HISTOFLUOR® ANTI-ENDOMYSIAL AUTOANTIBODY (EmA) FLUORESCENT TEST SYSTEM

For in vitro Diagnostic Use
For Export Only
For Professional Use

INTENDED USE: This is an indirect fluorescent antibody test for the qualitative and semi-quantitative detection of anti-endomysial autoantibodies in human serum. This test system is to be used as an aid in the detection of antibodies associated with celiac disease and dermatitis herpetiformis.

SUMMARY AND EXPLANATION OF THE TEST

The autoantibodies found in humans are directed at a number of diverse antigens. Many of these antigens are highly conserved, so that virtually identical antigenic epitopes are found in the tissues of animals and humans. Thus, the use of animal tissues to detect these antibodies is a long-standing laboratory procedure. Many of the autoantibodies detected on animal tissue are closely associated with specific autoimmune diseases in humans. Detection and quantitation of specific autoantibodies allow for the diagnosis and monitoring of specific autoimmune diseases (1).

PRINCIPLE OF THE TEST

The Immuno Concepts Fluorescent Test System uses the indirect fluorescent antibody technique first described by Weller and Coons (2). Patient samples are incubated with antigen substrate to allow specific binding of autoantibodies to cell components. If autoantibodies are present, a stable antigen-antibody complex is formed. After washing to remove non-specific and unbound antibodies, the substrate is incubated with an anti-human antibody conjugated to fluorescein. When results are positive, there is the formation of a stable three-part complex consisting of fluorescent antibody bound to human autoantibody, which is bound to cellular antigen. This complex can be visualized with the aid of a fluorescent microscope. In positive samples, the cells will show an apple-green fluorescence with a staining pattern characteristic of the particular antigen distribution within the cells. If the sample is negative for autoantibodies, the cells will not show a clearly discernible pattern of fluorescence.

SYSTEM COMPONENTS - MATERIALS PROVIDED

Use: All components come ready to use with no aliquoting or reconstitution required (except the PBS buffer which must be dissolved in deionized or distilled water before use).

Storage: All components can be stored under refrigeration at 2-10°C. After reconstitution, PBS buffer should be stored in screw cap containers at 2-25°C. Mounting medium and coverslips may be stored at room temperature (18-25°C).

Stability: All components remain stable at least 12 months from date of manufacture. Do not use any component beyond its expiration date.

REACTIVE REAGENTS
Substrate Slides SLIDE: Catalog No. 12004-01, 12008-01. Thin (approximately 4-5 micrometer) sections of primate distal esophagus.

Anti-Endomysial Antibody Specific Positive Control CONTROL| +: Catalog No. 12021-01. Ready-to-use dropper vial containing 1.0 ml positive human control serum with antibody specific to endomysial antigens. This serum demonstrates a positive anti-endomysial staining pattern on the primate tissue substrate.

Negative Control Serum CONTROL| - : Catalog No. 12031. Ready-to-use dropper vial containing 1.0 ml negative human control serum. This control may demonstrate weak fluorescent staining, but it shows no discernable pattern.

Fluorescent Antibody Reagent CONJ|FITC: Catalog No. 12009-01 (9.0 ml), 12075-01 (23 ml). Goat anti-human IgA conjugated to fluorescein isothiocyanate (FITC). Reagent comes ready-to-use in precision dropper bottles with 9.0 ml for every 10 slides in complete test kits.

NON-REACTIVE COMPONENTS
PBS Buffer Powder PWDR|PBS: Catalog No. 1011. Phosphate-buffered saline powder (0.01 M, pH 7.4 ± 0.2). Each pouch contains sufficient buffer powder to make 1 liter. (One pouch of buffer powder is supplied for every five slides in complete test kits.)

> **Preparation:** Dissolve one pouch of buffer powder in 1 liter of deionized or distilled water, cover, and store between 2-25°C for up to four weeks or until signs of contamination or other visible changes occur.

Semi-permanent Mounting Medium SOLN|MM: Catalog No. 1111. Ready-to-use dropper vial containing 5.0 ml glycerol-based mounting medium. This reagent may be stored at room temperature (18-25°C).

Coverslips CVSLP: Catalog No. 1042. Each packet contains ten 24x64 mm No. 1 glass coverslips. Coverslips may be stored at room temperature (18-25°C).

Additional Materials Required - but not provided

Volumetric pipettes to deliver 20-25 µl volumes
Coplin jars or staining dishes
Squeeze bottle or Pasteur pipettes
Serological pipettes
One-liter containers (for PBS buffer)
Deionized or distilled water
Test tubes to prepare serum dilutions
Bibulous paper or paper towels
Chamber for incubation
Disposable gloves
Lab timer
Fluorescent microscope equipped with 495 nm exciter filter and 515 nm barrier filter

Precautions

1. All human source materials used for this product have been tested and found to be negative (not repeatedly reactive) for antibodies to Human Immunodeficiency Virus-1 (HIV-1), Human Immunodeficiency Virus-2 (HIV-2), hepatitis C virus (HCV), and for hepatitis B surface antigen (HBsAg) by FDA approved methods. However, no test method can offer complete assurance that HIV-1, HIV-2, hepatitis C, hepatitis B, or other infectious agents are absent. Thus, all kit materials should be handled in the same manner as potentially infectious materials. All animal source materials used for this product have been sourced from the USA or USDA approved facilities.
2. All patient samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual: *Biosafety in Microbiological and Biomedical Laboratories, 1999 Edition.*
3. Dilution of the components or substitution of components other than those provided in this system may yield

inconsistent results.

4. Sodium azide (0.09%) is used as a preservative in some reagents. Sodium azide may react with lead or copper plumbing and form explosive metal azide salts. When disposing of reagents, flush with ample volumes of tap water to prevent potential residues in plumbing. Sodium azide is a poison and may be toxic if ingested.
5. This kit is for *in vitro* diagnostic use.
6. In the event hemolyzed or lipemic sera must be used, heat inactivate sera 30 minutes at 56°C for optimal results. Microbially contaminated sera should not be used.
7. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
8. Avoid splashing or generation of aerosols at all times.
9. Incubation times and temperatures other than those specified may give erroneous results.
10. Cross contamination of reagents or samples may give false results.
11. Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use.
12. Bring all reagents, slides, and specimens to room temperature (18-25°C) prior to use.
13. Wear disposable gloves when handling specimens and reagents, and wash hands thoroughly afterwards.
14. Microbial contamination of reagents or samples may give false results.
15. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.

Specimen Collection

Collection: Serum is the preferred specimen. Approximately 5 ml of whole blood should be collected aseptically by venipuncture using a sterile vacuum collection tube or other suitable collection system. Allow blood to clot at room temperature (18-25°C). Serum should be separated from the clot by centrifugation as soon as possible to minimize hemolysis.

Interfering Substances: Sera exhibiting a high degree of hemolysis, icterus, lipemia, or microbial growth should not be used because these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Storage: Sera may be stored at 2-10°C up to one week. If testing is further delayed, sera should be stored frozen at –20°C or lower. Serum should not be stored in a self-defrosting refrigerator or freezer.

CAUTION: Repeated freeze/thawing of patient samples may yield false positive or false negative results.

Interpretation of Results

QUALITY CONTROL

Positive, negative, and PBS controls should be tested one time per run. The positive control should show bright apple-green fluorescence in the appropriate tissue structures with a clearly discernible pattern characteristic of the control serum that was used. The negative control should show low intensity, nonspecific, dull green fluorescence in the tissue, but with no discernible pattern of staining. The PBS control is used to observe non-specific staining by the antibody reagent, and should not exhibit any green fluorescence. If the controls do not appear as described, the test is invalid and should be repeated.

It is important that the intensity of fluorescence not be confused with the presence or absence of antibodies. The key factor to consider in determining whether a given dilution of serum is positive is the appearance of a clearly discernible pattern, irrespective of the intensity of the fluorescent staining.

Some of the many factors that can affect your results may include, but are not limited to:

1. The type of light source used. Mercury light sources will produce greater excitation energy at 495 nm than Quartz/Halogen. The 50-watt, 100-watt, and 200-watt Mercury light sources differ little in excitation energy at 495 nm. The 100-watt Quartz/Halogen light sources will produce greater excitation energy at 495 nm than 50-watt Quartz/Halogen.
2. The condition and age of the light source. This is particularly true for Mercury light sources, which generally exhibit a gradual reduction in excitation energy at 495 nm prior to burning out. This gradual reduction in excitation energy can result in a significant loss in sensitivity over several weeks. This problem can be avoided by keeping a time log. For best results, replace 50-watt mercury bulbs at 100 hours, and 100 or 200-watt mercury bulbs at 200 hours.

Quartz/Halogen light sources generally do not exhibit a gradual reduction in excitation energy prior to burning out.

3. The type of exciter filter used. Interference exciter filters provide greater sensitivity over a much narrower wavelength than absorption exciter filters. Refer to your fluorescent microscope manual or sales representative for more information.
4. Proper alignment of the microscope light path. Refer to your fluorescent microscope manual for instructions.
5. The numerical aperture of the objective. With incident light fluorescence (Epi), fluorescence is increased exponentially as the numerical aperture (NA) of the objective is increased additively. This may cause a 40X objective with a NA of 0.65 to read one or more dilutions lower than a 40X objective with a NA of 0.85. The numerical aperture is printed on the side of the objective.
6. Suppression filters. Suppression filters reduce specific wavelengths of excitation and may be used in reducing sensitivity. Refer to your fluorescent microscope manual or sales representative for more information.
7. Precision and accuracy of dilution technique, equipment, and performance of the test procedures.

INTERPRETATION OF PATIENT RESULTS
100X total magnification is recommended for screening positive/negative, while 200X total magnification is recommended for pattern recognition.

Negative: A serum is considered negative if staining is less than or equal to the negative control well with no clearly discernible pattern. The tissue may demonstrate weak staining but with no clearly discernible pattern.

Positive: A serum is considered positive if the tissue shows a clearly discernible pattern of staining.

FLUORESCENT INTENSITY

Fluorescent intensity may be semi-quantitated by following the guidelines for fluorescent antibody reagents established by the Centers for Disease Control and Prevention, Atlanta, Georgia (CDC).

- 4+ Brilliant yellow-green (maximal fluorescence): clear-cut outline.
- 3+ Less brilliant yellow-green fluorescence: clear-cut outline.
- 2+ Definite pattern but dim fluorescence.
- 1+ Very subdued fluorescence.

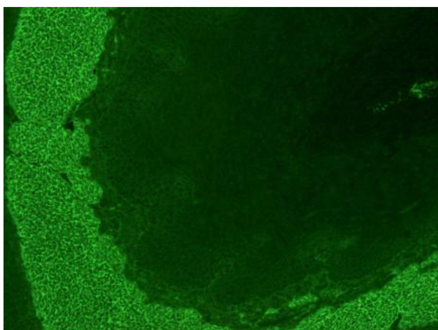
A standard slide for the determination of these fluorescent intensities, FITC QC Slide™, catalog number 1900, is available from Immuno Concepts, N.A. Ltd.

REPORTING OF RESULTS

Screening: Results should be reported as positive or negative at the 1:5 dilution, and the staining pattern should be reported.

PATTERN DETECTION

Anti-Endomysial Antibodies (EMA): A fine network of fibers is seen surrounding smooth muscle cells in the muscularis mucosa
Antigen: Tissue transglutaminase in the endomysial sheath surrounding the smooth muscle cells.
Disease Association: Endomysial antibodies are seen in 95-100% of untreated patients with celiac disease.

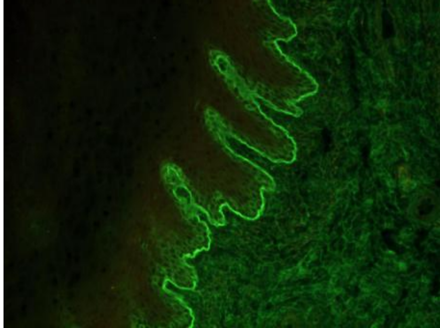


Anti-Endomysial Antibodies

Anti-Basement Membrane Antibodies: Prominent staining of the basement membrane area along the dermal-epidermal junction.

Antigen: The antigen has been identified as a 180 kDa transmembrane protein (BP 180).

Disease Association: These antibodies are associated with bullous or blistering pemphigoid, and have been detected in approximately 70% of patients with this disease.

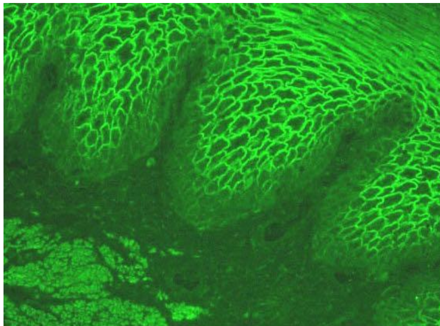


Anti-Basement Membrane Antibodies

Antibody to Intracellular Cement: Staining of the intracellular material in the stratified epithelial layer of the esophagus.

Antigen: The antigen has been identified as a 130 kDa desmoglein 3 protein.

Disease association: These antibodies are associated with the active form of pemphigus vulgaris, and have been detected in approximately 90% of patients with this disease.

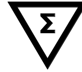


Antibody to Intracellular Cement

Limitations of the Test

1. Diagnosis cannot be made on the basis of tissue antibody detection alone. The physician must interpret these results in conjunction with the patient's history and symptoms, the physical findings, and other diagnostic procedures.
2. Treatment should not be initiated on the sole basis of a positive test for antibodies. Clinical indications, other laboratory findings, and the physician's clinical impression must be considered before any treatment is initiated.
3. Although a positive reaction may be highly suggestive of disease, it should not be considered diagnostic but rather viewed as a part of the overall clinical history of a patient.
4. Staining patterns often change with progressive titration of sera. This phenomenon is generally due to the presence of more than one disease condition.
5. Because of the many options available in fluorescent microscopes, it is recommended that light sources, filters, and optics be standardized when comparing patient titers between laboratories.

In the event of damage to the protective packaging, please contact Immuno Concepts prior to use.

	Manufacturer		Authorized Representative in the European Community
	Temperature Limitation		Contains Sufficient for <n> tests
	Consult Instructions for Use		In Vitro Diagnostic Medical Device

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HISTOFLUOR® ANTI-ENDOMYSIAL AUTOANTIBODY (EmA) FLUORESCENT TEST PROCEDURE

NOTE: If the laboratory is using an automated sample processing system, the procedure and recommendations of the manufacturer of the processor should be followed. The slide processing system should be programmed for the appropriate sample dilutions, dispense volumes, and incubation times as outlined below

1. **RECONSTITUTE BUFFER (PBS)**
   Dissolve contents of one buffer pouch in one liter of deionized or distilled water. The PBS buffer may be covered and stored at 2-10°C up to four weeks.

2. **DILUTE PATIENT SAMPLES**
   Screening: Dilute patient samples to 1:5 by adding 0.100 ml (100 µl) serum to 0.400 ml (400 µl) reconstituted PBS.

3. **PREPARE SUBSTRATE SLIDES** (30-40 µl/well)
   Remove slide(s) from pouch(es) and place control sera on control wells as follows: Invert control dropper bottle and squeeze gently until drop is visible at the tip. Gently touch the drop to appropriate control well while avoiding direct contact of dropper tip with slide surface. Add 1 drop (30-40 µl) patient sample to the numbered wells.
   NOTE: For general screening, the anti-endomysial positive control is recommended. For semi-quantitative titering, select the positive control illustrating the most similar pattern of fluorescence to the screening sample (e.g. for patient sample yielding an anti-endomysial pattern of fluorescence in screening, use anti-endomysial positive control).
   CAUTION: DIRECT CONTACT OF DROPPER TIP WITH SLIDE SURFACE MAY RESULT IN DAMAGE TO THE ANTIGEN SUBSTRATE.

4. **INCUBATE SLIDES** (30 ± 5 minutes at room temperature, i.e. 18-24°C)
   Place slide(s) into a moist covered chamber (a petri dish with moistened paper toweling will be adequate). Incubate, with lid in place, for 30 minutes (± 5 minutes) at room temperature (18-24°C).

5. **PBS RINSE**
   Remove slide(s) from incubator tray and rinse briefly with PBS using a squirt bottle, Pasteur, or serological pipette. Do not squirt buffer directly on the wells.
   NOTE: To avoid cross contamination on the slides, direct PBS stream along midline of slide, tilting first toward the upper row of wells followed by tilting toward the lower row of wells.

6. **PBS WASH** (10 minutes)
   Wash slide(s) 10 minutes with PBS in a slide staining dish or Coplin jar. This wash may be extended 10-30 minutes with no variability in final test results. Discard PBS wash solution after use.

7. **FLUORESCENT ANTIBODY REAGENT** (Cover the wells with 12-14 drops)
   Remove one slide at a time from PBS. Tap slide on its side against bibulous paper or paper toweling to remove excess buffer. Immediately return slide to the incubation chamber and cover the wells completely using fluorescent antibody reagent; begin by placing a drop over each well. Repeat for each slide. Fluorescent antibody reagent has been titered to compensate for residual buffer remaining on the slide after rinsing.
   NOTE: It is important that slide wells do not dry during this procedure or damage to the substrate may occur.
   DO NOT BLOT OR DRY THE SLIDE IN ANY MANNER OR ALLOW SLIDE TO SIT WITHOUT FLUORESCENT ANTIBODY REAGENT FOR LONGER THAN 15 SECONDS.

8. **INCUBATE SLIDES** (30 ± 5 minutes at room temperature, i.e. 18-24°C)
   Place lid on incubation chamber and cover with a paper towel to prevent exposure to light if the chamber is not opaque. Allow slide(s) to incubate 30 minutes (± 5 minutes) at room temperature (18-24°C).

9. **PBS RINSE**
   Remove slide(s) from incubator tray and rinse briefly with PBS. Do not squirt buffer directly on the wells.

10. **PBS WASH** (10 minutes)
    Wash slide(s) 10 minutes with PBS in a slide staining dish or Coplin jar. This wash may be extended 10-30 minutes with no variability in final test results when counterstain is not used.

11. **MOUNT COVERSLIP**
    Remove one slide at a time from PBS. Tap slide on its side against bibulous paper or paper toweling to remove excess buffer.
    DO NOT BLOT OR DRY THE SLIDE IN ANY MANNER OR ALLOW TO SIT WITHOUT COVERSLIP FOR LONGER THAN 15 SECONDS. Add enough semi-permanent mounting medium to cover the wells of each slide. Carefully place coverslip in position, avoiding air pockets, by gently lowering coverslip from one end of the slide to the other.
    NOTE: Excess mounting medium on slide may result in high background fluorescence, due to light scattering, or lack of clear resolution of cells (blurred image). Excess mounting medium may be removed from slide by gently blotting coverslip with bibulous or lens paper while avoiding any direct movement of the coverslip.

FOR TECHNICAL ASSISTANCE:
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