Abbott AXSYM SYSTEM

en

AUSAB

REF 7A39

49-1396/R15

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Read Highlighted Changes
Revised March, 2010

AUSAB

Hepatitis B Surface Antigen (Recombinant) (Subtypes ad and ay)

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

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

Key to symbols used

REF	List Number	STANDARD CAL A	Standard Calibrator (A-F)
IVD	*In Vitro* Diagnostic Medical Device	REACTION VESSELS	Reaction Vessels
LOT	Lot Number	MATRIX CELLS	Matrix Cells
	Expiration Date	SAMPLE CUPS	Sample Cups
2°C 8°C	Store at 2-8°C	REAGENT PACK	Reagent Pack
15°C 30°C	Store at 15-30°C		Consult instructions for use
	Caution	CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
	Manufacturer		

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

Abbott

NAME

AxSYM AUSAB

INTENDED USE

AxSYM AUSAB is a microparticle enzyme immunoassay (MEIA) for the quantitative determination of antibody to Hepatitis B Surface Antigen (anti-HBs) in human serum or plasma. It is intended for the measurement of anti-HBs following hepatitis B virus (HBV) infection or vaccination.

SUMMARY AND EXPLANATION OF TEST

AxSYM AUSAB is a Microparticle Enzyme Immunoassay which has been developed to measure the amount of antibody to the Hepatitis B Surface Antigen (anti-HBs) present in human serum or plasma.

Anti-HBs assays are often used to monitor the success of hepatitis B vaccination. 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 producing anti-HBs titers and in preventing HBV infection.[2-4]

Assays for anti-HBs are also used to monitor the convalescence and recovery of hepatitis B infected individuals. The presence of anti-HBs after acute HBV infection and loss of HBsAg can be a useful indicator of disease resolution. Detection of anti-HBs in an asymptomatic individual may indicate previous exposure to HBV.

Samples with anti-HBs concentrations less than 10.0 mIU/mL are considered nonreactive by the AxSYM AUSAB assay. Samples with anti-HBs concentrations greater than or equal to 10.0 mIU/mL are considered reactive.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM AUSAB is based on Microparticle Enzyme Immunoassay (MEIA) technology.

Sample and all AxSYM AUSAB reagents required for one test are pipetted by the Sampling Probe into various wells of a reaction vessel (RV) in the Sampling Center. The RV is immediately transferred into the Processing Center. Further pipetting is done in the Processing Center by the Processing Probe.

The reactions occur in the following sequence:

- Sample and Recombinant Hepatitis B Virus Surface Antigen (rHBsAg) Coated Microparticles are combined in one RV well.
- When anti-HBs is present in the sample, it binds to the coated microparticles forming an antibody-antigen complex in the reaction mixture.
- A portion of the reaction mixture is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.
- Biotinylated rHBsAg is dispensed onto the matrix cell forming an antigen-antibody-antigen complex.
- The Anti-Biotin:Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds to any microparticle-bound antigen-antibody-antigen complex.
- The matrix cell is washed to remove materials not bound to the microparticles.
- The substrate, 4-Methylumbelliferyl Phosphate, is added. The alkaline phosphatase-labeled conjugate catalyzes the removal of a phosphate group from the substrate, yielding the fluorescent product, 4-Methylumbelliferone. This fluorescent product is measured by the MEIA optical assembly.

The concentration of anti-HBs in the sample is determined using a previously generated calibration curve. If the concentration of the sample is greater than or equal to 10.0 mIU/mL, the sample is considered reactive for anti-HBs.

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

REAGENTS

Reagent Pack, 100 Tests

AxSYM AUSAB Reagent Pack (7A39-22)

- 1 Bottle (4.9 mL) Hepatitis B Surface Antigen (Recombinant) (Subtypes ad and ay) Coated Microparticles in TRIS buffer with protein stabilizers. Minimum concentration: 0.05%. Preservative: Sodium Azide. (Reagent Bottle 2)
- 1 Bottle (13.0 mL) Anti-Biotin (Rabbit):Alkaline Phosphatase Conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.05 µg/mL. Preservative: Sodium Azide. (Reagent Bottle 1)
- 1 Bottle (12.6 mL) Hepatitis B Surface Antigen (Recombinant) (Subtypes ad and ay): Biotin Conjugate in TRIS buffer with animal sera (Calf, Rabbit) is prepared with recalcified human plasma. Minimum concentration: 1 µg/mL. Preservative: Sodium Azide. (Reagent Bottle 3)
- 1 Bottle (17.8 mL) Dilution Reagent. Recalcified human plasma, sufficient for testing of 50 dilutions. Preservative: Sodium Azide. (Reagent Bottle 4)

Calibrators

AxSYM AUSAB Standard Calibrators (7A39-01)

6 Bottles (4 mL each) of AxSYM AUSAB Standard Calibrators are prepared with recalcified human plasma. Calibrator A is nonreactive for anti-HBs. Calibrators B through F are reactive for anti-HBs. Preservative: Sodium Azide.

The AxSYM AUSAB Standard Calibrators have the following concentrations of anti-HBs:

Standard Calibrator	Anti-HBs Concentration* mIU/mL
STANDARD CAL A	0
STANDARD CAL B	10
STANDARD CAL C	50
STANDARD CAL D	100
STANDARD CAL E	500
STANDARD CAL F	1000

* The AxSYM AUSAB primary calibrators are manufactured by dilution and referenced to the World Health Organization (WHO) 1st International Reference Preparation of anti-hepatitis B immunoglobulin at each concentration level.

Controls

AxSYM AUSAB Controls (7A39-10)

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

CONTROL – The Negative Control is nonreactive for anti-HBs.

CONTROL + The Positive Control is reactive for anti-HBs.

The AxSYM AUSAB controls have the following concentrations of anti-HBs:

Control	Color	Anti-HBs Concentration* mIU/mL***	Anti-HBs Acceptable Range mIU/mL
CONTROL –	Natural	0	0 - 2
CONTROL +	Blue**	80	60 - 100

* The AxSYM AUSAB primary controls are manufactured by dilution and referenced to the World Health Organization (WHO) 1st International Reference Preparation of anti-hepatitis B immunoglobulin at each concentration level.

** Dye: Acid Blue No. 9

*** The AxSYM AUSAB reporting unit is factory set to mIU/mL. An alternate result unit IU/L may be selected for reporting results (Assay Parameter 45).

Other reagents

AxSYM AUSAB Dilution Reagent (7A39-50)

DILUTION REAGENT 1 Bottle (100 mL) AxSYM AUSAB Dilution Reagent. Recalcified human plasma. Preservative: Sodium Azide.

AxSYM Probe Cleaning Solution (9A35-05)

PROBE CLEANING SOLUTION 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% Tetraethylammoniumhydroxide (TEAH).

Solution 1 (MUP) (8A47-04)

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

Solution 3 (Matrix Cell Wash) (8A81-04)

SOLUTION 3 MATRIX CELL WASH 4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M Sodium Chloride in TRIS Buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

Solution 4 (Line Diluent) (8A46)

SOLUTION 4 LINE DILUENT 1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M Phosphate Buffer. Preservatives: Sodium Azide and Antimicrobial Agent.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use

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

Safety Precautions

- **CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources 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.[5] Biosafety Level 2[6] or other appropriate biosafety practices[7,8] should be used for materials that contain or are suspected of containing infectious agents.
- The human plasma used in the Biotin Conjugate is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, anti-HIV-1/HIV-2, and anti-HBs.
- The human plasma used in the Dilution Reagent is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, anti-HIV-1/HIV-2, and anti-HBs.
- The human plasma used in the Calibrator A and the Negative Control is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV anti-HIV-1/HIV-2, and anti-HBs.
- The human plasma used in Calibrators B-F and the Positive Control is reactive for anti-HBs, and nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2.
- 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 splashes, air bubbles, foaming and require inspection and removal of bubbles before loading. If bubbles are present, refer to the AxSYM System Operations Manual, Section 9: Service and Maintenance, Subsection: Daily Maintenance.
- **Do not use Solution 1 (MUP) beyond the expiration date or a maximum of fourteen 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 to air may compromise performance.**
- Do not use kits beyond the expiration date or a maximum of 112 cumulative hours on-board the AxSYM System.
- Do not mix reagents from different reagent packs. Do not mix reagent packs and calibrators from different lots.
- Avoid microbial contamination of samples 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.
- Inadequate adherence to package insert instructions may result in erroneous results.
- Use accurately calibrated equipment.
- Use caution in handling patient samples to prevent cross contamination.

Additional safety and handling precautions and limitations for the reagent packs, calibrators, controls, patient samples and other reagents are described in the AxSYM System Operations Manual, Sections 7 and 8.

Storage Instructions

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The AxSYM AUSAB Reagent Pack, Standard Calibrators, Dilution Reagent and Controls must be stored at 2-8˚C. The AxSYM AUSAB Reagent Pack, Calibrators, Controls, and Dilution Reagent may be used immediately after removal from the refrigerator. Do not freeze the AxSYM AUSAB reagents.

  The AxSYM AUSAB Reagent Pack may be on-board the AxSYM System for a maximum of 112 cumulative hours. After 112 hours, the reagent pack must be discarded. Refer to the AxSYM System Operations Manual, Sections 2 and 5 and Appendices, for further information on tracking on-board time.

  Solution 1 (MUP) must be stored at 2-8˚C. It may be on-board the AxSYM System for a maximum of fourteen days. After fourteen days, it must be discarded.

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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 Positive or Negative Control value is out of the expected range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and must be retested. Assay recalibration may be necessary.

INSTRUMENT PROCEDURE

Assay File Installation

The AxSYM AUSAB Assay file must be installed on the AxSYM System from the software disk, 9A15-04, or higher, prior to performing AUSAB assays. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM AUSAB Assay Parameters

The default assay parameters for the AxSYM AUSAB assay are listed below. These parameters can be printed, displayed, and edited according to the procedure in the AxSYM System Operations Manual, Section 2. To print the ASSAY PARAMETERS, press PRINT*. Assay parameters that can be edited contain a (>) symbol. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameter screen.

* Current assay file contains parameters for Master Calibrators. This information is not applicable as of 31 July 2010.

Assay Parameters	
1	Long Assay Name (English): AUSAB
6	Abbrev Assay Name (English): AUSAB
11	Assay Number: 118
12	Assay Version: 3
13	Calibration Version: 00
14	Assay File Revision: *
15	Assay Enabled > ON
17	Assay Type: MEIA
18	Standard Cal Reps > 2
21	Cal A Concentration: 0.0
22	Cal B Concentration: 10.0
23	Cal C Concentration: 50.0
24	Cal D Concentration: 100.0
25	Cal E Concentration: 500.0
26	Cal F Concentration: 1000.0
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Standard Cal
45	Selected Result Concentration Units > mIU/mL
46	Selected Result Decimal Places > 1
64	Max Intercept-Max MUP intercept: 12000.0000
65	Min Intercept-Min MUP intercept: 1100.0000
66	Upper limit for NRMSE for low rates: 9999.9900
67	Upper limit for NRMSE for high rates: 0.5000
68	Max Rate-Max rate used to check Min MUP Intercept: 1700.0000
69	Min Rate-Rate cutoff for NRMSE and Corr. Coef: 8.0000
70	Min correlation coefficient for low rates: 0.9700
71	Min correlation coefficient for high rates: 0.9700
72	MUP T Delay-Time delay following MUP: 2.2000
80	Interpretation Option to use > 1
84	Hold results with POS interpretation > OFF
85	Hold results with NEG interpretation > OFF
86	Hold results with GRY interpretation > OFF
91	Low Range Neat: 0.0
92	High Range Neat: 1000.0
96	Low Range Dil1: 0.0
97	High Range Dil1: 25000.0
117	Negative Interpretation Cutoff >10.0

NOTE: Parameter 46 must not be edited below 1 decimal place.

NOTE: Parameter 45 can be edited to the alternate result unit IU/L.

NOTE: A grayzone feature is available. To utilize the grayzone option, parameter 117, Negative Interpretation Cutoff, must be edited to the desired value within the range of 5.0 to 9.9 mIU/mL. Specimens with mIU/mL values equal to the selected value and less than 10.0 are indicated as "GZ-NONREACTIVE" or "GZ-NEGATIVE" in the interpretation field. The report options are selected in parameter 80, Interpretation Option to Use.

The AxSYM AUSAB values available for parameter 80 (Interpretation Option to use) are:

	POS Interp	NEG Interp	GRY Interp
1.	REACTIVE	NONREACTIVE	GZ-NONREACTIVE
2.	REACTIVE	NEGATIVE	GZ-NEGATIVE
3.	REACTIVE	BLANK	BLANK
4.	BLANK	BLANK	BLANK

Refer to the AxSYM System Operations Manual for a detailed description of Instrument Procedures.

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

- Serum (including serum collected in separator tubes) or plasma (collected in sodium heparin, sodium citrate, ACD, CPDA-1, or EDTA) may be used in the AxSYM AUSAB Assay. Use the ratio of anticoagulant (quantity) to sample (volume) that is recommended by the manufacturer.
- The AxSYM System does not have the capability to verify sample type. It is the responsibility of the operator to verify the correct sample type(s) is(are) used in the AxSYM AUSAB assay.
- Samples containing fibrin, particulate matter or red blood cells may give inconsistent or erroneous results and must be completely clotted and centrifuged prior to testing.
- Inspect all samples for splashing, air bubbles and foaming. If necessary, remove bubbles prior to analysis using a new applicator stick for each sample. Refer to the AxSYM System Operations Manual, Section 7.
- Specimens with obvious microbial contamination should not be used.
- All patient samples to be tested in Primary Tubes must be centrifuged to remove red cells or particulate matter. Each sample that requires repeat testing, or that has been frozen and thawed, must be transferred to a centrifuge tube and centrifuged at a minimum of 10,000 RCF* for 10 minutes. Transfer clarified sample to a sample cup for testing.

  WARNING: AxSYM System Software Version 3.0 and higher offers an Auto Retest/Auto Dilution feature. Due to requirements discussed above, this feature must not be used.
- Samples from heparinized patients may be partially coagulated and erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the sample prior to heparin therapy.
- Samples may be stored on the clot or red blood cells for up to 7 days at 2-8˚C prior to being tested.
- If the assay will be performed after 7 days, the sample must be removed from the clot or red blood cells and stored frozen (-10˚C or colder).
- Performance differences observed were within assay variability for AxSYM AUSAB when 15 nonreactive and 15 reactive samples were subjected to 6 freeze/thaw cycles; however, multiple freeze/thaw cycles should be avoided. Samples must be mixed thoroughly after thawing and centrifuged prior to use, as described in this section, to remove particulate matter and to ensure consistency in the results.
- When shipped, samples must be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical samples and etiologic agents. Samples may be shipped either refrigerated on wet ice (2-8˚C) or frozen on dry ice (-10˚C or colder). Prior to shipment under ambient conditions, samples must be removed from the clot or red blood cells.
- All samples (patient samples, 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 more detailed discussion of on-board storage constraints.
- Do not use heat-inactivated samples.
- Performance has not been established for cadaver samples or body fluids such as urine, saliva, semen or amniotic fluid.
- Performance differences observed were within assay variability when AxSYM AUSAB Calibrators were tested with elevated levels of bilirubin (0-20 mg/dL), hemoglobin (0-500 mg/dL) or lipids (0-2000 mg/dL).

Sample Volume

Sample volume required to perform a single AUSAB test on the AxSYM System varies according to the different sample containers. For sample cups, the minimum sample volume required is 210 µL. For every additional AUSAB test performed from the same sample container, an additional 160 µL of sample will be required.

* RCF = Relative Centrifugal Force

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 AUSAB dilution is 94 µL. For every additional AUSAB dilution performed from the same container, an additional 44 µL of sample will be required. Refer to the AxSYM System Operations Manual, Section 5.

The sample cup minimum volume will be calculated by the AxSYM System. It will be displayed on the Order screen at the time the test(s) is(are) ordered, and printed in the Orderlist Report.

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 of the Positive or Negative Control into each respective sample cup.

For sample volume requirements in primary or aliquot tubes and calibrator and control volume requirements for multiple AxSYM AUSAB reagent lots, refer to the AxSYM System Operations Manual, Sections 5 and 6.

AxSYM AUSAB PROCEDURE

Materials Required

- 7A39-22 — AxSYM AUSAB Reagent Kit, containing:
  - AxSYM AUSAB **REAGENT PACK**
  - 100 **REACTION VESSELS**
  - 100 **MATRIX CELLS**
- 7A39-10 — AxSYM AUSAB Controls
- 7A39-01 — AxSYM AUSAB Standard Calibrators
- 7A39-50 — AxSYM AUSAB **DILUTION REAGENT**
- 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**

Materials Required But Not Provided

- Pipettes or pipette tips
- Cotton-tipped applicators

CAUTION:

- When manually dispensing samples, verify that dispensing equipment does not introduce cross contamination and that it delivers the specified sample volume. Use a separate pipette or pipette tip for each sample.
- 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.

The AxSYM system "Automatic Sample Retest" feature must not be used due to the AxSYM AUSAB assay requirement to centrifuge all samples prior to repeat testing.

1. Check for sufficient on-board inventory of matrix cells and bulk solutions, and for 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 AUSAB Calibrators, AUSAB Controls, and/or patient samples as required. Assign or modify the sample segment position (S/P) for each sample, as necessary.

   Refer to the Quality Control Procedures section in this package insert for calibration and control requirements.

   Calibration:

   Dispense 11* drops of the A - F Calibrators into individual sample cups.

   * When more than one AxSYM AUSAB reagent lot is on-board the AxSYM System, multiply calibrator and control volumes by the number of lots.

   Controls:

   Perform quality control by testing Positive and Negative Controls (one test each). Dispense 9* drops each of the Positive and Negative Controls into individual sample cups.

   * When more than one AxSYM AUSAB reagent lot is on-board the AxSYM System, multiply calibrator and control volumes by the number of lots.

   Patient Samples:

   Ensure that sufficient volume is present in the sample cups or tubes. The sample cup minimum volume is 210 µL for the first AUSAB test plus 160 µL for each additional 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 pressing PRINT. The printout contains sample placement information and minimum sample cup volume requirements for all tests ordered. When using Host Order Query, the Order screen information and the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5: Operating Instructions, Subsection: Ordering Patient Samples, for a description of the Host Query Option.
4. Place the sample segments containing the ordered samples into the Sample Carousel.
5. Open Reagent Bottle 4 containing the Dilution Reagent. Remove any droplets of Dilution Reagent on the inside of the cap with a cotton-tipped applicator. Failure to do so may result in contamination of the Reagent Pack Actuator.
6. Place the AUSAB Reagent Pack into the Reagent Pack Carousel.
7. Ensure that reaction vessels (RVs) are present on the RV Carousel. Additional RVs may be added as needed.
8. Press RUN. All entries on the Orderlist screen are transferred to the Order Status screen for sample processing.
9. Review the results to determine whether dilution of the sample is required. See the Sample 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.
10. When testing is completed, close the Reagent Bottle 4, remove the samples and the AxSYM AUSAB Reagent Pack from the Sampling Center. Store at 2-8˚C.

    NOTE: When using the on-board reagent stability tracking feature, the operator must perform a scan when removing any pack from the system in order to maintain the validity of the reagent stability timer.

Refer to Sections 5 and 6 of the AxSYM System Operations Manual for a detailed explanation of performing assay calibration and sample testing procedures.

SAMPLE DILUTION PROCEDURES

Automated Dilution Protocol

Patient samples with anti-HBs concentrations reported as greater than 1,000.0 mIU/mL (>1000.0 mIU/mL) may be diluted using the Automated Dilution Protocol. A sample volume of 150 µL is required unless the test is performed STAT. The minimum volume for a STAT AUSAB dilution test is 94 µL. For every additional AUSAB dilution performed from the same container, an additional 44 µL of sample will be required. The AxSYM System automatically calculates the concentration of the diluted sample and reports the result. Refer to Section 5 of the AxSYM System Operations Manual for additional information on ordering sample dilutions.

Manual Dilution Protocol

Patient samples with anti-HBs concentrations reported as greater than 25,000.0 mIU/mL (>25000.0 mIU/mL) by the Automated Dilution Protocol may be diluted using a manual dilution of 1:25. Add 10 µL of the patient sample to 240 µL of the AxSYM AUSAB Dilution Reagent. Repeat the Automated Dilution Protocol using this manually diluted sample. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

$$\text{Final Sample Concentration} = \text{Reported Concentration} \times \text{Manual Dilution Factor}$$

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Sample + Volume of Dilution Reagent)}}{\text{Volume of Sample}}$$

QUALITY CONTROL PROCEDURES

Calibration

The AxSYM AUSAB assay must be calibrated using a Standard Calibration (6-point) procedure.

Standard Calibration

To perform an AxSYM AUSAB Standard Calibration, test Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of both the Positive and Negative Controls 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:

- A reagent pack with a new lot number is used for an assay.
- Either of the AxSYM AUSAB Control values is out of its specified range.

Refer to the AxSYM System Operations Manual, Section 6, for additional information.

The operator must verify that the AxSYM AUSAB control values are within the acceptable ranges specified in this package insert (see **Controls** section).

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error code 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 minimum control requirement for an AxSYM AUSAB assay is a single sample of each of the Positive and Negative Controls tested once every 24 hours, each day of use. Controls may be placed in any position in the Sample Carousel.

The AxSYM AUSAB Control values must be within the acceptable ranges specified in this package insert (see **Controls** section). If a control value is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

If the quality control procedures in your laboratory require more frequent use of controls, follow those procedures.

Fluorescence Background Acceptance Criteria

Quality control with regards 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 AxSYM System Operations Manual, Section 2, for further information on parameter files.

RESULTS

Calculations

AxSYM AUSAB generates a calibration curve using a 4-parameter logistic curve fit. The amount of anti-HBs in samples is determined using the calibration curve.

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

- Samples with concentrations less than 10.0 mIU/mL are nonreactive by the AxSYM AUSAB assay.
- Samples whose concentrations are greater than or equal to 10.0 mIU/mL are reactive.

Refer to the Sample Dilution Procedures section of this package insert for calculation of diluted sample results.

Nonspecific reactives may be obtained with this, or any other highly sensitive immunoassay. The most common source of nonspecific reactives is particulate matter in the patient specimen, particularly fibrin clots and cellular material.

For indications following vaccination, national recommendations should be followed.

LIMITATIONS OF THE PROCEDURE

- For the diagnosis of acute or chronic hepatitis B viral infection, AxSYM AUSAB reactivity should be correlated with patient history and other hepatitis markers.
- Performance has not been established using cadaver samples or body fluids such as urine, saliva, semen or amniotic fluid.
- Do not use heat-inactivated samples.
- Frozen samples and those containing particulate matter or red blood cells must be centrifuged prior to running the assay.
- The AxSYM System "Automatic Sample Retest" feature must not be used due to the AxSYM AUSAB assay requirement to centrifuge all samples prior to repeat testing.
- Samples from heparinized patients may be partially coagulated. Erroneous results could occur due to the presence of fibrin. To prevent this phenomenon, draw the sample prior to heparin therapy.

NOTE: Quantitative values obtained using alternate assays (i.e. RIA, EIA or MEIA) may not be equivalent and cannot be used interchangeably. A new baseline using AxSYM AUSAB should be established when monitoring vaccinees.

EXPECTED VALUES

In a random population of 721 blood donor samples, 46 (6.4%) were reactive (≥ 10.0 mIU/mL) by AxSYM AUSAB. In a population reactive for anti-HBs (≥ 10.0 mIU/mL by IMx AUSAB), AxSYM AUSAB detected anti-HBs in 204/204 (100%).

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Assay reproducibility was determined during the clinical evaluation of AxSYM AUSAB. The five member panel was run in replicates of three, four times per day, over two days on each of three clinical lots. Calculations were made with a variance component analysis, using a nested analysis of variance model.[9] The results from these three clinical lots are summarized in Table 1.**

TABLE 1
Reproducibility of AxSYM AUSAB

Panel Member	N	Mean [mIU/mL]	Deviation from Mean* [mIU/mL]	%	Intra-Run SD	Intra-Run %CV	Inter-Run SD	Inter-Run %CV	Total SD	Total %CV
1	72	4.0	± 1.5	± 37.5	0.3	6.9	0.3	7.2	0.5	11.7
2	72	27.7	± 5.4	± 19.5	1.2	4.5	1.5	5.5	1.8	6.3
3	72	155.2	± 26.4	± 17.0	6.7	4.3	7.6	4.9	8.8	5.7
4	72	294.0	± 65.7	± 22.1	18.8	6.4	19.8	6.7	21.9	7.5
5	72	598.8	± 148.2	± 24.7	38.7	6.5	48.3	8.1	49.4	8.2
Positive Control	48	72.9	± 10.5	± 14.5	3.2	4.4	3.2	4.4	3.5	4.8

* based on a 99.73% (± 3 SD) Confidence Interval

** Representative performance data are shown. Results obtained at individual laboratories may vary.

Applying a confidence interval of ± 3 standard deviations, 9,973 of 10,000 replicates of e.g. panel member 3 would be expected to be within ± 17.0% of the mean.

Measuring Range

The AxSYM AUSAB measuring range is 2 mIU/mL - 1000 mIU/mL (defined by the limit of detection and the maximum of the calibration curve).

For samples exceeding the measuring range refer to the **SAMPLE DILUTION PROCEDURE** section.

Correlation

AxSYM AUSAB was compared to IMx AUSAB during the clinical evaluation. The results of these tests are shown in Table 2.*

TABLE 2
Comparison of AxSYM AUSAB with IMx AUSAB

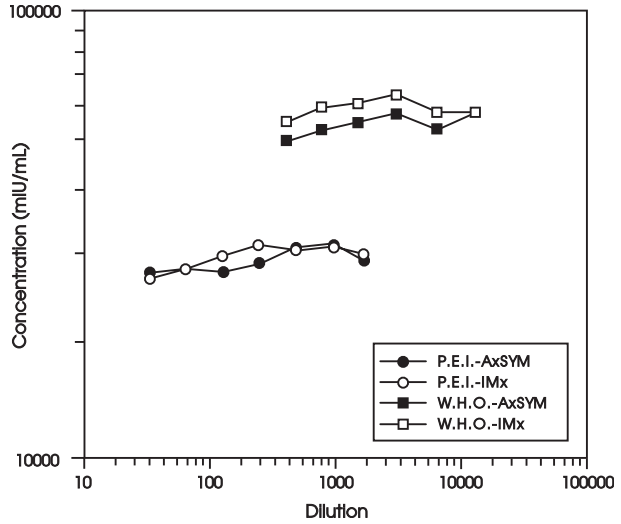
Method	Number of Observations	Intercept	Slope	Correlation Coefficient
IMx AUSAB	204	-9.90	1.18	0.986

* Representative performance data are shown. Results obtained at individual laboratories may vary.

Dilution Linearity

The PEI and WHO Reference Standards were diluted serially in AxSYM AUSAB Dilution Reagent. Each dilution was tested in duplicate by AxSYM AUSAB and IMx AUSAB. The original concentration of each standard was calculated by multiplying the result for each dilution in the range of 10 mIU/mL to 1000 mIU/mL by the dilution factor. The result for each dilution is plotted in Figure 1.*

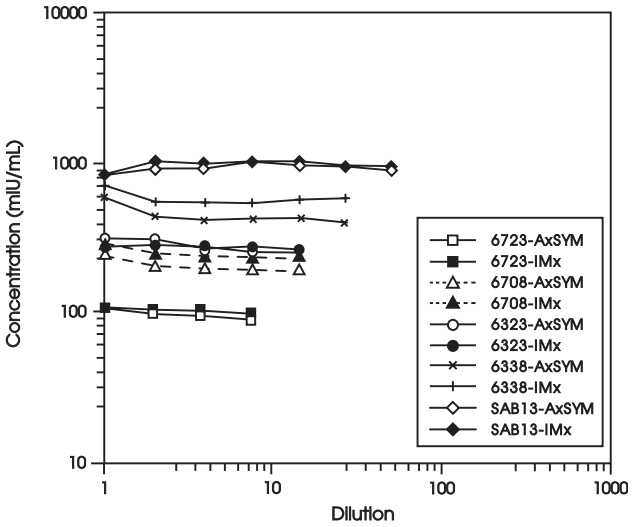
Figure 1
Dilution Linearity of Anti-HBs Standards



* Representative performance data are shown. Results obtained at individual laboratories may vary.

Samples from five individuals vaccinated with hepatitis B vaccine were diluted serially in AxSYM AUSAB Dilution Reagent. The dilutions were tested in duplicate by AxSYM AUSAB and IMx AUSAB. The original concentration of each sample was calculated by multiplying the result of each dilution in the range of 10 mIU/mL to 1000 mIU/mL by the dilution factor. The result for each dilution is shown in Figure 2.*

Figure 2
Dilution Linearity of Anti-HBs Vaccinee Samples



* Representative performance data are shown. Results obtained at individual laboratories may vary.

Specificity

The specificity of AxSYM AUSAB was determined by testing samples from three blood bank populations. Of 673 donor samples previously found nonreactive for anti-HBs by IMx AUSAB, 673 (100%) were nonreactive by AxSYM AUSAB. (Representative performance data are shown. Results obtained at individual laboratories may vary).

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ABBOTT
Max-Planck-Ring 2
65205 Wiesbaden
Germany
+49-6122-580

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