ALPHADIA IFA Anti-EmA (ME) IgA

IMMUNOFLUORESCENCE ASSAY
FOR THE DETECTION
OF ANTI - ENDOMYSIAL IgA
ANTIBODIES IN HUMAN SERUM

CAT # AD EMO48	48 TESTS
CAT # AD EMO96	96 TESTS

FOR IN VITRO DIAGNOSTIC USE
CONS : 2 - 8°C

ALPHADIA sa/nv
DIAGNOSTIC PRODUCTS
AVENUE VESALE 26 - B1300 WAVRE
BELGIUM
TEL : 32 (0) 10 24 26 49
FAX : 32 (0) 10 24 55 99
contact@alphadia.be

INTRODUCTION

Indirect fluorescent assay (IFA) for anti-EmA IgA 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. There is a small percentage of IgG EmA that will be negative IgA when screened. 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. 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.

PRINCIPLES

The primary test reaction involves circulating EmA IgA antibodies present in the patient's serum 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 that removes all unbound human antibody. The reagent used in the secondary reaction is a fluorescein labeled anti-human IgA 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.

KIT COMPONENTS

Monkey Esophagus (endomysial section)
12 x 4 or 8 wells
FITC Antihuman IgA conjugate w/Evans blue
1 or 2 x 3 ml
EmA IgA positive control : 1 x 1 ml
Negative control : 1 x 1 ml
Mounting medium : 1 x 3 ml
PBS Buffer : 2 x 1 liter
Blotters : 12 x 4 or 8 wells
Coverslips (22 x 70 mm) : 1 x 12 each
Note : liquid reagents contains the preservative Proclin 300 (< 0.1%).

MATERIALS PROVIDED

1. FITC Conjugate No CAM2 (3 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 class.
2. The antigen slides of monkey esophagus sections No M1061 must be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.
3. EmA positive control N° PCEA (1 ml) should be stored at 2-8°C upon receipt. Check label for specific expiration date.
4. Universal negative control N° NC (1 ml) should be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.
5. Buffer pack N° PBS - 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 N° TMM3 is stable when stored at 2-8°C. Check label for specific expiration date.

Note : all kit components are available separately.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-pipettor of 2-20µl range.
2. Timer (60 min range).
3. Graduated glass pipettes.
4. 1 L graduated cylinder.
5. Staining dish.
6. Moist chamber.
7. Clean containers for diluted buffers.
8. Distilled or deionized water.
9. Fluorescent microscope.
10. Lint free paper towel.

PRECAUTIONS

1. **Caution** : all blood products should be treated as potentially infectious. Source human serum for the controls provided in this kit were found to be non-reactive for Hepatitis-B surface antigen (HbsAg) and Human Immunodeficiency Virus (HIV) 1 and 2 antibody when tested in accordance with tested in accordance with current FDA required tests. No known test methods can offer total assurance that products derived from human blood will not transmit HIV, Hepatitis or other potentially infectious agents. Therefore, these reagents and all patient's specimens should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual - Biosafety in Microbiology and Biomedical Laboratories - 1984 or latest edition.
2. For in vitro diagnostic use only.
3. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
4. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
5. Do not use reagents past their expiration date.
6. All reagents must be at room temperature (21-26℃) before running the assay. Temperature will affect the results of the assay.
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 artefacts.
8. Use only distilled or deionized water and clean glassware.
9. Use separate pipette tips for each sample, control and reagent to avoid cross contamination.
10. Negative and positive controls must be run with each assay.
11. No assurance is given that these reagents are free of microbial or fungal contamination.
12. Incubation times and temperatures other than those specified may give erroneous results.

STABILITY AND STORAGE

1. The kits should be stored at 2-8℃.
2. Rehydrated PBS buffer remains stable for 60 days at 2-8℃.

SAMPLE COLLECTION

A whole blood sample should be collected by qualified personnel using approved aseptic venipuncture techniques. The samples may be stored at 2-8℃ if testing is to be done within 5 days. If stored longer, they should be frozen at -20℃ or lower. Do not use a frost-free freezer, which may allow the specimens to go through freeze-thaw cycles that may denature the IgA antibody and cause spurious results. Microbially contaminated, hyperlipemic, hemolytic, or heat treated samples should not be used. Clarify serum samples containing visible particulate matter by centrifugation.

PREPARATION OF REAGENTS AND SAMPLES

1. Bring all reagents to room temperature before use.
   Remove the amount of slides to be used for the day's testing. Remove the slide from the foil pouch by tearing at notch. Carefully remove slide from the pouch and avoid touching the well areas. The slide is ready-to-use.
2. Prepare PBS buffer by pouring the contents of the package into a container and add deionized or distilled water to bring up to 1.0
   liter final volume. Mix thoroughly.
   Store 1x solution at 2-8℃.
3. Positive and negative controls are ready-to-use. Do not dilute further.
4. FITC conjugate is ready-to-use liquid. The conjugate is stable at 2-8℃ until the labeled expiration date. The conjugate may be aliquoted into small amounts for extended storage and frozen (< -20℃) once.
5. Patient's sera : <u>Screening</u> : dilute each patient serum 1:10 (1+9) with PBS. Prepare dilutions (10µl + 90µl) in test tubes.
   <u>Titration</u> : set up doubling dilutions of serum starting at 1:10 (i.e. 1:10, 1:40, 1:80, 1:160 etc)
   Mix all dilutions by vortexing on low speed.
6. Assign each well of the slide the appropriate
   sample.

ASSAY PROCEDURE

Screening : dilute the serums 1:10 in PBS
Titration : set up doubling dilutions of serum starting at 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 patient serum (20 to 30µl) and controls into the designated the antigen wells.

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

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

5. Soak in PBS for five minutes. Repeat an additional five minutes 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 (20-30µl) 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.

QUALITY CONTROL
1. Positive and negative serum controls must be included in each assay run 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.
5. If any of the controls do not meet the efined conditions, the run is invalid and atient results should not be reported.

INTERPRETATION OF RESULTS
Positive : A positive result is reported when staining of the endomysium connective tissue around the smooth muscle fibers in the monkey esophagus is considered positive. Patients reactions should be compared with the positive control contained in the kitIgA 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.
Negative A serum is considered negative for EmA if the fluorescence is less than 1+ at 1:10 dilution.

LIMITATIONS OF PROCEDURE
1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.
2. The antibody titer obtained from individual samples do not necessarily correlate with disease severity and should not be reported as such. Antibodies from different patients may have different avidities. Paired sera run at the same time under the same conditions will give a better indication of the disease process.
3. The results obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures. Low positive samples should be evaluated in light of clinical symptoms and results of testing for other autoimmune antibodies, such as gliadin IgG and IgA, tissue transglutaminase (tTG), or biopsy. Confirmation of results is advised by redrawing and retesting the patient.

4. Only if test instructions are rigidly followed will optimum results be archieved.
5. Use fresh serum or samples frozen only once and thawed. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.

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