Introduction:

Indirect fluorescent assay (IFA) for anti-endomysial antibodies has proven to be a good method to screen for Celiac disease. Endomysial antibodies of the IgA subclass (IgA EmA) react with the reticulin component of the endomysium of the smooth muscle in primate esophagus tissue. These antibodies can be found in 60-70% of patients with Dermatitis herpetiformis (DH) on a non-restricted diet and in almost 100% of patients with Celiac disease (CD) and gluten-sensitivity enteropathy with partial or subtotalling villous atrophy.[1,2,5] There is a small percentage of IgG EmA that will be negative IgA when screened.[5] A negative result exhibited by a patient with overt clinical symptoms may need to be considered for IgG testing.

It is recommended to perform an anti-Gliadin test in order to reach the maximum specificity of the test. It has been demonstrated that serum IgA endomysial antibodies were found in the majority (87%) of patients with untreated Celiac disease and approximately 70% of anti-Gliadin (IgA AGA) positive patients. The R1 anti-Reticulin (R1-ARA) appears to be a less reliable marker with less than 50% being positive. These findings were based on the same patient population.[5] It should be noted that a strict adherence to a gluten-free diet will greatly effect the antibody results in most patients. IgA AGA and R1-ARA will normally disappear after one year while IgA EmA may persist at a lower titer. In this way the test may have prognostic value in monitoring strict adherence to diet.[4]

Principles:

The primary test reaction involves circulating EmA antibodies present in the patient's serum which attach to their homologous EmA antigens. This occurs during the incubation period while the serum covers the antigen surface. A secondary reaction then follows a rinsing period which removes all unbound human antibody. The reagent used in the secondary reaction is a fluorescein labeled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under the appropriate fluorescent microscope for various morphological patterns of EmA fluorescence which can be visually identified.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1527EL (3.0 ml) is to be stored at 2-8 C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label. This reagent will react with the human IgA Immunoglobulin classes.
2. The antigen slides of monkey esophagus (endomysial section) sections must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.
3. EmA positive control No. 5305L (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.

Note: All kit components are available separately. Please see the current SCIMEDX Corporation Catalog for more details.

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 1:10 in PBS. **Titration:** set up doubling dilutions of serum starting at 1:10, 1:10, 1:20, 1:40, 1:80, 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 (25-30 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:

Staining of the endomysium around the smooth muscle fibers in the monkey esophagus is considered positive. Patients reactions should be compared with the positive control contained in the kit.

IgA SMA reactivity should be considered and eliminated before reporting a positive EmA. IgA SMA stains only the myofibril and not the network

between them in which the endomysial antigen is found.

Limitations of Procedure:

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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SCIMEDX CORPORATION
400 Ford Road
Denville, NJ 07834 USA
Tel: 800.221.5598 Tel: 973.625.8822
Fax: 973.625.8796
www.scimedx.com

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