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 corresponds 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 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. 1501L (3.0 ml)/1533L (5.0 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. The antigen slides of rat striated muscle must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

3. Anti-striated positive control No. 5802L (1.0 ml) should be stored at 2-8 C upon receipt. Check label for specific expiration date.
4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.
5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stable at room temperature storage for 5 years. The reconstituted Buffer does not contain preservatives and should be stored at 2-8 C. Care should be taken to avoid contamination.
6. Mounting Medium No. 1610 is stable when stored at 2-8 C. Check label for specific expiration date.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (500 ml)
Distilled H_20
Fluorescence Microscope
Paper Towels - lint free

Reagent Preparation:

1. Buffer Pack No. 1601. Rehydrate buffer with 1 liter of sterile distilled water.

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 2-8 C if it is to be analyzed within a few 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, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

Test Instruction:

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

1. 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.
2. Place a drop of diluted serum (20 to 30 µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24 C).
4. 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 well.
5. Wash in PBS for five minutes. Repeat using fresh PBS.
6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. **Do not allow tissue to dry.** Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.
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 I

Striated Muscle Antibodies and Disease State

Patient Population	% Anti-Str Abs
1. All MG patients	40%
2. MG with Thymoma	90-100%
3. MG without Thymoma	30%
4. Thymoma without MG	25%

*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 (HB_sA_g) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_sA_g or 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.

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