Anti-Endomysial Antibody Test System

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 test, antigen, conjugate or technique may be at fault.
3. The positive serum controls should result in bright 3+ to 4+ fluorescence. If these controls show 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 non-specific 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
1. Staining of the endomysium around the smooth muscle fibres in the monkey oesophagus is considered positive. Patients reactions should be compared with the positive control contained in the kit.
2. IgA SMA reactivity should be considered and eliminated before reporting a positive EmA. IgA SMA stains only the myofibrils and not the network between them in which the endomysial antigen is found.

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

LITERATURE REFERENCES

INSTRUCTIONS FOR USE

Anti-Endomysial Antibody (EMA) Test System

K4818E - 48 Tests
K9618E - 96 Tests
K5018E - 50 Tests
K0018E - 100 Tests
K25018E - 250 Tests

Also for:

Monkey oesophagus slides
S4205E - 4 well
S8205E - 5 well
S205E10 (10 x 5 well)

Endomysial IgA Positive control
C026N

INTENDED USE
The Bio-Diagnostics Anti-Endomysial Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of endomysial antibodies in human serum.

SUMMARY AND EXPLANATION
IFA for anti-endomysial antibodies has proven to be a good method to screen for Coeliac disease. Endomysial antibodies of the IgA subclass (IgA EmA) react with the reticulin component of the endomysium of the smooth muscle in primate oesophagus 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 coeliac disease (CD) and gluten-sensitivity enteropathy with partial or subtotaling 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.

PRINCIPLE OF THE TEST
The primary test reaction involves circulating anti-endomysial antibodies present in the patient's serum, which attach to their homologous antigens. This occurs during the incubation period whilst the serum covers the antigen surface. A secondary reaction then follows a rinsing period that removes the unbound human antibody. The reagent used in the secondary reaction is a fluorescein labelled antihuman globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope.
Anti-Endomysial Antibody Test System

WARNINGS AND 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) or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The controls included in the kit contain 0.1% sodium azide or 0.01% Thiomersal as a preservative. Although this is at a low concentration, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure 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 artefacts.

KIT CONTENTS

| SLIDE | Monkey oesophagus (endomysial section) substrate slides. (S4205E, S8205E, S5205E or S0205E) |
| CONJ | IgA FITC Conjugate: J527. This reagent contains antibodies that will react with the human IgA immunoglobulin class. |
| CONTROL + | Endomysial antibody Positive Control no: C026N / C026N-0.5 |
| CONTROL - | Universal Negative Control no: C000 |
| IF/DF/A PBS | Buffer Pack no: R002 |
| MM | Mounting Medium no: R005 |

Note: All kit components are available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED
Test tubes and rack or microtitre system Disposable pipettes Staining Dish and Slide Forceps Moisture Chamber Volumetric Flask (500 ml) Distilled Water Fluorescence Microscope Paper Towels – lint free

REAGENT PREPARATION

KEY FOR OTHER SYMBOLS

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Contains sufficient for &lt;n&gt; tests</th>
<th>RFU</th>
<th>Ready for use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature limitation</td>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
<td></td>
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Anti-Endomysial Antibody Test System

STORAGE AND STABILITY
The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents. Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION
Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS
Screening: dilute test serums 1/5 (1 part patient sample to 4 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.

Titration: set up doubling dilutions of serum starting at 1/10, (i.e. 1/10, 1/20, 1/40, 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 30µl) 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 18-24°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 onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Place a blower on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blower. Line up the wells to blower holes. Place slide on top of the blower. Wipe the back of the slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. Do not allow tissue to dry.
7. Deliver 1 drop (20-30µl) 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.