidence interval of 75.69% to 95.47%. Table 9 is a comparison of the results for hepatitis B vaccine recipients.

Table 9
Comparison of the AxSYM AUSAB Results With Reference Anti-HBs Results for Hepatitis B Vaccine Recipients

AxSYM AUSAB Result	Reference Anti-HBs Result		
	+		-
+	144	4	0
GZ	1	5	2
-	1	10	44

| = Indeterminate; GZ = Gray Zone

Percent Agreement for Hepatitis B Vaccine Recipients (Pre- and Post- Samples)

Forty matched serum specimens from 20 hepatitis B vaccine recipients (pre- and post- samples) were obtained. Pre-samples were negative for anti-HBs and total anti-HBc. Table 10 is a summary, by vaccination status, of the percent agreement between AxSYM AUSAB and the reference anti-HBs assay.

Table 10
Percent Agreement for Hepatitis B Vaccine Recipients (Pre- and Post- Samples)

Vaccination Status	Positive Percent Agreement (%)	95% Confidence Interval (%)	Negative Percent Agreement (%)	95% Confidence Interval (%)
Pre-vaccination	NA	NA	20/20 (100.00)	[83.16, 100.00]
Post-vaccination	18/18 (100.00)	[81.47, 100.00]	2/2 (100.00)	[15.81, 100.00]
Pre- and Post-vaccination	18/18 (100.00)	[81.47, 100.00]	22/22 (100.00)	[84.56, 100.00]

NA = Not Applicable

Clinical Performance in a Pediatric Population

The performance of AxSYM AUSAB in a pediatric population was evaluated by testing specimens from a surplus pediatric population (n=120) collected in Fall River, MA by a specimen vendor, and from the pediatric subjects (n = 82) from the increased risk and signs and symptoms population shown in the Clinical Performance section of this package insert. Positive and negative percent agreement between the AxSYM AUSAB assay and the reference anti-HBs assay were calculated. Due to insufficient quantity of sample to complete reference assay testing, 16 specimens were not included in the percent agreement calculations. Table 11 is a demographic summary of the surplus population by age range and gender with AxSYM AUSAB results. Positive percent agreement was 98.51% (66/67) with a 95% confidence interval of 91.96% to 99.96%. Negative percent agreement was 100.00% (37/37) with a 95% confidence interval of 90.51% to 100.00%.

Table 11
Demographic Summary With AxSYM AUSAB Results for a Surplus Pediatric Population

Age Range	Gender	AxSYM AUSAB Result			Total
		+	GZ	-	
		Number of Specimens	Number of Specimens	Number of Specimens	
> 1 Month to 2 Years	Female	5	0	0	5
	Male	13	0	2	15
> 2 to 12 Years	Female	13	1	11	25
	Male	8	3	14	25
> 12 to 19 Years	Female	16	1	11	28
	Male	13	1	8	22
Total		68 (56.67%)	6 (5.00%)	46 (38.33%)	120

GZ = Gray Zone

Table 12 is a demographic summary of the prospectively collected population by age range and gender with AxSYM AUSAB results. The negative percent agreement was 92.59% (25/27) with a 95% confidence interval of 75.71% to 99.09%. The positive percent agreement was 100.00% (55/55) with a 95% confidence interval of 93.51% to 100.00%.

Table 12
Demographic Summary With AxSYM AUSAB Results for a Prospectively Collected Pediatric Population

Age Range	Gender	AxSYM AUSAB Result			Total
		+	GZ	-	
		Number of Specimens	Number of Specimens	Number of Specimens	
> 18 to 21 Years	Female	25	0	10	35
	Male	32	0	15	47
Total		57 (69.51%)	0 (0.00%)	25 (30.49%)	82

GZ = Gray Zone

ANALYTICAL SENSITIVITY

Limit of Blank, Limit of Detection, and Limit of Quantitation

A study was conducted based on guidance from NCCLS EP17-A¹⁸ producing a Limit of Blank of 0.10 mIU/mL, a Limit of Detection of 0.30 mIU/mL, and a Limit of Quantitation of 2.50 mIU/mL for the AxSYM AUSAB assay.

LINEARITY

The AxSYM AUSAB assay has been demonstrated to be linear (within 20%) up to a concentration of 500 mIU/mL using dilutions of the WHO Standard with known concentrations.

WHO Linearity

A study was conducted to evaluate the linearity of the AxSYM AUSAB assay using dilutions of the World Health Organization Reference Standard for Anti-HBs. Passing-Bablok and least squares linear regression analyses were performed using the mean concentration of 18 replicates of each of 7 WHO Standard dilutions versus the expected concentration. The slope, Y-intercept, and correlation coefficient were determined and 95% confidence intervals were calculated. Predicted concentrations were calculated by multiplying the expected concentrations by the slope and adding the Y-intercept. The data are summarized in Tables 13 and 14.

Table 13
AxSYM AUSAB WHO Standard Linearity

Regression Type	n	Slope		Y-intercept		Correlation Coefficient
		Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate
Passing-Bablok	7	1.00	[0.92, 1.22]	0.00	[-10.34, 4.67]	0.99
Least Squares	7	0.96	[0.83, 1.10]	15.32	[-34.97, 65.61]	0.99

n = number of dilutions

Table 14
AxSYM AUSAB WHO Standard Dilution Linearity Study - Predicted Concentrations

Expected Concentration (mIU/mL)	n	Mean Observed Concentration (mIU/mL)	Passing-Bablok Predicted Concentration (mIU/mL)	Least Squares Predicted Concentration (mIU/mL)
0	18	0.0	0.0	15.3
10	18	10.0	10.0	24.9
50	18	50.7	49.9	63.4
100	18	99.6	99.9	111.5
250	18	305.2	249.7	255.9
500	18	546.1	499.4	496.5
800	18	741.2	799.1	785.1

Specimen Dilution Linearity

A study was conducted to evaluate the linearity performance of the AxSYM AUSAB assay using serial dilutions of HBV natural infection specimens (recovered) and HBV vaccinee specimens (vaccinees). A linear regression analysis was performed for each specimen using the expected and observed concentrations. The slope, Y-intercept, and correlation coefficient were determined and 95% confidence intervals were calculated. The data are summarized in Table 15.

Table 15
AxSYM AUSAB Specimen Dilution Linearity

Group	Sample	n	Slope		Y-intercept		Correlation Coefficient	
			Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Recovered	1	8	1.00	[0.86, 1.15]	41.73	[-6.88, 90.34]	0.99	[0.94, 1.00]
	2	8	1.01	[0.92, 1.10]	14.59	[-6.78, 35.96]	1.00	[0.98, 1.00]
	3	7	1.01	[0.95, 1.07]	1.44	[-3.00, 5.88]	1.00	[0.99, 1.00]
	4	8	1.01	[0.88, 1.14]	16.73	[-6.21, 39.68]	0.99	[0.95, 1.00]
Vaccinees	1	8	1.01	[0.82, 1.21]	39.96	[-11.59, 91.52]	0.98	[0.90, 1.00]
	2	7	1.01	[0.91, 1.12]	12.63	[-15.23, 40.50]	1.00	[0.97, 1.00]
	3	8	1.03	[0.86, 1.20]	26.92	[-23.24, 77.09]	0.99	[0.92, 1.00]
	4	7	0.98	[0.92, 1.04]	14.54	[-0.67, 29.74]	1.00	[0.99, 1.00]

n = number of serial dilutions

ANALYTICAL SPECIFICITY

Cross-reactivity

The AxSYM AUSAB assay was evaluated for potential cross-reactivity by testing specimens from individuals with medical conditions unrelated to HBV infection in comparison to an FDA-licensed anti-HBs reference assay. A total of 210 specimens from 15 different categories were tested. One hundred forty-five (145) specimens were nonreactive (69.0%), 1 was gray zone (0.5%), and 64 were reactive (30.5%) by AxSYM AUSAB. The data are summarized in Table 16.

Table 16
Cross-reactivity of AxSYM AUSAB in Specimens From Individuals With Medical Conditions Unrelated to HBV

Specimen Category ^a	Number of Specimens Tested	Reference Anti-HBs Assay					
		+			-		
		AxSYM AUSAB			AxSYM AUSAB		
		+	GZ	-	+	GZ	-
Hepatitis A Virus	10	5	0	0	0	0	5
Hepatitis C Virus	15	3	0	0	0	1	11
Human Immunodeficiency Virus	15	2	0	0	0	0	13
Human T-Lymphotropic Virus	10	2	0	1	0	0	7
Cytomegalovirus	10	5	0	0	0	0	5
Epstein-Barr Virus	10	7	0	1	0	0	2
Herpes Simplex Virus	15	7	0	3	0	0	5
Rubella	10	2	0	0	0	0	8
Systemic Lupus Erythematosus	15	4	0	0	0	0	11
Rheumatoid Factor Positive	12	2	0	0	0	0	10
Human Anti-mouse Antibody Positive	5	1	0	0	0	0	4
Elevated IgM	9	0	0	0	0	0	9
Influenza Vaccine Recipients	15	4	0	0	0	0	11
Toxoplasmosis	9	4	0	0	0	0	5
Nonviral Liver Disease	50	16	0	3	0	0	31
Total (%)	210	64/210 (30.5%)	0/210 (0.0%)	8/210 (3.8%)	0/210 (0.0%)	1/210 (0.5%)	137/210 (65.2%)

GZ = Gray Zone

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

Interference

The AxSYM AUSAB assay was evaluated for interference by testing the potentially interfering substances listed in Table 17 and Table 18. Testing was performed using human serum nonreactive for anti-HBs supplemented with anti-HBs positive plasma to a target concentration of 8 mIU/mL or 12 mIU/mL. Separate aliquots were spiked with each interferent at the level listed below and tested in the AxSYM AUSAB assay. Results were compared to an unspiked control.

Table 17
Evaluation of Potentially Interfering Substances in AxSYM AUSAB (8 mIU/mL)

Potential Interferent	Target Spike Level	Mean Concentration (mIU/mL)			%Bias
		Control (Unspiked)	Spiked	Difference	
Hemoglobin	500 mg/dL	6.7	3.9	-2.8	-41.35 ^a
Total Bilirubin (unconjugated)	20 mg/dL	6.4	4.8	-1.6	-25.32 ^a
Total Protein	12 g/dL	7.5	8.1	0.7	8.95
Triglycerides	3,000 mg/dL	6.3	7.0	0.7	11.36

^a While the % bias is greater than 20% for the hemoglobin and total bilirubin samples, the negative samples shifted to a lower negative value when spiked with these interferents.

Table 18
Evaluation of Potentially Interfering Substances in AxSYM AUSAB (12 mIU/mL)

Potential Interferent	Target Spike Level	Mean Concentration (mIU/mL)			%Bias
		Control (Unspiked)	Spiked	Difference	
Hemoglobin	500 mg/dL	11.3	10.5	-0.7	-6.45
Total Bilirubin (unconjugated)	20 mg/dL	10.6	8.8	-1.8	-16.95
Total Protein	12 g/dL	11.5	13.1	1.7	14.44
Triglycerides	3,000 mg/dL	10.5	11.4	0.9	8.08

TUBE TYPE MATRIX COMPARISON

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

- Glass: serum and serum separator
- Plastic: serum, serum separator, and sodium heparin

On average, the tube types evaluated showed less than a 10% difference when compared to the control tube type (glass serum). The distribution of the percent differences per tube type is listed in the following table.

Table 19
Sample Type (Serum and Plasma) Study of AxSYM AUSAB Distribution of % Differences by Sample Type

Evaluation Tube Type	Distribution of %Differences		
	0% to ≤ 10%	> 10% to ≤ 20%	> 20%
Glass Serum Separator	59.2% (29/49)	32.7% (16/49)	8.2% (4/49)
Plastic Serum	59.2% (29/49)	32.7% (16/49)	8.2% (4/49)
Plastic Serum Separator	67.3% (33/49)	20.4% (10/49)	12.2% (6/49)
Plastic Sodium Heparin	65.3% (32/49)	22.4% (11/49)	12.2% (6/49)

Note: A negative bias was observed for all tube types when compared to serum in glass.

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