**ANTI SKELETAL ANTIBODY IFA TEST SYSTEM**

**INTENDED USE**

These reagents are intended for use in the detection and quantitation of IgG antibody in human sera to striated skeletal muscle antigens by the indirect fluorescent antibody (IFA) procedure. The Anti Skeletal Antibody IFA Test System is not to be used for diagnostic purposes and is intended to be used only when the actual diagnosis is based on an established method or procedure including clinical findings. For Export Only. The test system is for Professional Use Only.

**SUMMARY AND PRINCIPLES**

Demonstration of striated muscle antibody by utilizing the indirect fluorescent antibody method enables serologic assessment or possible detection of muscular diseases. The presence of a histochemically defined skeletal antibody in conjunction with a positive acetylcholine receptor site assay is helpful in the confirmatory diagnosis of myasthenia gravis (MG). Similar antibodies can be found using cardiac tissue. Patients with rheumatic fever, myocardial infarction and a variety of postcardioitory states may demonstrate antibodies detected using both skeletal and cardiac tissue. The absence of anti-striated antibodies effectively excludes thymoma (see table below). The utilization of frozen sections of monkey skeletal muscle is the recommended substrate for IFA.

<table>
<thead>
<tr>
<th>Table: Disease State and Striated Muscle Antibodies</th>
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<tbody>
<tr>
<td>Patient Population</td>
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<tr>
<td>All MG patients</td>
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<tr>
<td>MG with Thymoma</td>
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<tr>
<td>MG without Thymoma</td>
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<tr>
<td>Thymoma without MG</td>
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The indirect fluorescent antibody test is used for the detection of human IgG antibody to the antigens of striated skeletal muscle. Tissue is placed in the wells of specially prepared microscope slides. Dilutions of patient sera are placed on the wells where antibody, if present, binds to the antigen. The reaction is visualized through the use of a conjugate. The conjugate is fluorescein isothiocyanate (FITC) labeled, anti-human IgG (gamma chain specific). Excitation of the FITC by ultraviolet (UV) light causes this dye to emit longer, visible, wavelengths of light in the yellow-orange portion of the color spectrum. The conjugate will bind with human IgG antibodies attached to the antigens causing fluorescence when viewed through a microscope equipped with a UV light source.

**PRECAUTIONS**

1. Follow the procedure instructions exactly as they appear in this insert to ensure valid results.
2. Thimerosal (Merthiolate), used as a preservative in some of the reagents, may be toxic if ingested, inhaled or absorbed through skin and is a reproductive hazard.
3. Some components contain less than 0.1% sodium azide, which is toxic if ingested, inhaled or absorbed through skin and is a reproductive hazard.
4. The phosphate buffered saline and mounting medium found in this kit are irritating to the eyes, respiratory system and skin.
5. Some components in this kit contain 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitisation by skin contact.
6. The conjugate in this kit contains 0.01% Evans’ Blue. Evans’ Blue is a possible carcinogen and may cause reproductive harm.
7. WARNING - POTENTIAL BIOHAZARDOUS MATERIAL. Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of antibody to HIV, as well as HBsAg, and found to be negative (were not repeatedly reactive). Because no test method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent, these human control reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1999 (18).
8. Slides and reagents should be stored at +2 to +8°C until used.
9. Do not use components beyond their expiration date.
10. Handle slides by the edge since direct pressure on the antigen wells may damage the antigen.
11. Once the procedure has started, do not allow the wells to dry.

**STORAGE AND STABILITY**

1. Monkey Skeletal Muscle 4 Well Slides (Prod #10-5804): Store at +2 to +8°C. Slides are stable until their expiration date on the product label.
2. Striated Positive Control (Prod #10-5802): Store at +2 to +8°C. Refer to expiration date on label.
3. Autimmune Negative Control (Prod #10-1607): PBS is stable at room temperature in its non-reconstituted form. Refer to label for expiration date. PBS contains no preservative and should be stored at +2 to +8°C after it is reconstituted. Discard if turbidity develops.
4. FITC IgG Conjugate with Evans’ Blue, Primate Adsorbed (Prod #10-1502): Store at +2 to +8°C. Refer to expiration date on label.
5. Phosphate Buffered Saline, pH 7.5 (Prod #10-1607): PBS is stable at room temperature in its non-reconstituted form. Refer to label for expiration date. PBS contains no preservative and should be stored at +2 to +8°C after it is reconstituted. Discard if turbidity develops.
6. FITC Mounting Medium, pH 7.5 (Prod #10-1610): Store at +2 to +8°C. Refer to expiration date on label.

**SPECIMEN COLLECTION**

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2°C to 8°C if it is to be analyzed with 4-7 days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, additions of a preservative such as 0.01% thimerosal (merthiolate) or 0.1% sodium azide is strongly recommended. The CLSI provides recommendations for storing blood specimens (Approved Standard Procedure for the Handling and Processing of Blood Specimens, H18-A2 2005) (17).

**PREPARATION OF CONTROLS**

Include the positive, negative, and PBS controls in each run.

1. The positive control serum (Prod #10-5802) is standardized to demonstrate a positive reaction when used undiluted. Include in the test undiluted positive control.
2. The negative control serum (Prod #10-1201) is standardized to demonstrate a negative reaction when used undiluted. Include in the test undiluted negative control.
3. A PBS control may be run to establish that the conjugate is free from non-specific staining of the antigen substrate.
PREPARATION OF SPECIMENS

Prepare the 1:40 screening dilution of patient sera by mixing 0.05 mL of serum into 1.95 mL of PBS. Note: Samples screening positive at 1:40 should be titrated to end-point by preparing two-fold serial dilutions starting at 1:40. Mix equal volumes of diluted serum and PBS for subsequent two-fold dilutions.

TEST PROCEDURE

1. Remove the number of slides needed from the sealed pouches and mark them with a marking pen as necessary.
2. Add controls and diluted serum (approximately 25 µL) to wells.
3. Incubate slides in a moist chamber at room temperature for 30 minutes.
4. After incubation with sera the slides should be tapped onto a piece of paper toweling in such a way as to prevent the serum of one well coming into contact with any of the other wells. Direct a gentle stream of PBS over the slide using a wash bottle. Do not aim the stream of PBS directly onto the wells.
5. Place the slides in a wash chamber filled with PBS for 5 minutes. Replace wash chamber with fresh PBS and wash slides for another 5 minutes.
6. Remove the slides from the PBS and place, antigen side up, on a dry paper towel. Carefully place the 4 well blotters over the slide, positioned so as not to come into contact with the reaction wells. Hold one edge of the blotters with one hand to keep the blotters in place and apply sufficient gentle pressure with the microscope slide roller to remove the moisture surrounding antigen wells. DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
7. Using dispenser provided, deliver 1 drop of conjugate per antigen well. The conjugate dispenser is provided with a calibrated tip and allows quantitative delivery of reagents from the storage bottle. To use, wipe the tip with a paper towel, invert the bottle and squeeze gently to release one drop. If the tip contains an air bubble, tap the bottle gently to remove air bubble which will ensure precise drop delivery.
8. Incubate slides as described above (#5).
9. Rinse, wash and blot slides as described above (#4, #5, #6). DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
10. Place 2 to 3 drops of mounting medium on slide and cover with a coverslip avoiding air bubbles.
11. Read slides with a fluorescence microscope.

READING SLIDES

1. Do not attempt to read the slides before the microscope has been switched on for at least 5 minutes.
2. Read slides within one hour. Slides may be read within 24 hours if stored refrigerated in a moist chamber. Allow refrigerated slides to warm to room temperature before reading.
3. The slides should be examined at a total magnification of 400X.
4. Drying may disturb the most peripherally situated antigen in the well, therefore disregard these reactions.
5. The staining intensity may vary, however, the degree of staining is based on the overall appearance of the antigen.
6. Record reaction intensity at each dilution using the following criteria:
   - 2± to 4± = moderate to strong yellow-green fluorescence
   - 1± = Weak but definite yellow-green fluorescence
   - Negative = Vaguely visible or no fluorescence
7. Read the controls before proceeding to the test sera.
8. The titer is the reciprocal of the highest dilution showing 1± or greater fluorescence.

QUALITY CONTROL

1. The positive control serum must demonstrate a positive fluorescence or the test is invalid.
2. The negative control serum must demonstrate the absence of yellow-green specific fluorescence or the test is invalid.
3. Reading of test serum end-points with each microscope assembly must be made with reference to the reactivities of the control sera with the slides and conjugate provided.
4. The PBS control, if included, must demonstrate the absence of yellow-green specific fluorescence or the test is invalid.

INTERPRETATION OF RESULTS

Titers of less than 1:40 are reported as negative. Titers of greater than or equal to 1:40 should be titrated to endpoint and reported as positive. Titers of 1:30 or greater are suggestive of MG.

LIMITATIONS OF PROCEDURE

1. Light sources, total magnification, objective lenses, and ocular lenses influence intensity of staining. Variations in intensities may be observed when different microscope assemblies are used. Testing of sera should not be attempted unless the positive control serum gives the expected titer within one two-fold dilution and the negative control yields negative results.
2. The accuracy in the test often depends on the competency of the operator.
3. The patient clinical data and other laboratory tests should be carefully reviewed by a medical authority before a diagnosis is made.
4. No diagnosis should be based upon a single serologic test result, since various host factors must be taken into consideration.
5. A positive skeletal muscle test along with a positive test for AChR antibodies is helpful in confirming the diagnosis of myasthenia gravis.
6. Positive striated skeletal antibodies can be found in rheumatic fever, myocardial infarction and a variety of post cardiotomy states.
Consult Instructions for Use

Product Number
Lot Number
In Vitro Diagnostic Medical Device
Authorized Representative in the European Community
Use By
Caution, consult accompanying documents

Temperature limitation

Manufacturer

Irritant - Precaution

Negative Control
Striated Muscle Positive Control
Conjugate
Phosphate Buffered Saline
Mounting Medium
Coverslips
Blotters, 4 Well
Slide, 4 Well

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