HBsAg Confirmatory

This package insert must be read carefully prior to 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.

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Key to symbols used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>List Number</td>
</tr>
<tr>
<td>IVD</td>
<td>For In Vitro Diagnostic Use</td>
</tr>
<tr>
<td>ICD</td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td>MUP</td>
<td>Solution 1 MUP</td>
</tr>
<tr>
<td>MCV</td>
<td>Solution 3 Matrix Cell Wash</td>
</tr>
<tr>
<td>LDI</td>
<td>Solution 4 Line Diluent</td>
</tr>
<tr>
<td>PCE</td>
<td>Probe Cleaning Solution</td>
</tr>
<tr>
<td>SC</td>
<td>Sample Cups</td>
</tr>
</tbody>
</table>

CAUTION: Handle human sourced materials as potentially infectious. Consult instructions for use. (Infection Risk)

See REAGENTS section for a full explanation of symbols used in reagent component naming.
AxSYM® HBsAg Confirmatory is a Microparticle Enzyme Immunoassay (MEIA) used for confirmation of the presence of Hepatitis B Surface Antigen (HBsAg) in human serum, plasma or other body fluids by means of specific antibody neutralization. It is intended to be used for confirmation of samples found to be repeatedly reactive by AxSYM HBsAg (V2).

**SUMMARY AND EXPLANATION OF THE TEST**

AxSYM HBsAg Confirmatory uses the principle of specific antibody neutralization to confirm the presence of HBsAg in samples found to be repeatedly reactive. Antigen to Hepatitis B Surface Antigen (Human) (anti-HBs) is incubated with a sample. If HBsAg is present in the sample, it will be neutralized by the antibody. The neutralized HBsAg is blocked from binding to the anti-HBs coated microparticles in a test for HBsAg. A reduction of signal occurs when compared to the signal of a paired sample that has not been treated with the antibody reagent. A sample is considered to be positive if its reactivity in the AxSYM HBsAg Confirmatory test procedure is neutralized by the addition of antibody reagent and the reduction in signal is 50% or greater.

**BIOLOGICAL PRINCIPLES OF THE PROCEDURE**

AxSYM HBsAg Confirmatory is based on the Microparticle Enzyme Immunoassay (MEIA) technology. In addition to the AxSYM HBsAg Confirmatory kit, this assay requires the use of the AxSYM HBsAg (V2) Reagent Kit and AxSYM HBsAg Positive Control. AxSYM HBsAg Confirmatory differs from AxSYM HBsAg (V2) in that the sample is automatically pretreated with the AxSYM HBsAg Confirmatory Reagent A [Antibody to Hepatitis B Surface Antigen (Human) (anti-HBs)] or Reagent B [Recalculated plasma (Human), nonreactive for anti-HBs]. If HBsAg is present in the sample, it will be bound by Reagent A. The neutralized HBsAg is blocked from binding to the antibody coated microparticles in the HBsAg assay. Sample and all AxSYM HBsAg Confirmatory and AxSYM HBsAg (V2) 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 assay principle involves two steps: pretreatment of the sample and HBsAg testing.

The reactions occur in the following sequence:

- Patient samples are tested undiluted and with an automated 1:500 dilution procedure. The dilution is performed by the AxSYM System with Dilution Reagent.
- The sample and its 1:500 dilution are each pipetted into two RVs. For each sample and dilution, Reagent A is added to one RV and Reagent B is added to the other.
- When HBsAg is present in the sample, it is neutralized by the antibody in Reagent A.
- Anti-HBs Coated Microparticles and Biotinylated Anti-HBs solution are added to the reaction mixture.
- Any nonneutralized HBsAg in the sample binds to the Anti-HBs Coated Microparticles and Biotinylated Anti-HBs, forming an antibody-antigen-antibody 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.
- The Anti-Biotin-Alkaline Phosphatase Conjugate is dispensed onto the matrix cell and binds with any microparticle-bound antibody-antigen-antibody 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-Methylumbelliferyl Phosphate. This fluorescent product is measured by the MEIA optical assembly.

The presence of nonneutralized HBsAg in the sample is determined by comparing the rate of formation of fluorescent product to a cutoff rate determined from the AxSYM HBsAg Confirmatory Index Calibration. If the rate of formation of fluorescent product in the nonneutralized sample (incubated with Reagent A) is greater than or equal to the Cutoff, and the rate of the neutralized sample (incubated with Reagent A) is reduced by at least 50%, the sample is considered confirmed positive for HBsAg. For further information regarding MEIA technology, refer to the AxSYM System Operations Manual, Section 3.

**REAGENTS**

AxSYM HBsAg Confirmatory Kit, 20 Tests (7A40-60)

- **REAGENT A**
  - 1 Bottle (1 mL) REAGENT A: Antibody to Hepatitis B Surface Antigen (Human) nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HCV and anti-HIV-1/HIV-2. Minimum concentration (anti-HBs): 430 mIU/mL. Preservative: Sodium Azide. Dye: Violet (Acid Red No. 33 and Acid Blue No. 9).
- **REAGENT B**
  - 1 Bottle (1.5 mL) REAGENT B: Recalculated plasma (Human), nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HBs, anti-HCV and anti-HIV-1/HIV-2. Preservative: Sodium Azide. Dye: Yellow (Acid Yellow No. 23).
- **DILUTION REAGENT**
  - 1 Bottle (18 mL) DILUTION REAGENT: Recalculated plasma (Human) nonreactive for HBsAg, HIV RNA or HIV-1 Ag, anti-HBs, anti-HCV and anti-HIV-1/HIV-2. Preservative: Sodium Azide.

**WARNINGS AND PRECAUTIONS**

**CAUTION:** This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the Reagents section of this package insert. Components sourced from human blood have been tested and found to be nonreactive for antibodies to HCV and HIV-1/HIV-2 and nonreactive for HBsAg and HIV RNA or HIV-1 Ag. Reagent B and the Dilution Reagent are nonreactive for antibodies to HBs, and Reagent A is reactive for antibodies to HBs. No known test method can offer complete assurance that products derived from human sources or inactivating 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. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents. These precautions include, but are not limited to the following:

1. Wear gloves when handling specimens or reagents.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
4. Clean and disinfect all spills of specimens or reagents using a suitable disinfectant.
5. Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local, state and federal regulations.

**SAFETY PRECAUTIONS**

- All components of this product contain Sodium Azide. For a specific listing, refer to the Reagents section of this package insert. The components containing Sodium Azide are classified per applicable European Community (EC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.
  - **R22** Harmful if swallowed.
  - **R32** Contact with acids liberates very toxic gas.
  - **S35** This material and its container must be disposed of in a safe way.
  - **S36** Wear protective clothing.
  - **S46** If swallowed, seek medical advice immediately and show this container or label.

**HANDLING PRECAUTIONS**

- AxSYM HBsAg Confirmatory 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.
- Do not mix reagents from different reagent packs. Do not mix reagent packs 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.
Sections 7 and 8.

Pack, confirmatory kit, index calibrators, controls, patient samples and additional safety and handling precautions and limitations for the reagent.

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. Transfer of as little as 1 µl from one HBsAg positive sample may contaminate adjacent negative samples or HBsAg Confirmatory reagents.

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

STORAGE INSTRUCTIONS

The AxSYM HBsAg Confirmatory kit must be stored at 2-8°C. The AxSYM HBsAg Confirmatory kit may be used immediately after removal from the refrigerator. Do not freeze the AxSYM HBsAg Confirmatory reagents.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When an AxSYM HBsAg Positive Control value (S/N or % Neutralization or S/CO) or Index Calibrator (S/N) 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 HBsAg Confirmatory Assay contains 3 files: HBSAGCNF, NEUT (Ratio) and NEUTDIL (Ratio). The files must be installed on the AxSYM System from the software disk, 9A55-03, or higher, prior to performing HBsAg Confirmatory assays. The AxSYM HBsAg Assay File must also be installed on the AxSYM System from the software disk, 9A16-03, or higher, prior to performing HBsAg Confirmatory Assay, in order for the HBsAg (V2) reagent pack to be recognized by the AxSYM System. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM HBsAg Confirmatory Assay Parameters

The default assay parameters for the AxSYM HBsAg Confirmatory 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. The 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.

**Assay Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long Assay Name (English):</td>
<td>HBsAg_Confirm</td>
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<tr>
<td>Abbrev Assay Name (English):</td>
<td>HBsAgCnf</td>
</tr>
<tr>
<td>Assay Number:</td>
<td>112</td>
</tr>
<tr>
<td>Assay Version:</td>
<td>2</td>
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<tr>
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<td>ON</td>
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<td>Assay Type:</td>
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<td>Cal A Concentration:</td>
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<td>Default Dilution Protocol&gt;</td>
<td>REAGENT_B</td>
</tr>
<tr>
<td>Default Calibration Method&gt;</td>
<td>Index Cal</td>
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<tr>
<td>Selected Result Concentration Units&gt;</td>
<td>S/N</td>
</tr>
<tr>
<td>Selected Result Decimal Places&gt;</td>
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<td>Max Intercept-Min MUP Intercept:</td>
<td>10000.0000</td>
</tr>
<tr>
<td>Min Intercept-Min MUP Intercept:</td>
<td>1200.0000</td>
</tr>
<tr>
<td>Upper limit for NRMSE for low rates:</td>
<td>9999.9990</td>
</tr>
<tr>
<td>Upper limit for NRMSE for high rates:</td>
<td>0.5000</td>
</tr>
<tr>
<td>Max Rate-Max rate used to check Min MUP Intercept:</td>
<td>50.0000</td>
</tr>
<tr>
<td>Min Rate-Rate cutoff for NRMSE and Corr. Coef:</td>
<td>8.0000</td>
</tr>
<tr>
<td>Min correlation coefficient for low rates:</td>
<td>0.9500</td>
</tr>
<tr>
<td>Min correlation coefficient for high rates:</td>
<td>0.9700</td>
</tr>
<tr>
<td>MUP Delay-Time delay following MUP:</td>
<td>2.6000</td>
</tr>
<tr>
<td>Interpretation Option to use&gt;</td>
<td>1</td>
</tr>
<tr>
<td>Hold results with POS interpretation&gt;</td>
<td>ON</td>
</tr>
<tr>
<td>Hold results with NEG interpretation&gt;</td>
<td>OFF</td>
</tr>
</tbody>
</table>

**NOTE:** For software disk, 9A55-03, parameter 45 can be edited to the alternate result units S/CO.

**NOTE:** Parameter 44 cannot be edited to another value.

**NOTE:** Parameter 46 must not be edited below 2 decimal points.

Refer to the AxSYM System Operations Manual for detailed discussion of Instrument Procedure.

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 HBsAg Confirmatory 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) are used in the AxSYM HBsAg Confirmatory 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 must be transferred to a centrifuge tube and centrifuged (minimum of 10,000 RCF* for 10 minutes) prior to confirmatory testing. 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 14 days at 2-8°C prior to being tested.
- If the assay will be performed after 14 days, the sample must be removed from the clot or red blood cells and stored frozen (-10°C or colder).
- 15 nonreactive and 15 reactive samples showed no qualitative performance differences when 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 under ambient conditions, refrigerated on wet ice (2-8°C) or frozen on dry ice (-10°C or colder).
- 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 sample storage constraints.
- Do not use heat-inactivated samples.
- The assay was designed and validated for use with human serum or plasma from individual patient and donor specimens. Pooled specimens should not be used since the accuracy of their test results has not been validated.
- Performance has not been established for cadaver samples or body fluids such as urine, saliva, semen or amniotic fluid.
- No qualitative performance differences were observed when HBsAg Negative and Positive Controls were tested with elevated levels of bilirubin (0-20 mg/dL), hemoglobin (0-250 mg/dL) or lipids (0-1000 mg/dL).

* Relative Centrifugal Force
SAMPLE/REAGENT VOLUME
Minimum sample and reagent volumes required to confirm one to five HBsAg repeatedly reactive samples on the AxSYM System are described in the following table:

<table>
<thead>
<tr>
<th>Sample Segment Position</th>
<th>Reagent Color</th>
<th>Minimum Volume Required (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Reagent A</td>
<td>Violet</td>
<td>100 150 200 250 300 350</td>
</tr>
<tr>
<td>2 Reagent B</td>
<td>Yellow</td>
<td>150 200 250 300 350 400</td>
</tr>
<tr>
<td>3 Dilution Reagent</td>
<td>Natural</td>
<td>0 500 875 1250 1625 2000</td>
</tr>
<tr>
<td>4 Index Calibrator</td>
<td>Green</td>
<td>270 270 270 270 270 270</td>
</tr>
<tr>
<td>5 Positive Control</td>
<td>Blue</td>
<td>250 250 250 250 250 250</td>
</tr>
<tr>
<td>6 Patient Sample</td>
<td></td>
<td>275 275 275 275 275 275</td>
</tr>
<tr>
<td>7 Patient Sample</td>
<td></td>
<td>275 275 275 275 275 275</td>
</tr>
<tr>
<td>8 Patient Sample</td>
<td></td>
<td>275 275 275 275 275 275</td>
</tr>
<tr>
<td>9 Patient Sample</td>
<td></td>
<td>275 275 275 275 275 275</td>
</tr>
<tr>
<td>10 Patient Sample</td>
<td></td>
<td>275 275 275 275 275 275</td>
</tr>
</tbody>
</table>

* 270 µL = 9 drops
** 250 µL = 7 drops

AxSYM HBsAg CONFIRMATORY PROCEDURE
Materials Required
- 7A40-60 AxSYM HBsAg Confirmatory Reagent Kit
- 7A44-22 AxSYM HBsAg (V2) Reagent Kit
- 7A44-10 AxSYM HBsAg Controls
- 8A47-04 SOLUTION 1 [MUP]
- 8A81-04 SOLUTION 3 [MATRIX CELL WASH]
- 9A45-04 SOLUTION 4 [LINE DILUENT]
- 9A55-04/ 9A55-05 AxSYM PROBE CLEANING SOLUTION
- 8A76-01 SAMPLE CUPS
- 1B06-01 AxSYM HBsAg Confirmatory Template

Materials Required But Not Provided
- Pipettes or pipette tips

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.
- The AxSYM HBsAg Confirmatory assay is able to detect and neutralize HBsAg from at least 0.6 ng/ml to greater than 1,000,000 ng/ml in patient specimens. Sample dilutions are required to enable neutralization in the upper concentration range.

Assay Procedure
When testing samples with AxSYM HBsAg Confirmatory, the following points should be noted:
- Each sample must be tested both undiluted and with the automated 1:500 dilution.
- All sample dilutions must be made with the AxSYM HBsAg Confirmatory Dilution Reagent.
- Specific sample segment position (S/P) configuration is required. Use the HBsAg Confirmatory Template as a guide for reagent and sample placement.
- HBsAg Confirmatory assay reagents (Reagent A, Reagent B, and Dilution Reagent) are automatically ordered by the AxSYM System when HBsAgCnf is selected. These reagents must be manually pipetted into sample cups and placed in positions 1-3 of the sample segment.
- Tests must not be assigned to positions 1-3 for any HBsAg Confirmatory segment. These segment positions (S/P) must not appear on the Orderlist Report printout.
- The Index Calibrator is ordered in two ways:
  1. When a calibration is required, the Index Calibrator is ordered using the Calibration Order screen. Refer to the Quality Control Section for calibration requirements.
  2. When an AxSYM HBsAg Confirmatory calibration has been accepted and stored (ACTIVE), the Index Calibrator is ordered using the Patient Order screen on subsequent sample segments.
- The Positive Control and all patient samples are ordered using the Patient Order screen.
- One to five patient samples can be confirmed per Sample Segment. For more than five patient samples, set up additional sample segments with Reagent A, Reagent B, Dilution Reagent, Index Calibrator and Positive Control.
- An active calibration for the HBsAg lot in use must be placed in prior to running the HBsAg Confirmatory Assay.

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” and “Rerun” features must not be used due to the AxSYM HBsAg Confirmatory assay requirement to centrifuge all samples prior to 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. Centrifuge the samples (minimum 10,000 RCF for 10 minutes).
4. Order the HBsAg Index Calibrator, HBsAg Positive Control, and patient samples as follows:
   - Index Calibrator:
     For a Calibration:
     Select F4-CAL. Select HBsAgCnf assay. Assign S/P to position 4 of the chosen sample segment. The AxSYM System automatically orders two replicates with REAGENT_B. Do not simultaneously calibrate more than one AxSYM HBsAg (V2) reagent pack.
     For an Assay with an ACTIVE Calibration:
     Select F6-PATIENT. Assign S/P to position 4. Select HBsAgCnf assay. Select F4-DILS/REPS. Order two REPS with REAGENT_B.
   - Positive Control:
     Select F6-PATIENT. Assign S/P to position 5. Select HBsAgCnf assay. Select F4-DILS/REPS. Order one REP of REAGENT_B and one REP of REAGENT_A.
   - Patient Samples:
     Select F6-PATIENT. Assign S/P to positions 6-10 as needed, for up to 5 patients. Select HBsAgCnf assay. Select F4-DILS/REPS for each patient and order four tests: one with REAGENT_B, one with REAGENT_A, one with REAG_B_DIL and one with REAG_A_DIL.

NOTE: The operator may obtain an Orderlist Report by pressing PRINT. The printout contains sample placement information and minimum sample cup volume requirements for all tests ordered.
5. Place the HBsAg Confirmatory Template over the selected Sample Segment. Pipette each reagent and sample into a sample cup. Refer to the Sample/Reagent Volume section of this insert for required volumes. Place the sample cups into the assigned positions as indicated on the Template. Visually verify reagent color against the Template.
   - For the AxSYM System to correctly calculate the test results required for confirmation, this segment configuration must be followed.
6. Place the sample segment containing the Confirmatory reagents and ordered samples into the Sample Carousel.
7. Place the AxSYM HBsAg (V2) Reagent Pack into the Reagent Pack Carousel.
8. Ensure that reaction vessels (RVs) are present on the RV Carousel. Additional RVs may be added as needed.
9. Press RUN. All entries on the Orderlist screen are transferred to the Order Status screen for sample processing.
10. Review the results to determine whether retesting with a manual sample dilution is required.
11. When testing is completed, remove the samples and the AxSYM HBsAg (V2) Reagent Pack from the Sampling Center. Store at 2-8°C. Discard any Confirmatory Reagents, Index Calibrator or Positive Control remaining in the sample cups.

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.

SAMPLE DILUTION PROCEDURES
- Manual Sample Dilution
If a sample is reactive but is not neutralized using the automated 1:500 dilution, an additional dilution is required. Prepare a manual 1:50 dilution using a 20 µL sample plus 980 µL of the Dilution Reagent. This manually diluted sample is tested using the automated 1:500 dilution, resulting in a final dilution of 1:25,000.
* If desired 20 µL + 1 mL is acceptable (1:51 dilution).
- To Order Patient Samples Diluted 1:25,000
Select F6-PATIENT. Assign S/P to positions 6 - 10 as needed for up to 5 patients. Select HBsAgCnf assay. Select F4-DILS/REPS for each patient and order two tests: one with REAGENT_B_DIL and one with REAGENT_A_DIL.

Refer to Sections 5 and 6 of the AxSYM System Operations Manual for a detailed explanation of routine ordering procedures.
QUALITY CONTROL PROCEDURES

CALIBRATION
A minimum of two replicates of the AxSYM HBsAg (V2) Index Calibrator treated with Reagent B must be tested for AxSYM HBsAg Confirmatory Calibration.

One test of the AxSYM HBsAg Positive Control treated with Reagent A and one test of the AxSYM HBsAg Positive Control treated with Reagent B must be tested as a means of evaluating the assay calibration.

Once an AxSYM HBsAg Confirmatory calibration is accepted and stored (ACTIVE), all subsequent samples may be tested without further calibration unless a new lot of either the AxSYM HBsAg (V2) reagent pack or the AxSYM HBsAg Confirmatory kit is used.

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

The operator must verify that the AxSYM HBsAg control values are within the acceptable ranges specified in this package insert (See Interpretation of Results section).

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 minimum control requirement for each HBsAg Confirmatory sample segment is one test of the AxSYM HBsAg Positive Control treated with Reagent A, one test of the AxSYM HBsAg Positive Control treated with Reagent B and two tests of the AxSYM HBsAg (V2) Index Calibrator treated with Reagent B. See Interpretation of Results section for acceptable result ranges.

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 the AxSYM System Operations Manual, Section 2, for further information on parameter files.

RESULTS
The software evaluates performance of the assay by the following assay-specific criteria:
- Maximum acceptable calibrator %CV: 20.00
- Minimum acceptable Index Calibrator (rate): 3.00
- Maximum acceptable Index Calibrator (rate): 25.00

CALCULATIONS
The AxSYM System calculates the Cutoff Rate as 1.5 times the mean rate of the AxSYM HBsAg (V2) Index Calibrator treated with Reagent B.

Cutoff Rate = 1.5 x Mean Rate of the Index Calibrator Treated with Reagent B

There are three result reports for each sample* being tested. They are identified as REAGENT_A, REAGENT_B, and %NEUT reports for undiluted samples and REAG_A_DIL, REAG_B_DIL, and %NEUTDIL for 1:500 diluted samples.

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

For the Reagent A report:

\[
\frac{S/N(A)}{Cutoff Rate} = \text{Rate of Sample* Treated with Reagent A} \\
\frac{S/CO(A)}{Cutoff Rate} = \text{Rate of Sample* Treated with Reagent A} \\
\]

For the Reagent B report:

\[
\frac{S/N(B)}{Cutoff Rate} = \text{Rate of Sample* Treated with Reagent B} \\
\frac{S/CO(B)}{Cutoff Rate} = \text{Rate of Sample* Treated with Reagent B} \\
\]

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

\[
\text{Percent Neutralization} = \frac{S/N(B) - S/N(A)}{S/N(B) - S/N of the Index Calibrator Treated with Reagent B} \times 100
\]

For S/CO calculations, the percent neutralization (%NEUT or %NEUTDIL) for the sample* using the results of the Reagent A treated sample* [S/CO(A)] and Reagent B treated sample* [S/CO(B)] is calculated as follows:

\[
\text{Percent Neutralization} = \frac{S/CO(B) - S/CO(A)}{S/CO(B) - S/CO of the Index Calibrator Treated with Reagent B} \times 100
\]

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

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, Section 1 and 2.

INTERPRETATION OF RESULTS
Prior to the final interpretation of patient samples, the assay validity must be verified by the operator.

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

- The Calibration is “ACTIVE” (A Calibration report must be printed after each calibration.)
- The test result (S/N or S/CO value) of the Positive Control treated with Reagent B must be greater than or equal to an S/N of 7.00 or an S/CO of 4.66.
- The Positive Control %NEUT must be greater than 50% and have the interpretation of POSITIVE.
- For an assay with an ACTIVE calibration, the S/N or S/CO results for the Index Calibrator treated with Reagent B must be in the range S/N = 0.60 - 1.45 or S/CO = 0.40 - 0.95.

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

\[
\text{The INTRP field of the sample treated with Reagent B is REACTIVE} \quad \text{(S/N value is greater than or equal to 1.50 or S/CO value is greater than or equal to 1.00)}
\]

AND

the percent neutralization value is greater than or equal to 50.

If POSITIVE does not appear as an interpretation in either of the percent neutralization reports, proceed as follows for additional possible interpretations:

- When NEGATIVE appears in the INTRP field for an undiluted sample treated with Reagent B, the sample is nonreactive for HBsAg by AxSYM HBsAg Confirmatory.
- When REACTIVE appears in the INTRP field for an undiluted sample treated with Reagent B and the interpretation for the 1:500 diluted sample treated with Reagent B is NEGATIVE, the sample is repeat reactive, nonconfirming for HBsAg by AxSYM HBsAg Confirmatory.
- When REACTIVE appears in the INTRP field for both the undiluted and diluted sample treated with Reagent B and both percent neutralizations are less than 50.00, the AxSYM HBsAg Confirmatory testing must be repeated at a sample dilution of 1:25,000 as described in the Manual Sample Dilution section.

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

<table>
<thead>
<tr>
<th>INTRP field for Uncultured Sample</th>
<th>INTRP field for 1:500 Diluted Sample</th>
<th>REAGENT_B %NEUT</th>
<th>REAG_B_DIL %NEUT</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. REACTIVE POSITIVE NEGATIVE NONE</td>
<td>CONFIRMED POSITIVE CONFIRMED POSITIVE</td>
<td>REACTIVE NEGATIVE</td>
<td>REACTIVE POSITIVE</td>
<td>REACTIVE POSITIVE</td>
</tr>
<tr>
<td>2. REACTIVE POSITIVE REACTIVE POSITIVE</td>
<td>CONFIRMED POSITIVE</td>
<td>REACTIVE POSITIVE</td>
<td>REACTIVE POSITIVE</td>
<td>REACTIVE POSITIVE</td>
</tr>
<tr>
<td>3. REACTIVE POSITIVE NEGATIVE NONE</td>
<td>CONFIRMED POSITIVE</td>
<td>REACTIVE POSITIVE</td>
<td>REACTIVE POSITIVE</td>
<td>REACTIVE POSITIVE</td>
</tr>
<tr>
<td>4. NEGATIVE NEGATIVE NEGATIVE NONREACTIVE FOR HBsAg</td>
<td>NONREACTIVE</td>
<td>REACTIVE none</td>
<td>REACTIVE none</td>
<td>Repeat confirmatory testing with 1:25,000 dilution</td>
</tr>
<tr>
<td>5. REACTIVE none NEGATIVE NONREACTIVE</td>
<td>REACTIVE</td>
<td>none</td>
<td>none</td>
<td>Repeat confirmatory testing with 1:25,000 dilution</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes and in order to differentiate acute HBV infection from chronic HBV infection, the detection of HBsAg must be correlated with patient symptoms and other hepatitits B viral serological markers.
- It is recognized that presently available methods for the detection of hepatitis B surface antigen may not detect all potentially infectious units of blood or infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with hepatitis B. Nonreactive test results in individuals with prior exposure to hepatitis B may be due to antigen levels below the detection limit of this assay or lack of antigen reactivity to the antibodies used in this assay.
- Weak reactive results, not confirmed with undiluted sample, but confirmed in the 1:500 dilution, should be repeated to avoid erroneous interpretation.
- Sample of freshly vaccinated patients or sample contamination by HBsAg may result in a confirmation in the AxSYM HBsAg Confirmatory assay.
- This assay was designed and validated for use with human plasma or serum from individual patient and donor specimens. Insufficient data are available to interpret tests performed on pooled blood or processed plasma and products made from such pools. Testing of these specimens is not recommended.
- The AxSYM HBsAg Confirmatory Assay pipettes Reagent A, Reagent B and Dilution Reagent directly from the Sample Carousel. When the Sampling Probe moves to the Sample Carousel to pipette these reagents, the Sampling Center Motion Detection Light illuminates at the same time the Sample Carousel begins to move. THERE WILL NOT BE A DELAY BETWEEN LIGHT ILLUMINATION AND CAROUSEL MOVEMENT. If loading or unloading of the Sample Carousel is necessary while performing the AxSYM HBsAg Confirmatory Assay, press the PAUSE button to pause all activity in the Sampling Center. Wait for the Sample Carousel to come to a complete stop before loading or unloading (the system status field will display PAUSED).
- 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 specimens and those containing particulate matter or red blood cells must be centrifuged prior to running the assay.
- The AxSYM system “Automatic Sample Retest” and “Rerun” features must not be used due to the AxSYM HBsAg Confirmatory assay requirement to centrifuge all samples prior to 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.

BIBLIOGRAPHY

5. CDC, Guidelines for the prevention of transmission of Human Immunodeficiency Virus and Hepatitis B Virus to health-care and public-safety workers. MMWR 1989;38, (S-6); 1-65.

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