HIV Ag/Ab Combo

Customer Service
For additional product information, please contact your local customer service organization.

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.

Key to symbols used

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>List Number</td>
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<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot Number</td>
</tr>
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<td></td>
<td>Expiration Date</td>
</tr>
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<td></td>
<td>Store at 2-8°C</td>
</tr>
<tr>
<td></td>
<td>CAUTION: Consult accompanying documents</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td>REAGENT PACK</td>
<td>Reagent Pack</td>
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<tr>
<td>REACTION VESSELS</td>
<td>Reaction Vessels</td>
</tr>
<tr>
<td>MATRIX CELLS</td>
<td>Matrix Cells</td>
</tr>
<tr>
<td>SAMPLE CUPS</td>
<td>Sample Cups</td>
</tr>
<tr>
<td></td>
<td>Consult instructions for use</td>
</tr>
</tbody>
</table>

See REAGENTS section for a full explanation of symbols used in reagent component naming.
NAME
AxSYM HIV Ag/Ab Combo

INTENDED USE
AxSYM HIV Ag/Ab Combo is a microparticle enzyme immunoassay (MEIA) for the simultaneous qualitative detection of antibodies to human immunodeficiency virus type 1 and/or type 2 (HIV-1/HIV-2) and HIV p24 antigen in human serum or plasma. AxSYM HIV Ag/Ab Combo is used as an aid in the diagnosis of HIV infection. AxSYM HIV Ag/Ab Combo does not discriminate between HIV-1 and HIV-2 antibody reactivity or HIV p24 antigen reactivity.

SUMMARY AND EXPLANATION OF THE TEST
Acquired immunodeficiency syndrome (AIDS) is caused by two types of human immunodeficiency viruses, collectively designated as HIV. 1-7 HIV is the etiologic agent of AIDS. 1,3-7 HIV is transmitted by sexual contact, exposure to blood or blood products, and perinatal or perinatal infection of a fetus or newborn, respectively. 2 Antibodies against HIV are nearly always detected in AIDS patients and HIV infected asymptomatic individuals. 8,9 and HIV nucleic acid (RNA and/or proviral DNA) is always detected in AIDS patients and seropositive individuals. 9,10 Phenylogenetic analysis classifies HIV-1 into groups M (major), N (non-M, non-O), and O (outlier). 11 Group M viruses have spread throughout the world to cause the global AIDS pandemic. In contrast, groups N and O are relatively rare and endemic to west central Africa. 11-17 However, group O infections have been identified in Europe and the USA. 18-22 HIV-1 group M is composed of genetic subtypes (A, B, C, D, F, G, H, J, and K) and circulating recombinant forms (CRFs). 5,23 The geographic distribution and regional predominance of HIV-1 subtypes and CRFs vary. All subtypes and many recombinant strains exist in Africa with CRF02_AG the predominant strain in west and west central Africa, subtypes A, C, and D predominant in eastern central Africa, and subtype C predominant in southern Africa. 5,23-25 HIV-1 subtype B is the predominant subtype in the USA, Europe, Japan, and Australia. However, a significant percentage of new HIV-1 infections in Europe are caused by non-B subtypes. 29,30 In Asia, subtype C is found in India, and CRF01_AE (formerly called subtype E) and subtype B are found in Thailand and southeast Asia. 31 South America predominately has subtypes B and F. 32,33

Human immunodeficiency virus type 2 (HIV-2) is similar to HIV-1 in its structural morphology, genomic organization, cell tropism, in vitro cytopathogenicity, transmission routes and ability to cause AIDS. 6-8 However, HIV-2 is less pathogenic than HIV-1, and HIV-2 infections have a longer latency period with slower progression to disease, lower viral titers, and lower rates of vertical and horizontal transmission. 34-37 HIV-2 is endemic to west Africa but HIV-2 infections, at a low frequency compared to HIV-1, have been identified in the USA, Europe, Asia, and other regions of Africa. 35,37 HIV-2 is classified into genetic subtypes A-G with most infections caused by subtypes A and B. 38,39

The key immunogenic protein and antigenic target for serodetection of HIV infection is the viral (HIV) transmembrane protein (TMP). Antibodies against the TMP (anti-TMP) consistently are among the first to appear at seroconversion of HIV infected individuals. 5,39-43 The anti-TMP response remains relatively strong throughout the course of the disease, as evidenced by the near universal presence of antibodies against the TMP in asymptomatic and symptomatic stages of HIV infection. 9,39-44 TMPs from HIV-1 groups M and O and HIV-2 are represented in AxSYM HIV Ag/Ab Combo on the solid phase and in the probe by three pairs of recombinant proteins and two synthetic peptides derived from native TMP sequences. The rationale for including three pairs of TMPs is derived from the genetic diversity within HIV-1 and between HIV-1 and HIV-2. 2 Serologic studies indicate that although HIV-1 and HIV-2 share multiple common epitopes in their core antigens, the envelope glycoproteins are much less cross-reactive. 7,45-49 Antibodies elicited against the TMP (or portions of the TMP) of a viral strain within one group or type may react well, poorly, or not at all with the TMP (or portions of the TMP) from a viral strain of a different group or type. 15,50-56 An example may be antibodies elicited against HIV-1 group M and HIV-2 group N. 11,12

Early after infection with HIV, prior to seroconversion, HIV antigen(s) may be detected in serum or plasma specimens. 55-63 The HIV structural protein most often used as the marker of antigenemia is the core protein, p24. The AxSYM HIV Ag/Ab Combo employs pairs of mouse monoclonal (anti-) p24 antibodies on microparticles and in the probe to detect HIV p24 antigen prior to seroconversion, thereby decreasing the seroconversion window and improving early detection of HIV infection.

Specimens that are initially reactive in the AxSYM HIV Ag/Ab Combo MEIA should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HIV-1 and/or HIV-2 antibodies and/or HIV p24 antigen. However, as for all enzyme immunoassays, AxSYM HIV Ag/Ab Combo may yield nonspecific reactions due to other causes, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be investigated further in sensitive, supplemental HIV specific tests, such as immunoblots, antigen tests, and HIV nucleic acid tests. Supplemental testing of repeat reactive specimens obtained from individuals at risk for HIV infection usually confirms the presence of HIV antibodies or HIV antigen, and HIV nucleic acid. A full differential diagnostic work-up for the diagnosis of AIDS and AIDS-related conditions includes an examination of the patient’s immune status and a clinical history.

BILOGICAL PRINCIPLES OF THE PROCEDURE
AxSYM HIV Ag/Ab Combo is based on MEIA technology utilizing recombinant HIV (E. coli) antigens and HIV p24 monoclonal (mouse) antibodies coated on microparticles to capture antibodies against HIV-1/ HIV-2 and HIV p24 antigen, respectively. Captured antibodies/antigen react with biotin-labeled recombinant antigens, peptides, and p24 monoclonal antibodies. The biotin-labeled complexes are detected using an anti-biotin: alkaline phosphatase conjugate. Sample and all AxSYM HIV Ag/Ab Combo reagents required for one test are pipetted by the sampling probe into designated wells of a reaction vessel (RV) in the sampling center. The RV is immediately transferred into the processing center. Further pipetting is done in the processing center by the processing probe. The reactions occur in the following sequence:

- A reaction mixture is formed by combining specimen diluent, sample, and microparticles coated with recombinant antigens and HIV p24 monoclonal antibodies in the sample well of the reaction vessel.
- When human antibodies to HIV-1/2 or HIV p24 antigen are present in the sample, they bind to the coated microparticles, forming antigen-antibody complexes on the microparticles.
- A portion of the reaction mixture (containing microparticles) is transferred to the matrix cell.
- The matrix cell is washed to remove materials not bound to the microparticles.
- Biotinylated recombinant antigens, synthetic peptides, and HIV p24 monoclonal antibodies are dispensed onto the matrix cell, forming immune complexes composed of recombinant antigen-human antibody-biotinylated antigen or monoclonal antibody-p24 antigen-biotinylated monoclonal antibody.
- The matrix cell is washed to remove materials not bound to the microparticles.
- The anti-biotin: alkaline phosphatase conjugate is dispensed onto the matrix cell and binds with the immune complexes.
- The matrix cell is washed to remove materials not bound to the microparticles.
- The substrate, 4-Methylumbelliferyl phosphate, is added. The alkaline phosphatase-labeled conjugate catalyzes the removal of a phosphate group from the substrate, yielding the fluorescent product, 4-Methylumbelliferone. This fluorescent product is measured by the MEIA optical assembly.

The presence or absence of antibodies to HIV-1/2 and/or HIV p24 antigen in the sample is determined by comparing the rate of formation of fluorescent product to the cutoff rate which is previously calculated using the AxSYM HIV Ag/Ab Combo Index Calibrator. If the rate of formation of fluorescent product in the sample is greater than or equal to the cutoff rate, the sample is considered reactive for antibodies and/or p24 antigen. For further information regarding MEIA technology, refer to the AxSYM System Operations Manual, Section 3.

REAGENTS
REAGENT KIT, 100 TESTS
AxSYM HIV Ag/Ab Combo Reagent Pack (2G83-20)
- Bottle (6.8 mL) HIV-1/HIV-2 antigen/antibody coated microparticles in TRIS buffer. Minimum concentration: 0.025% solids. Preservative: sodium azide. (Reagent Bottle 1)
- Bottle (14.3 mL) biotinylated probes: HIV-1/HIV-2 antibodies and anti-HIV p24 antibodies in TRIS buffer with surfactant, protein blockers, stabilizers, and 10% goat serum. Minimum concentration: 100 ng/mL. Preservatives: sodium azide and ProClin 300. (Reagent Bottle 2)
• 1 Bottle (11.7 mL) anti-biotin (rabbit): alkaline phosphatase conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.05 μg/mL. Preservative: sodium azide. (Reagent Bottle 1)

• 1 Bottle (38.2 mL) Specimen Diluent in TRIS buffer with surfactant, protein blockers, and stabilizers. Preservatives: sodium azide and ProClin 300. (Reagent Bottle 4)

Index Calibrator

- **INDEX CAL** 1 Bottle (4.3 mL) AxSYM HIV Ag/Ab Combo Index Calibrator. Recalculated human plasma, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. Preservative: sodium azide. Dye: green (Yellow No. 23 and Blue No. 9).

CONTROLS

AxSYM HIV Ag/Ab Combo Controls: (NEG, PC1, PC2, VLPC) (2G83-11)

4 Bottles (8.0 mL each) of AxSYM HIV Ag/Ab Combo Controls.

- **CONTROL** - The Negative Control, prepared in recalculated human plasma, is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. Preservative: sodium azide.

- **POSITIVE CONTROL 1** - The HIV-1 Positive Control (PC1), prepared in recalculated human plasma (inactivated), is reactive for anti-HIV-1 and nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, and anti-HCV. Preservative: sodium azide.

- **POSITIVE CONTROL 2** - The HIV-2 Positive Control (PC2), prepared in recalculated human plasma (inactivated), is reactive for anti-HIV-2 and nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, and anti-HCV. Preservative: sodium azide.

- **VIRAL LYSE POSITIVE CONTROL** - The Viral Lysate Positive Control (VLPC) is purified HIV viral lysate prepared in TRIS buffer with stabilizers. Preservative: sodium azide.

AxSYM HIV Ag/Ab Combo Controls: (NEG, PC1, VLPC) (2G83-12)

3 Bottles (8.0 mL each) of AxSYM HIV Ag/Ab Combo Controls.

- **CONTROL** - The Negative Control, prepared in recalculated human plasma, is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2. Preservative: sodium azide.

- **POSITIVE CONTROL 1** - The HIV-1 Positive Control (PC1), prepared in recalculated human plasma (inactivated), is reactive for anti-HIV-1 and nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, and anti-HCV. Preservative: sodium azide.

- **VIRAL LYSE POSITIVE CONTROL** - The Viral Lysate Positive Control (VLPC) is purified HIV viral lysate prepared in TRIS buffer with stabilizers. Preservative: sodium azide.

Control Color Dye Control Range S/CO

<table>
<thead>
<tr>
<th>Control</th>
<th>Color</th>
<th>Dye</th>
<th>Control Range S/CO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONTROL</strong></td>
<td>Natural</td>
<td>None</td>
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</tr>
<tr>
<td><strong>POSITIVE CONTROL 1</strong></td>
<td>Blue</td>
<td>Blue No. 9</td>
<td>1.00 - 10.00</td>
</tr>
<tr>
<td><strong>POSITIVE CONTROL 2</strong></td>
<td>Red</td>
<td>Red No. 33</td>
<td>1.00 - 10.00</td>
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<tr>
<td><strong>VIRAL LYSE POSITIVE CONTROL</strong></td>
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<td>Blue No. 9</td>
<td>1.00 - 10.00</td>
</tr>
</tbody>
</table>

AxSYM HIV Ag/Ab Combo Index Calibrator and Controls should be mixed by gentle inversion prior to use.

OTHER REAGENTS

AxSYM Probe Cleaning Solution (9A35-05)

- **PROBE CLEANING SOLUTION** 2 Bottles (220 mL each) AxSYM Probe Cleaning Solution containing 2% tetraethylammonium hydroxide (TEAH).

Solution 1 (MUP) (8A47-04)

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

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

- **SOLUTION 3 MATRIX CELL WASH** 4 Bottles (1000 mL each) Solution 3 (Matrix Cell Wash) containing 0.3 M sodium chloride in TRIS buffer. Preservatives: sodium azide and antimicrobial agents.

Solution 4 (Line Diluent) (8A46)

- **SOLUTION 4 LINE DILUENT** 1 Bottle (10 L) Solution 4 (Line Diluent) containing 0.1 M phosphate buffer. Preservatives: sodium azide and antimicrobial agent.

WARNINGS AND PRECAUTIONS

**For In Vitro Diagnostic Use.**

**SAFETY PRECAUTIONS**

**CAUTION:** This product contains human sourced infectious and/or potentially infectious components. Refer to the REAGENTS section of this package insert. HIV-1 Positive Control has been tested and found to be reactive for anti-HIV-1. HIV-2 Positive Control has been tested and found to be reactive for anti-HIV-2. Remaining components have been tested and found to be nonreactive for antibodies to HCV, HIV-1/HIV-2 and nonreactive for HBsAg and HIV-1 RNA or HIV-1 Ag. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.

- Some components of this product contain methylisothiazolones, which are components of ProClin and are classified per applicable European Community (EC) Directives as: Irritant (Xi). For a specific listing refer to the REAGENTS section of this package insert. The following are the appropriate Risk (R) and Safety (S) phrases:

  - R43 May cause sensitization by skin contact
  - S24 Avoid contact with skin
  - S35 This material and its container must be disposed of in a safe way
  - S37 Wear suitable gloves
  - S46 If swallowed, seek medical advice immediately and show this container or label

- All components of this product contain sodium azide. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

**HANDLING PRECAUTIONS**

- AxSYM HIV Ag/Ab Combo 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 reagent pack beyond the expiration date, or a maximum of 336 cumulative hours on-board the AxSYM System.

- Do not mix reagents from different reagent packs. Do not mix reagents, controls, and index calibrators from different lots. The reagent pack containing an associated index calibrator must be discarded together.

- 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 may indicate an error code and no result will be reported. For a description of the system error codes, refer to the AxSYM System Operations Manual, Section 10.

- Inadequate adherence to these package insert instructions may result in inconsistent results.

- Use accurately calibrated equipment.

- 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**

- Store at 2-8°C and should be stored at 2-8°C after receipt.
When stored and handled as directed, reagents are stable until expiration date. The AxSYM HIV Ag/Ab Combo Reagent Pack may be on-board the AxSYM System for a maximum of 336 cumulative hours (for example, 42 eight hour shifts). Recalibration may be required to obtain maximum onboard reagent stability. More frequent use of controls may be required to monitor reagent performance within the same lot. After 336 hours, the reagent pack and its associated index calibrator must be discarded. Refer to the AxSYM System Operations Manual, Sections 2, 5, and Appendix C, for further information on tracking on-board time.

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

- **15°C**

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

**INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS**

When AxSYM HIV Ag/Ab Combo Controls, Negative Control, Positive Control 1, Positive Control 2, or Viral Lysate Positive Control values are out of the expected range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and the 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 HIV Ag/Ab Combo must only be used with AxSYM System Software Version 3.60 or higher.

**Assay File Installation**

The AxSYM HIV Ag/Ab Combo Assay File Version 1.00.1 or higher must be installed on the AxSYM System from the assay disk, 2G84-01 or higher, prior to performing the AxSYM HIV Ag/Ab Combo assay. Refer to the AxSYM System Operations Manual, Section 2, for proper installation procedures.

**AxSYM HIV Ag/Ab Combo Assay Parameters**

The AxSYM HIV Ag/Ab Combo assay parameters are listed below. Values for the assay parameters that can be edited contain a (> symbol). These parameters can be displayed and edited according to the procedure in the AxSYM System Operations Manual, Section 2. In order to obtain values for the parameters with an asterisk (*), review the specific Assay Parameters screen. Press PRINT to print the assay parameters.

<table>
<thead>
<tr>
<th><strong>Assay Parameters</strong></th>
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<tbody>
<tr>
<td>1 Long Assay Name (English):</td>
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<tr>
<td>6 Abbrev Assay Name (English):</td>
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<tr>
<td>18 Standard Cal Reps &gt;</td>
<td>3</td>
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<tr>
<td>43 Default Dilution Protocol &gt;</td>
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<tr>
<td>44 Default Calibration Method &gt;</td>
<td>Index Cal</td>
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<tr>
<td>45 Selected Result Concentration Units &gt;</td>
<td>S/CO</td>
</tr>
<tr>
<td>46 Selected Result Decimal Places &gt;</td>
<td>2</td>
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<tr>
<td>64 Max Intercept-Max MUP intercept:</td>
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</tr>
<tr>
<td>65 Min Intercept-Min MUP intercept:</td>
<td>1850.0000</td>
</tr>
<tr>
<td>66 Upper Limit for NRMSE for low rates:</td>
<td>9999.9900</td>
</tr>
<tr>
<td>67 Upper Limit for NRMSE for high rates:</td>
<td>0.3900** or 0.5100***</td>
</tr>
<tr>
<td>68 Max Rate-Max rate used to check Min MUP Intercept:</td>
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</tr>
<tr>
<td>69 Min Rate-Rate cutoff for NRMSE and Corr. Coef.:</td>
<td>8.0000</td>
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</tbody>
</table>

**Assay Parameters**

<table>
<thead>
<tr>
<th><strong>Assay Parameters</strong></th>
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</thead>
<tbody>
<tr>
<td>70 Min correlation coefficient for low rates:</td>
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<tr>
<td>71 Min correlation coefficient for high rates:</td>
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<tr>
<td>72 MUP T Delay-Time delay following MUP:</td>
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<tr>
<td>75 Low Extreme Value &gt;</td>
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<tr>
<td>76 High Extreme Value &gt;</td>
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<tr>
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<tr>
<td>84 Hold results with POS interpretation &gt;</td>
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</tr>
<tr>
<td>85 Hold results with NEG interpretation &gt;</td>
<td>OFF</td>
</tr>
<tr>
<td>86 Hold results with GRY interpretation &gt;</td>
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<tr>
<td>91 Low Range Undiluted:</td>
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<tr>
<td>92 High Range Undiluted:</td>
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</table>

**** AxSYM HIV Ag/Ab Combo Assay File Version 1.00.1

*** AxSYM HIV Ag/Ab Combo Assay File Version 1.00.2 or higher

**NOTE:** Parameters 43, 44, and 45 cannot be edited. There are no other options for this assay.

The AxSYM HIV Ag/Ab Combo values available for Assay Parameter 80 (Interpretation Option to use) are:

- **POS Interp**: REACTIVE
- **NEG Interp**: NEGATIVE
- **GRY Interp**: GRAYZONE

To utilize the grayzone option, option 1 for parameter 80 (Interpretation Option to use) must be selected. Specimens with assay S/CO values equal to or greater than 0.90, and less than the cutoff are indicated as “GRAYZONE” in the interpretation field. (Refer to INTERPRETATION OF RESULTS section.)

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

**SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS**

- Human serum (collected in Kaolin-coated or serum separator tubes) or plasma (collected in EDTA, sodium heparin, lithium heparin, sodium citrate, ACD, CPD, CPD/Adsol, or CPDA-1) may be used in the AxSYM HIV Ag/Ab Combo assay. Follow the manufacturer’s processing instructions for serum and plasma collection tubes.
- The AxSYM System does not provide the capability to verify the sample type. It is the responsibility of the operator to verify that the correct sample type(s) is (are) used in the AxSYM HIV Ag/Ab Combo assay.
- This assay was designed and validated for use with human serum or plasma from individual patient and donor specimens. Pooled specimens must not be used since the accuracy of their test results has not been validated.
- Gravity separation is not sufficient for specimen preparation.
- All patient specimens to be tested in primary tubes must be centrifuged to remove red blood cells or particulate matter. Follow the tube manufacturer’s instructions for centrifugation.
- After specimens have been initially separated per the collection tube manufacturer’s instructions, they must be clarified by centrifugation at 150,000-300,000 g-minutes* if:
  - they contain clots, red blood cells, or particulate matter
  - they require repeat testing, or
  - they have been frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing.
  - g-minute = relative centrifugal force (RCF) x minutes spun

The following chart lists examples of acceptable time and force ranges that meet this criteria.

<table>
<thead>
<tr>
<th><strong>Time (minutes)</strong></th>
<th>RCF (g x)</th>
<th>RCF x Time (g-minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10000</td>
<td>150000</td>
</tr>
<tr>
<td>20</td>
<td>7500 - 12000</td>
<td>150000 - 240000</td>
</tr>
<tr>
<td>25</td>
<td>6000 - 12000</td>
<td>150000 - 300000</td>
</tr>
</tbody>
</table>

**NOTE:** The AxSYM System offers an Auto Retest/Auto Dilution feature. Due to the requirements discussed above, this feature must not be used with this assay.

- Centrifuged specimens with a lipid layer on top of the liquid must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen and not the lipemic material.
Perform AxSYM HIV Ag/Ab Combo calibration by testing three replicates of the Index Calibrator. Invert gently to mix and dispense at least 10 drops of the Index Calibrator into a sample cup. Do not simultaneously calibrate more than one AxSYM HIV Ag/Ab Combo reagent lot.
Controls
Perform quality control by testing Positive and Negative Controls (one test each). Invert gently to mix and dispense at least 8 drops each of the Positive and Negative Controls into individual sample cups.

* When more than one AxSYM HIV Ag/Ab Combo reagent lot is on board the AxSYM System, multiply the control volume by the number of lots.

Patient Specimens
Ensure that sufficient volume is present in the sample cups or tubes. The sample cup minimum volume is 194 μL for the first AxSYM HIV Ag/Ab Combo test plus 144 μL for each additional AxSYM HIV Ag/Ab Combo test. For volume requirements in primary or aliquot tubes, refer to the AxSYM System Operations Manual, Section 5.

NOTE: The operator may obtain an Orderlist Report by pressing PRINT. The printout contains sample placement information and minimum 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.

QUALITY CONTROL PROCEDURES
CALIBRATION
A minimum of three replicates of the AxSYM HIV Ag/Ab Combo Index Calibrator must be tested for an AxSYM HIV Ag/Ab Combo calibration. A single sample of both the Positive and Negative Controls must be tested as a means of evaluating the calibrated equipment.

NOTE: It is recommended that all three AxSYM HIV Ag/Ab Combo Positive Controls, Positive Control 1, Positive Control 2, and Viral Lysate Positive Control, be run to verify the calibration.

If the AxSYM HIV Ag/Ab Combo assay is used to evaluate HIV-2, the HIV-2 Positive Control must be tested every 24 hours.

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

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

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

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

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

RESULTS
CALCULATION
The AxSYM HIV Ag/Ab Combo assay protocol calculates the cutoff rate (CO) from the mean rate of three Index Calibrator replicates and stores the result. The cutoff rate is determined by adding 27.5 to the mean Index Calibrator rate.

Cutoff rate (CO) = Index Calibrator mean rate + 27.5

The AxSYM HIV Ag/Ab Combo assay protocol calculates a result based on the ratio of the sample rate (S) to the cutoff rate 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 HIV Ag/Ab Combo Results

<table>
<thead>
<tr>
<th>Initial Result (S/CO)</th>
<th>Instrument Flag</th>
<th>Interpretation</th>
<th>Retest Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 1.00</td>
<td>REACTIVE</td>
<td>Reactive</td>
<td>Retest in duplicate</td>
</tr>
<tr>
<td>0.90 to &lt; 1.00</td>
<td>GRAYZONE</td>
<td>Grayzone Reactive</td>
<td>Retest in duplicate</td>
</tr>
<tr>
<td>&lt; 0.90*</td>
<td>NEGATIVE</td>
<td>Negative</td>
<td>No retest required</td>
</tr>
</tbody>
</table>

* If parameter 80-option 2 is selected, NEGATIVE is designated as < 1.00 S/CO.

NOTE: Specimens that are reactive or grayzone on initial testing must be centrifuged according to directions in the SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS section of this package insert and retested in duplicate.
Reactive or Grayzone may give inconsistent results with the AxSYM HIV Ag/Ab Combo assay requirement to centrifuge prior to testing. The AxSYM System "Automatic Sample Retest" feature must not be used due to the AxSYM HIV Ag/Ab Combo assay requirement to centrifuge prior to testing. If either duplicate retest S/CO value is greater than or equal to 0.90, the specimen should be tested by supplemental tests. Analysis of follow up samples is recommended.

### SPECIFIC PERFORMANCE CHARACTERISTICS

#### PRECISION

Assay reproducibility was determined during the clinical evaluation of AxSYM HIV Ag/Ab Combo. A six-member panel and assay controls were run at 10 sites on 10 instruments in total, with the exception of the HIV-1 p24 panel which was tested at eight sites only. Nine sites tested two different lots of the controls and one site tested three different clinical lots of the controls. All samples were tested in triplicate in three runs. At one site, the panel and assay controls were tested on one clinical lot; at eight sites, they were tested on each of two clinical lots; and at one site, they were tested across four different clinical lots. The intra-assay and inter-assay standard deviation (SD) and percent coefficient of variation (%CV) were analyzed with a variance components analysis using a mixed analysis of variance model (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total (N)</th>
<th>Mean (S/CO)</th>
<th>SD</th>
<th>%CV</th>
<th>Inter-assay SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted anti-HIV-1</td>
<td>189</td>
<td>25.96</td>
<td>1.29</td>
<td>5.0</td>
<td>2.41</td>
<td>9.3</td>
</tr>
<tr>
<td>Undiluted anti-HIV-2</td>
<td>189</td>
<td>54.15</td>
<td>2.00</td>
<td>3.7</td>
<td>2.47</td>
<td>4.6</td>
</tr>
<tr>
<td>Diluted HIV-1 medium</td>
<td>189</td>
<td>11.85</td>
<td>0.54</td>
<td>4.6</td>
<td>0.74</td>
<td>6.3</td>
</tr>
<tr>
<td>Diluted HIV-2 medium</td>
<td>189</td>
<td>11.79</td>
<td>0.52</td>
<td>4.4</td>
<td>0.91</td>
<td>7.7</td>
</tr>
<tr>
<td>Anti-HIV-1 group O HIV-1 p24</td>
<td>189</td>
<td>25.96</td>
<td>1.59</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>414</td>
<td>0.38</td>
<td>0.02</td>
<td>4.5</td>
<td>0.02</td>
<td>5.5</td>
</tr>
<tr>
<td>Positive Control 1</td>
<td>414</td>
<td>3.84</td>
<td>0.21</td>
<td>5.3</td>
<td>0.25</td>
<td>6.6</td>
</tr>
<tr>
<td>Positive Control 2</td>
<td>414</td>
<td>3.55</td>
<td>0.17</td>
<td>4.7</td>
<td>0.22</td>
<td>6.2</td>
</tr>
<tr>
<td>Viral Lysate Positive Control</td>
<td>414</td>
<td>3.23</td>
<td>0.16</td>
<td>4.9</td>
<td>0.19</td>
<td>5.9</td>
</tr>
</tbody>
</table>

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

#### SPECIFICITY

Specificity is based on testing of random blood donors and hospitalized patient populations (serum and plasma specimens). Specificity is defined as the ability of the AxSYM HIV Ag/Ab Combo assay to detect randomly selected specimens as negative in populations at low risk for infection.

- **Specificity based on zero prevalence of antibodies to HIV-1 or HIV-2, and/or HIV-1 p24 antigen in random blood donors (7900 tested) is estimated to be 100.00% (7900/7900) for the AxSYM HIV Ag/Ab Combo assay (Table 2).**
- **Specificity based on low prevalence of antibodies to HIV-1 or HIV-2, and/or HIV-1 p24 antigen in a hospitalized population (1938 tested) is estimated to be 99.90% (1929/1938) for the AxSYM HIV Ag/Ab Combo assay (Table 2).**

The data from 7900 random blood donors from seven geographically distinct regions and 1938 hospitalized patients from six geographically distinct regions are summarized in Table 2. For the AxSYM HIV Ag/Ab Combo assay, the following were true:

- **True negative specimens**
- **False positive specimens**

### EXPECTED VALUES

In a random population of 7900 volunteer blood donor specimens, 10 (0.13%) were repeatedly reactive by AxSYM HIV Ag/Ab Combo. Among 7900 specimens from individuals with HIV-1 classified disease status, HIV-1 unknown disease status, HIV-1 subtyped, and unknown disease status for HIV-1 group O, HIV-1 p24 antigen, and HIV-2, the AxSYM HIV Ag/Ab Combo assay detected 100.00% as reactive.
Blood Donors (Total) 7900 8 0.10 10 a 0.13

42 specimens were confirmed by HIV-1 Western blot. These samples belong to the following categories: homosexual males (28), STD (5), hemophiliacs (6), and multiple transfusion (2).

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

AxSYM HIV Ag/Ab Combo was used to test 319 specimens containing potentially interfering substances. Results are shown in Table 3. This group contained specimens from the following categories: (viral infection) CMV, EBV, HSV, HAV, HBV, anti-HCV, anti-HTLV-I, anti-HTLV-II, rubella; (bacterial/other) fungal infection/anti-yeast (e.g., Candida albicans), toxoplasmosis, syphilis, E. coli; (autoimmune) rheumatoid factor (RF), autoimmune antibodies (ANA); (other conditions) pregnant females all trimesters, multiparous females, elevated IgG, elevated IgM, multiple myeloma, monoclonal gammapathy, flu vaccine recipients, and human antimmune antibodies (HAMA).

<table>
<thead>
<tr>
<th>Population Group (Site)</th>
<th>Specimens Tested (N)</th>
<th>Initially Reactive (N) (%)</th>
<th>Non-Confirmed Repeatedly Reactive (N) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Donors (Total)</td>
<td>7900</td>
<td>8</td>
<td>0.10</td>
</tr>
<tr>
<td>Hospitalized Patients</td>
<td>1938</td>
<td>11</td>
<td>0.57</td>
</tr>
</tbody>
</table>

a Two initial grayzone specimens were repeatedly reactive upon retest.

b Six samples, which were initially and repeatedly reactive, could be confirmed by HIV-1 Western blot. One sample, which was initially and repeatedly reactive, could be confirmed by HIV-1 PCR.

Sensitivity

The sensitivity for anti-HIV-1 (including anti-HIV-1 group O), HIV-1 p24 antigen, and anti-HIV-2 is expressed in terms of detection rate using confirmatory assay results (e.g., Western blot, HIV-1 p24 antigen, HIV-1 PCR) as a basis for comparison.

The ability of AxSYM HIV Ag/Ab Combo to detect antibodies to HIV-1/HIV-2 and/or HIV-1 p24 antigen in individuals clinically diagnosed with HIV-1 infection and classified disease status or from seropositive individuals (unknown disease status) is shown in Table 5.

- The HIV-1 antibody detection rate in a population of 615 HIV-1 antibody confirmed seropositive individuals is 100% (615/615). This rate includes 453 clinically diagnosed patients from different disease stages of HIV-1 infection and 55 HIV-1 subtyped samples.
- The HIV-2 antibody detection rate in a population of 108 HIV-2 antibody confirmed seropositive individuals is 100% (108/108).
- The HIV-1 p24 antigen detection rate in a defined (≥ 25 pg/mL) population of 50 HIV-1 p24 antigen confirmed positive individuals is 100% (50/50).
- The HIV-1 group O antibody detection rate in a population of 19 HIV-1 group O antibody confirmed positive specimens tested is 100% (19/19).

Table 2

Specificity Results Using Random Blood Donors and Hospitalized Patients

<table>
<thead>
<tr>
<th>Population Group (Site)</th>
<th>Specimens Tested (N)</th>
<th>Initially Reactive (N) (%)</th>
<th>Non-Confirmed Repeatedly Reactive (N) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Donors (Total)</td>
<td>7900</td>
<td>8</td>
<td>0.10</td>
</tr>
<tr>
<td>Hospitalized Patients</td>
<td>1938</td>
<td>11</td>
<td>0.57</td>
</tr>
</tbody>
</table>

a Two initial grayzone specimens were repeatedly reactive upon retest.

b Six samples, which were initially and repeatedly reactive, could be confirmed by HIV-1 Western blot. One sample, which was initially and repeatedly reactive, could be confirmed by HIV-1 PCR.

c Nine of the HIV-1 group O specimens were diluted 1:100, while the remaining 10 specimens were undiluted.

d All specimens were sourced from different geographical regions: Argentina, China, Ghana, Ivory Coast, Mozambique, Thailand, Uganda, USA, Zimbabwe, Belgium, Cameroon, Germany, Iran, Italy, Kenya, Korea, Netherlands, Nigeria, Poland, Spain, Syria, Togo, Turkey, Zaire, Romania, Arabia, Brazil, Ethiopia, France, Great Britain, Lebanon, and Russia.

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

AxSYM HIV Ag/Ab Combo was used to test 319 specimens containing potentially interfering substances. Results are shown in Table 3. This group contained specimens from the following categories: (viral infection) CMV, EBV, HSV, HAV, HBV, anti-HCV, anti-HTLV-I, anti-HTLV-II, rubella; (bacterial/other) fungal infection/anti-yeast (e.g., Candida albicans), toxoplasmosis, syphilis, E. coli; (autoimmune) rheumatoid factor (RF), autoimmune antibodies (ANA); (other conditions) pregnant females all trimesters, multiparous females, elevated IgG, elevated IgM, multiple myeloma, monoclonal gammapathy, flu vaccine recipients, and human antimmune antibodies (HAMA).

Table 3

Plasma Specimens Containing Potentially Interfering Substances

<table>
<thead>
<tr>
<th>Group Potentially Interfering Substances</th>
<th>Specimens Tested (N)</th>
<th>Initially Reactive (N) (%)</th>
<th>Non-Confirmed Repeatedly Reactive (N) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Donors (Total)</td>
<td>7900</td>
<td>8</td>
<td>0.10</td>
</tr>
<tr>
<td>Hospitalized Patients</td>
<td>1938</td>
<td>11</td>
<td>0.57</td>
</tr>
</tbody>
</table>

a Four samples, which were initially and repeatedly reactive, could not be confirmed by HIV-1/ HIV-2 Western blot, HIV-1 p24 antigen, and/or HIV-1 PCR. These samples belong to the following categories: E. coli (2), RF (1), monoclonal gammapathy (1). E. coli infections were detected in urine samples.

b One sample, which was initially and repeatedly reactive, could be confirmed by HIV-1 Western blot. This sample belongs to the following sample category: monoclonal gammapathy.

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

AxSYM HIV Ag/Ab Combo was used to test 319 specimens containing potentially interfering substances. Results are shown in Table 4. This group contains specimens from the following categories: hemodialysis, hemophiliacs, multiple transfusion recipient, homosexual males, intravenous drug users (IVDU), and sexually transmitted diseases (STD).

Table 4

Increased Risk Population

<table>
<thead>
<tr>
<th>Group</th>
<th>Specimens Tested (N)</th>
<th>Initially Reactive (N) (%)</th>
<th>Non-Confirmed Repeatedly Reactive (N) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Risk</td>
<td>179</td>
<td>42a</td>
<td>23.5</td>
</tr>
</tbody>
</table>

a 42 specimens were confirmed by HIV-1 Western blot. These samples belong to the following categories: homosexual males (28), STD (5), hemophiliacs (6), and multiple transfusion (2).

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

Sensitivity

The sensitivity for anti-HIV-1 (including anti-HIV-1 group O), HIV-1 p24 antigen, and anti-HIV-2 is expressed in terms of detection rate using confirmatory assay results (e.g., Western blot, HIV-1 p24 antigen, HIV-1 PCR) as a basis for comparison.

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- The HIV-1 p24 antigen detection rate in a defined (≥ 25 pg/mL) population of 50 HIV-1 p24 antigen confirmed positive individuals is 100% (50/50).
- The HIV-1 group O antibody detection rate in a population of 19 HIV-1 group O antibody confirmed positive specimens tested is 100% (19/19).

Table 5

Detection of Anti-HIV-1 (Groups M and O), Anti-HIV-2 and/or HIV-1 p24 Antigen in Serum or Plasma Specimens from Patients with Classified Disease Status and from Seropositive Individuals

<table>
<thead>
<tr>
<th>Group</th>
<th>HIV Infection Tested (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>453a</td>
</tr>
<tr>
<td>HIV-2</td>
<td>108</td>
</tr>
<tr>
<td>Unknown Disease Status</td>
<td>107</td>
</tr>
<tr>
<td>HIV-1</td>
<td>55b</td>
</tr>
<tr>
<td>HIV-1 Group O Status</td>
<td>HIV-1 Group O</td>
</tr>
<tr>
<td>HIV-1 p24 Ag</td>
<td>19c</td>
</tr>
</tbody>
</table>

a Specimens drawn from individuals in CDC classifications Stage A, Stage B, or Stage C.


c Nine of the HIV-1 group O specimens were diluted 1:100, while the remaining 10 specimens were undiluted.

d All specimens were sourced from different geographical regions: Argentina, China, Ghana, Ivory Coast, Mozambique, Thailand, Uganda, USA, Zimbabwe, Belgium, Cameroon, Germany, Iran, Italy, Kenya, Korea, Netherlands, Nigeria, Poland, Spain, Syria, Togo, Turkey, Zaire, Romania, Arabia, Brazil, Ethiopia, France, Great Britain, Lebanon, and Russia.

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

Sensitivity of the assay to detect antibodies to HIV-1 and/or HIV-2 was evaluated in sequential specimens from 22 seroconverting donors. These well-characterized specimens are commercially available from Boston Biomedica (BBI, Massachusetts, USA), North American Biologicals Inc. (Nabi, USA), Pyramid (USA) or Bioclinical Partners (BCP, Massachusetts, USA). The sensitivity of the assay in seroconverting donors is better than the methods of comparison. The AxSYM HIV Ag/Ab Combo assay was reactive 1-2 bleeds earlier than the method of comparison for 16 out of 22 seroconversion panels and equal in 4 out of 22 seroconversion panels. Two seroconversion panels were not detected by the method of comparison. Table 6 shows a subset of six representative seroconversion panels.
Table 6

<table>
<thead>
<tr>
<th>Donor</th>
<th>Analytical Sensitivity on AFSSAPS Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-1 p24</td>
</tr>
<tr>
<td>S2023</td>
<td>500</td>
</tr>
<tr>
<td>S2024</td>
<td>250</td>
</tr>
<tr>
<td>S2025</td>
<td>100</td>
</tr>
<tr>
<td>S2026</td>
<td>50</td>
</tr>
<tr>
<td>S2027</td>
<td>25</td>
</tr>
<tr>
<td>S2028</td>
<td>10</td>
</tr>
<tr>
<td>S2029</td>
<td>5</td>
</tr>
</tbody>
</table>

Analytical sensitivity (pg/mL) 16.7 14.5 21.2

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

BIBLIOGRAPHY


Phair JP. Human immunodeficiency virus antigenia. JAMA 1987;258:1218.


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