Introduction: Clinical and experimental evidence strongly suggests that circulating antibodies directed against acetylcholine receptor (AChR) and muscle cell antigens are important in the pathogenesis of myasthenia gravis.1-3 Several antibody mediated mechanisms have been implanted in the alteration of neuromuscular transmission, including (1) complement mediated destruction of motor and plates with consequent AChR loss, (2) blockage of the AChR active site preventing AChR access, or (3) alteration of AChR turnover.4,5 The positive response to thymectomy in patients with a short history of myasthenia gravis (MG) may be due to alteration of thymic cell populations that normally regulate antibody production.6,7

Principles:

Tests for detections of antibodies to AChR's and muscle cell antigens can be of diagnostic value.8 High titers of anti-AChR and anti-striated (Str) muscle antibodies define MG patients with thymoma.9 The absence of anti-AChR or anti-Str antibodies effectively excludes MG or thymoma respectively.10 Because antibody titer to either AChR or Str muscle correspond only approximately to clinical status, their detection does not have direct prognostic value.11-13

Radioimmunoassay is used for AChR antibody detection, whereas routine indirect immunofluorescence (IFA) is used for detection of anti-striated antibodies. Acetone fixed longitudinal sections of skeletal muscle is the substrate used for anti-striated antibody detection.14 Normal human sera can react with skeletal muscle in dilutions up to 1:30. A suggested screening dilution of 1:40 is recommended to increase specificity.10,15

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1502L (3.0 ml) with Evans Blue Counterstain is to be stored at 2-8 C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.

2. Test tubes and rack or microtiter system with pipettes

3. Staining Dish and Slide Forceps

4. Moist Chamber

5. Volumetric Flask (500 ml)

6. Distilled H2O

7. Reagent Preparation:


2. Reagents:

- Dilute serum
- Phosphate Buffered Saline
- Fluorescent Microscope
- Paper Towels - lint free

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Test Instruction:

1. Serum: In a microtiter plate, set up dilution of serum starting at 1:40, 1:80, 1:160, 1:320, etc.

2. Screening: dilute test sera 1:40 in PBS. Titration: set up doubling dilutions of serum starting at 1:40, 1:80, 1:160, 1:320, etc.

3. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.

4. Place a drop of diluted serum (20 to 30 µl) and controls over the antigen wells.

5. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24 C).

6. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the slide.

7. Deliver 1 drop (20-30 µl) of conjugate per antigen well. Repeat steps 3-6.

8. Place 4-5 drops of mounting medium on slide.

9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

Quality Control:

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.

2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.

3. The positive serum control should result in bright +3 to +4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.

4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

Results:

The IFA test will result in a cross-striation staining pattern of skeletal muscle. The percentages of patients with anti-striated antibodies vary with the clinical state (Table I).

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Striated Muscle Antibodies and Disease State</th>
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<tbody>
<tr>
<td>Patient Population</td>
<td>% Anti-Str Abs</td>
</tr>
<tr>
<td>1. All MG patients</td>
<td>40%</td>
</tr>
<tr>
<td>2. MG with Thymoma</td>
<td>90-100%</td>
</tr>
<tr>
<td>3. MG without Thymoma</td>
<td>30%</td>
</tr>
<tr>
<td>4. Thymoma without MG</td>
<td>25%</td>
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</tbody>
</table>

*Absence of anti-Str Abs effectively excludes thymoma.

Limitations of Procedure:

1. More than one mechanism may be involved in MG. Factors like sex, age, presence or absence of thymoma, other autoantibodies, HLA type, response to thymectomy and/or immunosuppressive drugs must be considered in addition to the detection of anti-AChR and anti-Str antibodies.

2. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.

Precautions:

1. All human components have been tested by radioimmunoassay for (HBsAg) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HBsAg, HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.

3. Do not use components beyond their expiration date.

4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.

5. For In Vitro Diagnostic Use.

6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.

7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.


12. F(ab1)2 reagents are not required if goat rather than rabbit antibodies are used to detect human surface immunoglobulin. Immunol 119: 1084-8, 1977.
