HISTOFLUOR® IgG ANTI-SKIN AUTOANTIBODY (ASA) FLUORESCENT TEST SYSTEM

For Research Use Only
For Professional Use

INTENDED USE: This is an indirect fluorescent antibody test for the qualitative and semi-quantitative detection of antibasement membrane, anti-intercellular substance and anti-endomysial autoantibodies in human serum. The optional component is to be use as an aid in the detection of antibodies associated with Pemphigus vulgaris and pemphigoid bullous.

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
Fluorescent Antibody Reagent CONJ\|FITC: Catalog No. 12009-10R (9.0 ml). Goat anti-human IgG 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.

Precautions

PRECAUTIONS:
Sodium azide (0.09%) is used as a preservative. 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.

Interpretation of patient Results

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.

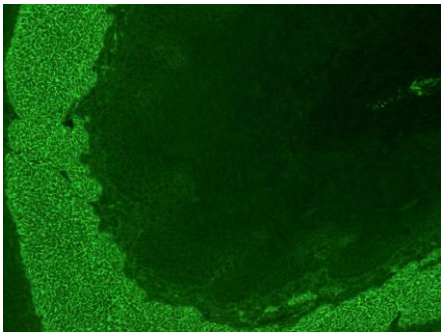
A standard slide for the determination of these fluorescent intensities, FITC QC Slide™, catalog number 1900, is available from Immuno Concepts, N.A. Ltd.

Reporting of Results

Screening: Results should be reported as positive or negative at the 1:10 dilution, and the staining pattern should be reported.

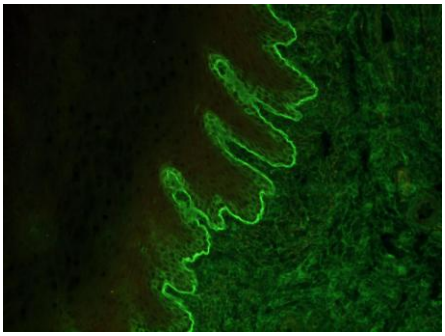
PATTERN DETECTION

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.



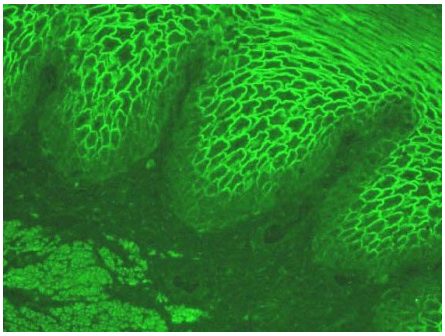
Anti-Endomysial Antibodies

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.

TEST PROCEDURE

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 2-25°C up to four weeks.

2. **DILUTE PATIENT SAMPLES**
   Screening: Dilute patient samples to 1:10 by adding 0.025 ml (25 µl) serum to 0.225 ml (225 µl) reconstituted PBS.

3. **PREPARE SUBSTRATE SLIDES** (approx. 50 µl/well)
   Remove slide(s) (12008-01) from pouch(es) and 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 (approx. 50 µl/well) patient sample to the numbered wells.
   CAUTION: DIRECT CONTACT OF DROPPER TIP WITH SLIDE SURFACE MAY RESULT IN DAMAGE TO THE ANTIGEN SUBSTRATE.

4. **INCUBATE SLIDES** (30 minutes at room temperature, i.e. 18-25°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 at room temperature (18-25°C).

5. **PBS RINSE**
   Remove slide(s) from incubator tray and rinse briefly with PBS using squirt bottle, Pasteur, or serological pipette. Do not squirt buffer directly on the wells.
   NOTE: To avoid cross contamination, direct PBS stream along midline of slide, tilting first toward rows 1-4 followed by tilting toward wells 5-8.

6. **PBS WASH** (10 minutes)
   Wash slide(s) in PBS in a Coplin jar for 10 minutes.

7. **FLUORESCENT ANTIBODY REAGENT**
   Remove one slide at a time from PBS and tap slide on its side against bibulous paper or paper toweling to remove excess PBS. Immediately return slide to the incubation chamber and cover the wells completely using Fluorescent IgG antibody reagent (12009-10); begin by placing a drop over each well. Repeat for each slide.
   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 minutes at room temperature, i.e. 18-25°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 at room temperature (18-25°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) in PBS in a Coplin jar for 10 minutes.

11. **MOUNT COVERSLIP**
    Remove one slide at a time from PBS and tap slide on its side against bibulous paper or paper toweling to remove excess PBS.
    DO NOT BLOT OR DRY THE SLIDE IN ANY MANNER OR ALLOW SLIDE TO SIT WITHOUT COVERSLIP FOR LONGER THAN 15 SECONDS. Add 4-5 drops of semi-permanent mounting medium along midline 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:
USA: 1-800-251-5115 Outside USA: 1-916-363-2649
Email: technicalsupport@immunoconcepts.com

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


Manufacturer


Contains Sufficient for <n> tests


Temperature Limitation

RUO Research Use Only


Consult Instructions for Use

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