Abbott AxSYM® System

In Vitro Test
List No. 8B88-10
XX-XXXX/RX

AxSYM CORE™ 2.0
Controls

ABBOTT LABORATORIES
Abbott Park, IL 60064 USA

Printed in USA

INTENDED USE

The AxSYM CORE™ 2.0 Controls are used for monitoring the performance of the AxSYM® System (reagent and instrument) when used for the qualitative detection of total antibodies to hepatitis B virus core antigen (anti-HBc) when using the AxSYM CORE 2.0 Reagent Kit. The performance of the AxSYM CORE 2.0 Controls has not been established with any other anti-HBc assays.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

- **CAUTION: This product contains human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[1] Biosafety Level 2[2] or other appropriate biosafety practices[3,4] should be used for materials that contain or are suspected of containing infectious agents.**
- The AxSYM CORE 2.0 Negative Control is nonreactive for anti-HBc, anti-HBs, HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.
- The AxSYM CORE 2.0 Positive Control is reactive for anti-HBc and 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 **MATERIALS PROVIDED** section of this package insert. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

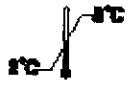
MATERIALS PROVIDED

2 Bottles (9 mL each) of AxSYM CORE 2.0 Controls prepared in recalcified human plasma.

- The Negative Control (1 Bottle) is recalcified anti-HBc negative human plasma. Preservative: 0.1% Sodium Azide.
- The Positive Control (1 Bottle) is recalcified anti-HBc positive human plasma in recalcified anti-HBc negative human plasma. Preservatives: 0.1% Sodium Azide and Antimicrobial Agent.

PREPARATION AND STORAGE

- Controls are liquid ready-to-use. No preparation is required.
- When stored and handled as directed, the controls are stable until the expiration date.
- The controls must be stored at 2-8°C and may be used immediately after removal from 2-8°C storage.
- Refer to the AxSYM CORE 2.0 assay reagent package insert for the maximum on board stability requirements.



Store at 2-8°C

QUALITY CONTROL PROCEDURES

Refer to the AxSYM CORE 2.0 assay package insert and the AxSYM System Operations Manual for additional information.

The minimum control requirement for the AxSYM CORE 2.0 assay is a single sample of each of the Negative and Positive Controls control level tested once every 24 hours each day of use for each reagent lot. Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control procedure.

PROCEDURE

- AxSYM CORE 2.0 Controls must be mixed by gentle inversion before use.
- To obtain the recommended volume requirements for the AxSYM CORE 2.0 Controls, hold the bottles **vertically** and dispense 6 drops of each control into each respective sample cup.

EXPECTED RESULTS

The AxSYM CORE 2.0 Controls have the following ranges:

Control	Color	Anti-HBc (Minimum Titer)	Control Range (S/CO)
Negative	Natural	Not Applicable	1.500 – 2.500
Positive	Blue[a]	1:1.25	0.005 – 0.800

[a] Dye: Acid Blue No. 9

LIMITATIONS

- Control values have not been established for assays other than the AxSYM CORE 2.0 assay. If the user wishes to use this control material with other assays, it is their responsibility to establish the appropriate ranges.
- Controls are in a serum matrix made from recalcified plasma. The user should provide alternate control material for plasma when necessary.
- The controls are not calibrators and should not be used for assay calibration.

BIBLIOGRAPHY

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

September 2006

Abbott AxSYM® System

AxSYM CORE 2.0
List No. 8B88
66-8430/R1

AxSYM CORE™ 2.0

Antibody to Hepatitis B Virus Core Antigen (Anti-HBc)

Customer Service
United States: 1-877-4ABBOTT

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

CAUTION:

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

ABBOTT LABORATORIES
Diagnostics Division
Abbott Park, IL 60064 USA

Printed in USA

NAME

AxSYM CORE™ 2.0

INTENDED USE

AxSYM CORE 2.0 is a microparticle enzyme immunoassay (MEIA) intended for the qualitative detection of total antibodies (IgG and IgM) to hepatitis B virus core antigen (anti-HBc) in adult and pediatric serum (including serum collected in serum separator tubes) or plasma (collected in potassium EDTA, sodium citrate, sodium heparin, lithium heparin, or plasma separator tubes containing lithium heparin). The assay is used as an aid in the diagnosis of acute, chronic, or resolved hepatitis B virus (HBV) infection 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 CORE 2.0 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-HBc determinations can be used as an indicator of current or past HBV infection. Anti-HBc is found in serum shortly after the appearance of hepatitis B surface antigen (HBsAg) in acute HBV infections. It will persist after the disappearance of HBsAg and before the appearance of detectable antibody to HBsAg (anti-HBs).[1-6] In the absence of information about any other HBV markers, it must be considered that an individual with detectable levels of anti-HBc may be actively infected with HBV or that the infection may have resolved.[7] Anti-HBc in some patients may be the only serological marker of HBV infection.[5,8-10]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

AxSYM CORE 2.0 is a two-step competitive/blocking MEIA. Anti-HBc in the sample blocks the binding of the anti-HBc (human):alkaline phosphatase conjugate to the rHBcAg coated on the microparticles, and the Specimen Diluent, which contains a reducing agent (dithiothreitol), minimizes nonspecific reactivity.[11-13] The AxSYM CORE 2.0 reagents and sample are pipetted in the following sequence:

SAMPLING CENTER

- Sample and all AxSYM CORE 2.0 reagents required for one test are pipetted by the Sampling Pipettor into various wells of a Reaction Vessel (RV).
- Specimen Diluent, Hepatitis B Virus Core Antigen (*E. coli*, Recombinant) (rHBcAg) Coated Microparticles, and sample are combined in one RV well.
- When anti-HBc is present in the sample, it binds to the rHBcAg coated microparticles, forming an antibody-antigen 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

- A portion of the reaction mixture is transferred to the Matrix Cell. The microparticles bind irreversibly to the glass fiber matrix.
- Antibody to Hepatitis B Virus Core Antigen (Human):Alkaline Phosphatase Conjugate is dispensed onto the Matrix Cell and binds with the rHBcAg antigenic sites on the microparticles which are not bound with anti-HBc from the sample.
- 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 is measured by the MEIA optical assembly.

The presence or absence of anti-HBc in the sample is determined by comparing the rate of formation of fluorescent product (S) to the cutoff rate (CO), which is calculated from a previous AxSYM CORE 2.0 Index Calibration. Samples with S/CO values of 0.001 to 0.800 are considered reactive by AxSYM CORE 2.0. Samples with S/CO values of 1.200 to 3.000 are considered nonreactive by AxSYM CORE 2.0. Samples with S/CO values of 0.801 to 1.199 are considered grayzone by AxSYM CORE 2.0 and should be retested in duplicate. Samples with S/CO values greater than 3.000 are considered invalid by AxSYM CORE 2.0 and should be retested once using a single sample.

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

REAGENTS

REAGENT PACK, 100 TESTS

AxSYM CORE 2.0 Reagent Pack (8B88-20)*

- 1 Bottle (3.7 mL) Specimen Diluent. Dithiothreitol in acetate buffer. Minimum concentration: 70 mM. (Reagent Bottle 1)
- 1 Bottle (4.0 mL) Hepatitis B Virus Core Antigen (*E. coli*, Recombinant) Coated Microparticles in TRIS buffer with protein (0.94% bovine) stabilizer. Minimum concentration: 0.03% solids. Preservative: 0.1% Sodium Azide. (Reagent Bottle 2)
- 1 Bottle (11.5 mL) Antibody to Hepatitis B Virus Core Antigen (Human):Alkaline Phosphatase Conjugate in TRIS buffer with protein (0.94% bovine) stabilizer. Minimum concentration: 0.2 µg/mL. Preservatives: 0.1% Sodium Azide and Antimicrobial Agents. (Reagent Bottle 3)

Index Calibrator

1 Bottle (8 mL) AxSYM CORE 2.0 Index Calibrator is recalcified anti-HBc negative human plasma. Preservative: 0.1% Sodium Azide. Dye: Green (Acid Yellow No. 23 and Acid Blue No. 9).

* 8B88-66 includes the AxSYM CORE 2.0 Reagent Pack and Index Calibrator (100 tests), Reaction Vessels (100 each), and Matrix Cells (100 each).

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AxSYM CORE 2.0 Controls (8B88-10) (sold separately)

2 Bottles (9 mL each) of AxSYM CORE 2.0 Controls

- The Negative Control (1 Bottle) is recalcified anti-HBc negative human plasma. Preservative: 0.1% Sodium Azide.
- The Positive Control (1 Bottle) is recalcified anti-HBc positive human plasma in recalcified anti-HBc negative human plasma. Preservatives: 0.1% Sodium Azide and Antimicrobial Agent.

The AxSYM CORE 2.0 Controls have the following ranges:

Control	Color	Anti-HBc (Minimum Titer)	Control Range (S/CO)
Negative	Natural	Not Applicable	1.500 – 2.500
Positive	Blue[a]	1:1.25	0.005 – 0.800

[a] 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. 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. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[14] Biosafety Level 2[15] or other appropriate biosafety practices[16,17] should be used for materials that contain or are suspected of containing infectious agents.
- The AxSYM CORE 2.0 Index Calibrator is nonreactive for anti-HBc, anti-HBs, HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.
- The AxSYM CORE 2.0 Negative Control is nonreactive for anti-HBc, anti-HBs, HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HIV-1/HIV-2, and anti-HCV.
- The AxSYM CORE 2.0 Positive Control is reactive for anti-HBc and 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 CORE 2.0 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 CORE 2.0 Reagent Pack beyond the expiration date.
- Do not use AxSYM CORE 2.0 Reagent Pack beyond a maximum of 112 cumulative hours on board the AxSYM System.
- Do not mix reagents from different Reagent Packs. Do not mix reagents and index calibrators from different lots.
- Avoid microbial contamination of specimens and reagents. Use of disposable pipettes or pipette tips is recommended.
- Avoid chemical contamination of reagents and equipment.
- Ensure that sufficient sample volume is present. If sample volume is insufficient, the AxSYM System will give an error code and no result will be reported. For a description of the system error codes, refer to the AxSYM System Operations Manual, Section 10.
- Use caution in handling patient specimens to prevent cross contamination.

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 CORE 2.0 Reagent Pack, Index Calibrator, and Controls must be stored at 2-8°C. They may be used immediately after removal from the refrigerator. Index Calibrator and Controls should be returned to 2-8°C storage immediately after use.

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

The AxSYM CORE 2.0 Reagent Pack may be on board the AxSYM System for a maximum of 112 cumulative hours; for example, 14 eight-hour shifts. After 112 hours, the Reagent Pack and associated Index Calibrator must be discarded. Refer to the AxSYM System Operations Manual, Sections 2 and 5, for further information on tracking onboard time.

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

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

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INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When an AxSYM CORE 2.0 Negative or Positive Control value is out of the expected range, it may indicate deterioration of the reagents or errors in technique. The test results of associated specimens are invalid and these specimens must be retested. Assay recalibration may be necessary. Refer to the AxSYM System Operations Manual, Section 10, for further troubleshooting information.

INSTRUMENT PROCEDURE

NOTE: AxSYM CORE 2.0 must only be used with AxSYM System Software Version 3.60 or higher.

ASSAY FILE INSTALLATION

The AxSYM CORE 2.0 assay file must be installed on the AxSYM System from the AxSYM CORE 2.0 Assay Disk, List No. 3C33-01 or higher, prior to performing the AxSYM CORE 2.0 assay. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

AxSYM CORE 2.0 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 CORE 2.0 assay are listed below.

	Assay Parameters
1	Long Assay Name (English): CORE_2
6	Abbrev Assay Name (English): CORE_2
11	Assay Number: 128
43	Default Dilution Protocol > UNDILUTED
44	Default Calibration Method > Index Cal
45	Selected Result Concentration Units > S/CO
80	Interpretation Option to use > 1

NOTE: 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 potassium EDTA, sodium citrate, sodium heparin, lithium heparin, or plasma separator tubes) may be used with the AxSYM CORE 2.0 assay. Follow the manufacturer's processing instructions for serum or plasma collection tubes.
- 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.
- 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 CORE 2.0 assay.
- This assay was designed for use with human serum or plasma from individual patient specimens. Pooled specimens must not be used.
- Gravity separation is not sufficient for specimen preparation. Specimens containing clots, red blood cells, or particulate matter may give inconsistent results and must be clarified by centrifugation prior to testing.
- **All patient specimens to be tested in Primary Tubes must be centrifuged to remove red blood cells or particulate matter. Follow the manufacturer's instructions for centrifugation.**
- **Specimens must be transferred to a centrifuge tube and centrifuged at a Relative Centrifugal Force (RCF) of 10,000 x g for 10 minutes if:**
  - **they still contain clots, red blood cells, or particulate matter after being centrifuged according to the collection tube manufacturer's instructions, or**
  - **they require repeat 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 the 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.[18]
  - 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.[19]
  - Specimens that are not tested within the specified time period listed above must be removed from the clot or red blood cells and stored frozen (-20°C or colder).
  - Specimens may be subjected to up to 3 freeze/thaw cycles prior to being tested. Specimens must be mixed **thoroughly** after thawing, by LOW speed vortexing or by gentle inversion, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.
- Specimens may be shipped at -20°C or colder (dry ice), 2-8°C (wet ice), or 15-30°C 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, all samples (patient specimens, controls, and index calibrator) should be tested within 3 hours of being placed on board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of onboard sample storage constraints.

SAMPLE VOLUME

The sample volume required to perform a single AxSYM CORE 2.0 test on the AxSYM System varies according to the type of sample container used. For sample cups, a ROUTINE test and a STAT test each require 236 µL. For every additional AxSYM CORE 2.0 test performed (ROUTINE or STAT) from the same sample container, an additional 186 µL of sample is required.

The sample cup minimum volume for both ROUTINE and STAT tests is calculated by the AxSYM System. They are displayed on the Order screen at the time the test(s) is(are) ordered. The sample cup STAT minimum volume is printed on the Orderlist Report. When using the 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 CORE 2.0 Index Calibrator and Controls, hold the bottles **vertically** and dispense 9 drops of Index Calibrator or 6 drops (per replicate) of each control into each respective sample cup.

For sample volume requirements in Primary or Aliquot Tubes, and for calibrator/control volume requirements for multiple AxSYM CORE 2.0 reagent lots, refer to the AxSYM System Operations Manual, Section 5.

AxSYM CORE 2.0 PROCEDURE

MATERIALS PROVIDED

- 8B88-66 AxSYM CORE 2.0 Reagent Kit, containing:
  - AxSYM CORE 2.0 Reagent Pack
  - AxSYM CORE 2.0 Index Calibrator
  - 100 Reaction Vessels
  - 100 Matrix Cells

MATERIALS REQUIRED BUT NOT PROVIDED

- 8B88-10 AxSYM CORE 2.0 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

CAUTION:

- Mix the AxSYM CORE 2.0 Index Calibrator and Controls by gentle inversion prior to use.
- When manually dispensing samples into sample cups, verify that dispensing equipment does not introduce cross contamination and delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9.

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 CORE 2.0 Index Calibrator, AxSYM CORE 2.0 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.

   Index Calibration

   Perform AxSYM CORE 2.0 calibration by testing 2 replicates of the Index Calibrator. Invert gently to mix and dispense at least 9 drops of the Index Calibrator into a sample cup. Do not simultaneously calibrate more than one AxSYM CORE 2.0 reagent lot.

   Controls

   Perform quality control by testing the Negative and Positive Controls (one test each). Invert gently to mix and dispense at least 6* drops each of the Negative and Positive Controls into individual sample cups.

   * When more than one AxSYM CORE 2.0 reagent lot is on board the AxSYM System, multiply the control volume by the number of lots.

   Patient Specimens

   Ensure that sufficient volume is present in the sample cups or tubes. The sample cup minimum volume is 236 µL for the first AxSYM CORE 2.0 test plus 186 µL for each additional AxSYM CORE 2.0 test. For volume requirements in Primary or Aliquot Tubes, refer to the AxSYM System Operations Manual, Section 5.

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

4. Place sample segments containing the ordered samples into the Sample Carousel.
5. Place the AxSYM CORE 2.0 Reagent Pack into the Reagent Pack Carousel.
6. Ensure that RVs are present on the RV Carousel. Additional RVs may be added as needed.

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7. Press RUN. All entries on the Orderlist screen are transferred to the Order Status screen for sample processing.
8. Review the results to determine whether retesting is required.
9. When testing is completed, remove the samples and the AxSYM CORE 2.0 Reagent Pack from the Sampling Center. Store reagent pack at 2-8°C.

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

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

QUALITY CONTROL PROCEDURES

CALIBRATION

A minimum of 2 replicates of the AxSYM CORE 2.0 Index Calibrator must be tested for an AxSYM CORE 2.0 calibration. A single sample of both the Negative and Positive Controls must be tested as a means of evaluating the assay calibration. Once the AxSYM CORE 2.0 calibration is accepted and stored, all subsequent samples may be tested without further calibration unless one or more of the following occur:

- A reagent pack with a new lot number is used.
- Either of the AxSYM CORE 2.0 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 additional information on:

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

The operator must verify that the AxSYM CORE 2.0 Control values are within the ranges specified in this package insert. Refer to the **REAGENTS** section of this package insert for AxSYM CORE 2.0 Control ranges.

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

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

The performance of the Abbott AxSYM CORE 2.0 Controls has not been established with any other anti-HBc assays.

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 the CLSI document C24-A2[20], *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)[21].

The minimum control requirement for an AxSYM CORE 2.0 assay is a single sample of each of the Negative and Positive Controls tested once every 24 hours, each day of use for each reagent lot. Controls may be placed in any position in the Sample Carousel.

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

The operator must verify that the AxSYM CORE 2.0 Control values are within the ranges specified in this package insert. Refer to the **REAGENTS** section of this package insert for AxSYM CORE 2.0 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.

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 parameter files.

RESULTS

CALCULATION

The AxSYM System calculates the mean rate of the Index Calibrator replicates and stores the result. The cutoff rate is then determined by dividing the Index Calibrator mean rate by 2.

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

The AxSYM CORE 2.0 assay protocol calculates a result based on the ratio of the sample rate (S) to the cutoff rate (CO) for each sample and control.

S/CO = Sample Rate / Cutoff Rate

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 CORE 2.0 Results

Initial Result (S/CO)	Instrument Interpretation	Instrument Flag	Interpretation	Retest Procedure
0.001 to 0.800	REACTIVE		Reactive	No Retest Required
0.801 to 1.199	GRAYZONE[c]		Gray Zone	Recentrifuge; Retest in Duplicate[a]
1.200 to 3.000	NONREACTIVE		Nonreactive	No Retest Required
> 3.000		>	Invalid	Recentrifuge; Retest Single Sample Once[a,b]

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

[b] If the retested specimen gives a valid result, follow the appropriate retest procedure for either reactive, gray zone, or nonreactive results. If the specimen is found to be repeatedly invalid, another specimen should be obtained.

[c] An "Instrument Interpretation" of Grayzone (GZ) should be interpreted as a Re-Test Zone

Final AxSYM CORE 2.0 Results

AxSYM CORE 2.0			
Initial Interpretation	**Retest Results**	**Final Result**	**Interpretation**
Reactive	No Retest Required	Reactive	Presumptive evidence of anti-HBc.
Gray Zone	Both of the duplicate retests are ≤ 1.000 S/CO or Initial result and at least one retest result are ≤ 1.000 S/CO.		
	Both of the duplicate retests are > 1.000 S/CO or Initial result and at least one retest result are > 1.000 S/CO.	Nonreactive	Anti-HBc is not detected.
Nonreactive	No Retest Required		

LIMITATIONS OF THE PROCEDURE

- **WARNING: Not intended for use in screening blood, plasma, or tissue donors.** The effectiveness of AxSYM CORE 2.0 for use in screening blood, plasma, or tissue donors has not been established.
- Current methods for the detection of anti-HBc may not detect all potentially infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with HBV.

For diagnostic purposes and in order to differentiate acute HBV infection from chronic HBV infection, anti-HBc reactivity should be correlated with patient history and the presence of other hepatitis markers. Reactive results do not discriminate between acute or chronic HBV infections.

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, 58.91% (1,256/2,132) were individuals, living in the United States, who were 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 47.45% Caucasian, 36.39% African American, 12.82% Hispanic, 1.51% Asian, 0.32% American Indian/Alaska Native, with the remaining 1.51% represented by other ethnic groups. The population was 63.06% female and 36.94% male ranging in age from 18 to 75 years. AxSYM CORE 2.0 was reactive in 15.45% (194/1,256) 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 CORE 2.0 reactive results observed from each location. Table 2 is a summary of the percent AxSYM CORE 2.0 reactive results by age range and gender.

Table 1
AxSYM CORE 2.0 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 CORE 2.0 Reactive Results Observed From Each Location
Site 1, Galveston, TX	58.52 (735/1,256)	17.41 (128/735)
Site 2, Dallas, TX	4.22 (53/1,256)	20.75 (11/53)
Site 3, Miami, FL	3.74 (47/1,256)	19.15 (9/47)
Site 4, St. Petersburg, FL	3.03 (38/1,256)	23.68 (9/38)
Site 5, Chicago, IL	0.16 (2/1,256)	50.00 (1/2)
Site 6, Denver, CO	2.39 (30/1,256)	23.33 (7/30)
Specimen Vendor Location:		
Colton, CA	5.97 (75/1,256)	1.33 (1/75)
Plymouth, MA	7.32 (92/1,256)	18.48 (17/92)
High Point, NC	14.65 (184/1,256)	5.98 (11/184)

Table 2
AxSYM CORE 2.0 Results by Age Range and Gender for Individuals at Increased Risk of HBV Infection

Age Range	Gender	AxSYM CORE 2.0 Result + Number of Specimens	AxSYM CORE 2.0 Result – Number of Specimens	Total
10 to 19	Female	0	13	13
	Male	1	10	11
20 to 29	Female	13	164	177
	Male	3	88	91
30 to 39	Female	20	164	184
	Male	12	87	99
40 to 49	Female	35	205	240
	Male	46	105	151
50 to 59	Female	21	113	134
	Male	34	64	98
60 to 69	Female	2	32	34
	Male	3	8	11
70 to 79	Female	3	4	7
	Male	1	2	3
Unknown[a]	Female	0	3	3
Total		**194 (15.45%)**	**1,062 (84.55%)**	**1,256**

[a] Age was not provided for three subjects.

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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-A2[22]. Testing was conducted at three clinical testing sites using three AxSYM CORE 2.0 reagent 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 CORE 2.0 Index Calibrator, Negative Control, and Positive Control. Panel members were prepared by adding anti-HBc positive plasma to anti-HBc negative serum. The data are summarized in Table 3.

Table 3
AxSYM CORE 2.0 5-day Precision—Three Reagent Master Lots, Three Clinical Testing Sites

Sample	Total No. Reps	Grand Mean S/CO	Within-Run		Within-Day		Within-Laboratory Precision (Total)			Precision with Additional Component of Between-Lot		Precision with Additional Component of Between-Site		Precision with Additional Components of Site and Lot (Overall)	
			SD	%CV	SD	%CV	SD	%CV	CL	SD	%CV	SD	%CV	SD	%CV
Panel 1	360	0.728	0.0282	3.9	0.0378	5.2	0.0386	5.3	5.8	0.1515	20.8	0.0402	5.5	0.1519	20.9
Panel 2	360	1.217	0.0431	3.5	0.0516	4.2	0.0556	4.6	5.0	0.1114	9.2	0.0592	4.9	0.1132	9.3
NC	360	1.918	0.0736	3.8	0.0830	4.3	0.0843	4.4	4.7	0.0849	4.4	0.0887	4.6	0.0887	4.6
PC	360	0.296	0.0138	4.6	0.0170	5.7	0.0185	6.3	6.9	0.0894	30.2	0.0217	7.3	0.0901	30.4

Sample	Total No. Reps	Grand Mean Rate	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
IC	360	818.98	29.914	3.7	32.439	4.0	33.936	4.1	4.5	114.309	14.0	50.637	6.2	119.729	14.6

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

Within-Laboratory Precision

A 20-day precision study was conducted based on guidance from CLSI EP5-A2[23]. Testing was conducted at Abbott Laboratories using two AxSYM CORE 2.0 reagent lots, one control lot, and two AxSYM instruments. Testing included two precision runs per day (a minimum of two hours apart) for each reagent lot, on each instrument, on each of 20 days. Each precision run included two replicates of each of the two panel members and the AxSYM CORE 2.0 Index Calibrator, Negative Control, and Positive Control. Panel members were prepared by adding anti-HBc positive plasma to anti-HBc negative serum. The data are summarized in Table 4.

Table 4
AxSYM CORE 2.0 20-day Precision

Sample	Total No. Reps	Grand Mean S/CO	Within-Run		Within-Day		Within-Laboratory Precision (Total)			Precision with Additional Component of Between-Lot		Precision with Additional Component of Between-Instrument	
			SD	%CV	SD	%CV	SD	%CV	CL	SD	%CV	SD	%CV
Panel 1	320	0.783	0.0219	2.8	0.0283	3.6	0.0324	4.1	4.5	0.1477	18.9	0.0534	6.8
Panel 2	320	1.216	0.0365	3.0	0.0400	3.3	0.0503	4.1	4.5	0.1520	12.5	0.0881	7.2
NC	320	1.873	0.0556	3.0	0.0611	3.3	0.0714	3.8	4.1	0.1177	6.3	0.1311	7.0
PC	320	0.326	0.0120	3.7	0.0137	4.2	0.0159	4.9	5.3	0.0469	14.4	0.0248	7.6

Sample	Total No. Reps	Grand Mean Rate	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
IC	320	827.51	24.399	2.9	25.220	3.0	30.265	3.7	4.0	35.167	4.2	57.668	7.0

NC = Negative Control, PC = Positive Control, IC = Index Calibrator, Reps = 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 CORE 2.0 with serum specimens from 1,784 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 in the United States at specimen collection sites located in Galveston, TX (43.55%); Dallas, TX (5.72%); Miami, FL (3.76%); St. Petersburg, FL (2.69%); Chicago, IL (3.64%); and Denver, CO (5.89%), or were obtained from a specimen vendor at the following three locations: Colton, CA (6.45%); Plymouth, MA (17.99%); and High Point, NC (10.31%) (Population 1). Specimens were also prospectively collected in Vietnam from 299 individuals at increased risk of HBV infection and individuals with signs and symptoms of hepatitis infection (Population 2).

Population 1 was Caucasian (53.76%), African American (28.64%), Hispanic (13.62%), Asian (2.07%), and American Indian/Alaska Native (0.39%), with the remaining 1.51% represented by other ethnic groups. The population was 54.88% female and 45.12% male and ranged in age from 18 to 83 years. Testing of these specimens occurred at clinical testing sites located in Port Jefferson, NY (43.55%); Dallas, TX (38.40%); and Raritan, NJ (18.05%).

Population 2 was Vietnamese (100.00%). The population was 53.18% female and 46.82% male and ranged in age from 18 to 68 years. Testing of these specimens occurred at a clinical testing site located in Raritan, NJ.

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.

The specimens were assigned an HBV classification (Tables 5 and 6), and the AxSYM CORE 2.0 results were compared to the reference anti-HBc results (Tables 7 and 8). Agreement of the AxSYM CORE 2.0 assay was assessed relative to the reference anti-HBc results (Tables 9 and 10).

Results of HBV Classification

Population 1 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 13 unique HBV reference marker patterns observed in the AxSYM CORE 2.0 clinical investigation.

Table 5
HBV Classification for Individuals at Increased Risk of HBV Infection and Individuals With Signs and Symptoms of Hepatitis Infection—Population 1

HBV Reference Markers					HBV Classification
Number of Specimens	HBsAg	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
1	+	–	–	–	Early Acute
2	+	+	+	–	Acute
20	+	–	+	–	Chronic
1	+	–	–	+	Chronic
2	+	–	+	I	Chronic
3	–	+	+	+	Recovering Acute
2	–	+	+	I	Early Recovery
158	–	–	+	+	Immune Due to Natural Infection
25	–	–	+	I	Distantly Immune/Anti-HBs Unknown
79	–	–	+	–	Distantly Immune/Anti-HBs Not Detected
474	–	–	–	+	Immune Due to HBV Vaccination
57	–	–	–	I	Unknown
960	–	–	–	–	Susceptible
1,784					**Total**

I = Indeterminate

Population 2 specimens were assigned an HBV classification using the positive (+) and negative (–) patterns for the four HBV reference markers. Table 6 is a summary of how these classifications were derived and the number of specimens in each classification. There were 10 unique HBV reference marker patterns observed in the AxSYM CORE 2.0 clinical investigation.

Table 6
HBV Classification for Individuals at Increased Risk of HBV Infection and Individuals With Signs and Symptoms of Hepatitis Infection—Population 2

HBV Reference Markers					HBV Classification
Number of Specimens	HBsAg	Anti-HBc IgM	Total Anti-HBc	Anti-HBs	
1	+	–	–	–	Early Acute
3	+	–	+	+	Chronic
119	+	–	+	–	Chronic
2	+	–	–	+	Chronic
3	+	–	+	I	Chronic
71	–	–	+	+	Immune Due to Natural Infection
5	–	–	+	I	Distantly Immune/Anti-HBs Unknown
15	–	–	+	–	Distantly Immune/Anti-HBs Not Detected
41	–	–	–	+	Immune Due to HBV Vaccination
39	–	–	–	–	Susceptible
299					**Total**

I = Indeterminate

Comparison of Results

Table 7 is a comparison of the AxSYM CORE 2.0 results to the reference anti-HBc assay results for Population 1 by HBV classification.

Table 7
Comparison of AxSYM CORE 2.0 Results With Reference Anti-HBc Results by HBV Classification—Population 1

	Reference Anti-HBc Result[a]				
	+		–		
	AxSYM CORE 2.0 Result[b]				
HBV Classification	+	–	+	–	Total
Early Acute	0	0	1[c]	0	1
Acute	2	0	0	0	2
Chronic	22	0	0	1	23
Recovering Acute	3	0	0	0	3
Early Recovery	2	0	0	0	2
Immune Due to Natural Infection	157	1[d]	0	0	158
Distantly Immune/Anti-HBs Unknown	25	0	0	0	25
Distantly Immune/Anti-HBs Not Detected	78	1[e]	0	0	79
Immune Due to HBV Vaccination	0	0	18[f]	456	474
Unknown	0	0	1	56	57
Susceptible	0	0	18[g]	942	960
Total	**289**	**2**	**38**	**1,455**	**1,784**

[a] Includes retesting performed according to the package insert as required.
[b] Includes retesting as required.
[c] This specimen was tested and determined to be positive for HBV DNA and anti-HBc by a second FDA-approved anti-HBc assay.
[d] This specimen was tested and determined to be positive for anti-HBc by a second FDA-approved anti-HBc assay.
[e] This specimen was tested and determined to be negative for HBeAg, anti-HBe, HBV DNA, and anti-HBc by a second FDA-approved anti-HBc assay.
[f] One specimen was tested and determined to be positive for anti-HBe; one specimen was equivocal for anti-HBe; one specimen was positive for HBV DNA; one specimen was positive for anti-HBc by a second FDA-approved anti-HBc assay.
[g] Two specimens were tested and determined to be positive for anti-HBc by a second FDA-approved anti-HBc assay; one specimen was positive for HBV DNA and anti-HBc by a second FDA-approved anti-HBc assay.

Note: Thirty-six (36) specimens with an initial grayzone result were retested in duplicate.

Table 8 is a comparison of the AxSYM CORE 2.0 results to the reference anti-HBc assay results for Population 2 by HBV classification.

Table 8
Comparison of AxSYM CORE 2.0 Results With Reference Anti-HBc Results by HBV Classification—Population 2

	Reference Anti-HBc Result[a]				
	+		–		
	AxSYM CORE 2.0 Result[b]				
HBV Classification	+	–	+	–	Total
Early Acute	0	0	0	1	1
Chronic	125	0	2[c]	0	127
Immune Due to Natural Infection	71	0	0	0	71
Distantly Immune/Anti-HBs Unknown	5	0	0	0	5
Distantly Immune/Anti-HBs Not Detected	15	0	0	0	15
Immune Due to HBV Vaccination	0	0	23[d]	18	41
Susceptible	0	0	4[e]	35	39
Total	**216**	**0**	**29**	**54**	**299**

[a] Includes retesting performed according to the package insert as required.
[b] Includes retesting as required.
[c] Both specimens were tested and determined to be positive for HBeAg and HBV DNA.
[d] Five specimens were tested and determined to be positive for anti-HBe; one specimen was equivocal for anti-HBe.
[e] One specimen was tested and determined to be positive for anti-HBe; one specimen was positive for anti-HBe and anti-HBc by a second FDA-approved anti-HBc assay.
Note: Ten (10) specimens with an initial grayzone result were retested in duplicate.

Percent Agreement

Table 9 is a summary, for each HBV classification, of the percent agreement between AxSYM CORE 2.0 and the reference anti-HBc assay for Population 1.

Table 9
Percent Agreement Between AxSYM CORE 2.0 Results and Reference Anti-HBc Results Summarized by HBV Classification—Population 1

HBV Classification	Positive Percent Agreement (%)	95% Confidence Interval (%)	Negative Percent Agreement (%)	95% Confidence Interval (%)
Early Acute	NA	NA	0/1 (0.00)	[0.00, 97.50]
Acute	2/2 (100.00)	[15.81, 100.00]	NA	NA
Chronic	22/22 (100.00)	[84.56, 100.00]	1/1 (100.00)	[2.50, 100.00]
Recovering Acute	3/3 (100.00)	[29.24, 100.00]	NA	NA
Early Recovery	2/2 (100.00)	[15.81, 100.00]	NA	NA
Immune Due to Natural Infection	157/158 (99.37)	[96.52, 99.98]	NA	NA
Distantly Immune/Anti-HBs Unknown	25/25 (100.00)	[86.28, 100.00]	NA	NA
Distantly Immune/Anti-HBs Not Detected	78/79 (98.73)	[93.15, 99.97]	NA	NA
Immune Due to HBV Vaccination	NA	NA	456/474 (96.20)	[94.06, 97.73]
Unknown	NA	NA	56/57 (98.25)	[90.61, 99.96]
Susceptible	NA	NA	942/960 (98.13)	[97.05, 98.89]
Overall	289/291 (99.31)	[97.54, 99.92]	1,455/1,493 (97.45)	[96.52, 98.19]

NA = Not Applicable

Table 10 is a summary, for each HBV classification, of the percent agreement between AxSYM CORE 2.0 and the reference anti-HBc assay for Population 2.

Table 10
Percent Agreement Between AxSYM CORE 2.0 Results and Reference Anti-HBc Results Summarized by HBV Classification—Population 2

HBV Classification	Positive Percent Agreement (%)	95% Confidence Interval (%)	Negative Percent Agreement (%)	95% Confidence Interval (%)
Early Acute	NA	NA	1/1 (100.00)	[2.50, 100.00]
Chronic	125/125 (100.00)	[97.09, 100.00]	0/2 (0.00)	[0.00, 84.19]
Immune Due to Natural Infection	71/71 (100.00)	[94.94, 100.00]	NA	NA
Distantly Immune/Anti-HBs Unknown	5/5 (100.00)	[47.82, 100.00]	NA	NA
Distantly Immune/Anti-HBs Not Detected	15/15 (100.00)	[78.20, 100.00]	NA	NA
Immune Due to HBV Vaccination	NA	NA	18/41 (43.90)	[28.47, 60.25]
Susceptible	NA	NA	35/39 (89.74)	[75.78, 97.13]
Overall	216/216 (100.00)	[98.31, 100.00]	54/83 (65.06)	[53.81, 75.20]

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NA = Not Applicable

Percent of Positive Specimens for Individuals Diagnosed With Acute or Chronic HBV Infection

AxSYM CORE 2.0 performance was evaluated by testing prospectively-collected serum specimens from individuals diagnosed with acute or chronic HBV infection. Table 11 is a summary of the percent of AxSYM CORE 2.0 positive specimens from subjects with documented acute or chronic HBV infection. Acute status was defined by four-marker HBV reference testing, and chronic status was defined by the presence of HBsAg for ≥ 6 months.

Table 11
Percent of Positive Specimens—Individuals Diagnosed With Acute or Chronic HBV Infection

Specimen Category	Number of Specimens	Number of Positive Specimens (%)	95% Confidence Interval (%)
Individuals Diagnosed With Acute HBV Infection	6	6 (100.00)	[54.07, 100.00]
Individuals Diagnosed With Chronic HBV Infection	43	43 (100.00)	[91.78, 100.00]
Total	**49**	**49 (100.00)**	**[92.75, 100.00]**

Clinical Performance in a Pediatric Population

The performance of AxSYM CORE 2.0 in a pediatric population was evaluated by testing specimens from a surplus pediatric population (n=100) collected in Fall River, MA, by a specimen vendor and from the pediatric subjects (n=108) from Population 1 and Population 2, as shown in the Clinical Performance section of this package insert. The positive and negative percent agreement between the AxSYM CORE 2.0 assay and the reference anti-HBc assay were calculated. The positive percent agreement for the surplus pediatric population was 66.67% (2/3) with a 95% confidence interval of 9.43% to 99.16%, and the negative percent agreement was 97.94% (95/97) with a 95% confidence interval of 92.75% to 99.75%. The positive percent agreement for prospectively collected pediatric population was 100.00% (24/24) with a 95% confidence interval of 85.75% to 100.00%, and the negative percent agreement was 95.24% (80/84) with a 95% confidence interval of 88.25% to 98.69%.

Table 12 is a demographic summary of the surplus population by age range and gender with AxSYM CORE 2.0 results.

Table 12
Demographic Summary With AxSYM CORE 2.0 Results for a Surplus Pediatric Population

Age Range	Gender	AxSYM CORE 2.0 Result		Total
		+	−	
		Number of Specimens	Number of Specimens	
> 2 to 12 Years	Female	2	27	29
	Male	0	21	21
> 12 to 19 Years	Female	1	33	34
	Male	1	15	16
Total		**4 (4.00%)**	**96 (96.00%)**	**100**

Table 13 is a demographic summary of the prospectively collected population (Population 1 and Population 2) by age range and gender with AxSYM CORE 2.0 results.

Table 13
Demographic Summary With AxSYM CORE 2.0 Results for a Prospectively Collected Pediatric Population

Age Range	Gender	AxSYM CORE 2.0 Result		Total
		+	−	
		Number of Specimens	Number of Specimens	
> 18 to 21 Years	Female	17	33	50
	Male	11	47	58
Total		**28 (25.93%)**	**80 (74.07%)**	**108**

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SEROCONVERSION DETECTION

The ability of the AxSYM CORE 2.0 assay to detect anti-HBc was evaluated by testing six seroconversion panels obtained from three commercial vendors. The results were compared to the results from FDA-licensed HBsAg and anti-HBc assays.

- Four of the six seroconversion panels demonstrated a change from HBsAg reactive to HBsAg nonreactive. In these four panels, anti-HBc was detected by AxSYM CORE 2.0 concurrent with HBsAg reactivity by the FDA-licensed HBsAg assay, or at a minimum, at the serial bleed following the last serial bleed reported as reactive by the FDA-licensed HBsAg assay. The remaining two seroconversion panels became HBsAg reactive and remained HBsAg reactive for all subsequent serial bleeds. In these two panels, anti-HBc was detected by AxSYM CORE 2.0 concurrent with HBsAg reactivity by the FDA-licensed HBsAg assay.
- Anti-HBc was detected by AxSYM CORE 2.0 two days earlier than the FDA-licensed anti-HBc assay in one panel, coincident with the FDA-licensed anti-HBc assay in four panels, and three days later than the FDA-licensed anti-HBc assay in one panel.

ANALYTICAL SPECIFICITY

A study was conducted to evaluate the potential for cross-reactivity in the AxSYM CORE 2.0 assay when used to test specimens from individuals with medical conditions unrelated to HBV infection. A total of 181 specimens from 19 different categories were tested. Specimens with an initial interpretation of reactive or gray zone were tested by an FDA-licensed anti-HBc assay. One hundred thirty-three specimens (73.5%) were nonreactive, 3 (1.7%) were gray zone, and 45 (24.9%) were reactive by AxSYM CORE 2.0. The data are summarized in Table 14.

Table 14
Cross-reactivity of AxSYM CORE 2.0
in Specimens from Individuals with Medical Conditions Unrelated to HBV

Category[a]	Number of Specimens Tested	AxSYM CORE 2.0		
		Nonreactive	Gray Zone	Reactive[b]
Hepatitis A Virus	8	3	2	3
Hepatitis C Virus	10	5	0	5
Human Immunodeficiency Virus	10	4	0	6
Human T-Lymphotropic Virus	9	1	0	8
Cytomegalovirus	10	6	0	4
Epstein-Barr Virus	10	6	0	4
Herpes Simplex Virus	10	8	0	2
Rubella	10	10	0	0
Systemic Lupus Erythematosus	10	9	0	1
Rheumatoid Arthritis Disease	10	5	0	5
Elevated IgG	10	10	0	0
Elevated IgM	10	10	0	0
Influenza Vaccine Recipients	10	9	0	1
HBV Vaccine Recipients	5	5	0	0
Toxoplasmosis	4	3	0	1
Alcoholic Liver Disease	10	10	0	0
Fatty Liver Disease	15	14	0	1
Obstructive Jaundice	15	13	1[c]	1
Hepatocellular Carcinoma	5	2	0	3
Total (%)	**181**	**133/181 (73.5%)**	**3/181 (1.7%)**	**45/181 (24.9%)**

[a] Information about age and gender of the individuals is not available.
[b] With the exception of three Rheumatoid Arthritis Disease specimens, all specimens reactive by AxSYM CORE 2.0 were repeatedly reactive by an FDA-licensed anti-HBc assay.
[c] Specimen could not be retested due to insufficient volume. The specimen was negative by an FDA-licensed anti-HBc assay.

Interference

At the concentrations listed below, total bilirubin (unconjugated), hemoglobin, total protein, and triglycerides showed less than 0.200 S/CO difference for samples targeted to 0.8 S/CO and less than 11% bias for samples targeted to 1.2 S/CO in the AxSYM CORE 2.0 assay.

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

Tube Type Matrix Comparison

The following tube types are acceptable for use with the AxSYM CORE 2.0 assay:

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

On average, the tube types evaluated showed less than an 11% 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 15
Sample Type (Serum and Plasma) Study of AxSYM CORE 2.0
Distribution of Percent Differences by Sample Type

	Distribution of %Differences		
Evaluation Tube Type	**0% To ≤10%**	**>10% to ≤ 20%**	**>20%**
Plastic Serum	85.4% (35/41)	14.6% (6/41)	0.0% (0/41)
Glass Serum Separator	92.7% (38/41)	7.3% (3/41)	0.0% (0/41)
Plastic Serum Separator	90.2% (37/41)	9.8% (4/41)	0.0% (0/41)
Plastic Plasma Separator	87.8% (36/41)	12.2% (5/41)	0.0% (0/41)
Plastic Sodium Citrate	80.5% (33/41)	17.1% (7/41)	2.4% (1/41)
Plastic Potassium EDTA	70.7% (29/41)	29.3% (12/41)	0.0% (0/41)
Plastic Sodium Heparin	90.2% (37/41)	4.9% (2/41)	4.9% (2/41)
Plastic Lithium Heparin	92.7% (38/41)	7.3% (3/41)	0.0% (0/41)

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