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 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

1. Reunala T. et al. IgA anti-endomysial antibodies in dermatitis herpetiformis: correlation with jejunal morphology, gluten-free diet and anti-gliadin antibodies. Br J Dermatol 1987;117:185-91.
2. Volta U, Lenzi M, Lazzari R, et al. Antibodies to gliadin detected by immunofluorescence and a micro-ELISA method; markers of active childhood and adult coeliac disease. Gut 1985;26:667-71.
3. Calabuig M, Torregosa R, Polo P, et al. Serological markers and celiac disease; a new diagnostic approach? J Pediatr Gastroenterol Nutr 1990;10:435-42.
4. Scott H, Ek J, Brandtzaeg P. Changes of serum antibody activities to various dietary antigens related to gluten withdrawal or challenge in children with coeliac disease. Int Archs Allergy Appl Immunol 1985;76:138-44.
5. Volta U, Molinaro N, Fusconi M et al. IgA Antiendomysial Antibody Test A Step Forward in Celiac Disease Screening. Dig. Diseases and Sci. Vol. 36, No 6, June 1991.
6. Kapuscinska, A., Zalewski, T., Chorzelski, T.P., et al. Disease Specificity and dynamics of Changes In IgA Class Anti-endomysial Antibodies in Celiac Disease. J Pediatr Gastroenterol Nutr 1987; 6:529-534.

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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 (endomysial sections)
S4205E - 4 well **S8205E/S8205E-IN** - 8 well
S5205E - 5 well **S0205E** - 10 well
S5205E10 (10 x 5 well) **S0205E25** (25 x 10 well)

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.

SUMARY 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 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.

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.

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WARNINGS AND PRECAUTIONS

1. The human components of the controls have been tested and found negative for HIV 1/2, HCV, HBsAg, HIV1 RNA, HBV DNA and HCV RNA and Syphilis by FDA approved tests. However, these tests cannot guarantee the absence of infectious agents. 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 these are at low concentrations, 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: C000N/C000N-0.5
IFA/DFA **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

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer — Contains sufficient for <n> tests — **RFU** Ready for use

Temperature limitation — **IVD** In vitro diagnostic medical device

Anti-Endomysial Antibody Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2ºC to +8ºC. 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 18-24ºC).
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. Carefully dry the back and edges of the slide using a paper towel. **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.