Introduction:

The in vitro detection of skin antibodies by the indirect immunofluorescent technique has been established as an aid in the diagnosis of skin and systemic diseases· The utilization of monkey esophagus, has been the recommended substrate for IFA. Monkey esophagus is used for the detection of both basement membrane antibodies and intercellular substance antibodies.[1,5] The intercellular substance antibody has been associated with the presence of a variety of disorders of the skin.[3] The detection of the basement membrane antibody has been associated with the presence of a variety of bullous pemphigoid autoimmune disorders of the skin.[3]

Principles:

The primary test reaction involves circulating anti-epidermal antibodies present in the patient's serum, which attach to their homologous epidermal antigens. This occurs during the incubation period while the serum covers the antigen surface. A secondary reaction then follows a rinsing period which removes all unbound human antibody. The reagent used in the secondary reaction is a fluorescein labeled anti-human globulin conjugate containing Evans Blue Counterstain. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under the appropriate fluorescent microscope for various morphological patterns of epidermal fluorescence which can be visually identified.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1502L (3.0 ml) (for use with Primate Substrates) 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 contains antibodies which will react with the human IgA, IgG and IgM immunoglobulin classes.
2. The antigen slides of monkey esophagus sections must be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.

3. ASA positive control No. 5303L (1.0 ml) for Basement Membrane reaction should be stored at 2-8 C upon receipt. Check label for specific expiration date.
4. ASA positive control No. 5302L (1.0 ml) for Intercellular Substance reaction should be stored at 2-8 C upon receipt. Check label for specific expiration date.
5. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.
6. Buffer Pack No. 1601 - 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.
7. Mounting Medium No. 1610 is stable when stored at 2-8 C. Check label for specific expiration date.

Note: All kit components are available separately. Please see the current SCIMEDX Corporation Catalog for more details.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (500 ml)
Distilled H_20
Fluorescence Microscope
Paper Towels - lint free

Reagent Preparation:

1. Buffer Pack No. 1601. Rehydrate buffer with 1 liter of sterile distilled water.

Specimen Collection:

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8 C if it is to be analyzed within a few days. Serum may be held

for 3 to 6 months by storage at -20 C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thimerosal or 0.095% sodium azide is strongly recommended.

Test Instruction:

Screening: dilute test serums 1:20 in PBS. **Titration:** set up doubling dilutions of serum starting at 1:20, 1:40, 1:80, 1:160, 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 30 µl) 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 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 on to the well.
5. Wash in PBS for five minutes. Repeat 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 (25-30 µl) 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 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 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.

Results:

1. Diffuse staining throughout the tissue is considered non-specific and should be considered a negative result.
2. Staining of the basement membrane (BM) of the epidermis is considered positive and is associated with 70% of bullous pemphigus cases.
3. Staining of the intercellular substance (ICS) of the prickle cell layer of the epidermis is considered positive 90% of pemphigus cases.
4. The titer is the highest dilution of the patient's serum, showing a weak 1+ fluorescence of the ICS or BM. Titers of 1:20 or greater are clinically relevant for both patterns.

CORRELATION OF BULLOUS DISEASE TO DIAGNOSTIC ASSAYS

	Biopsy Findings	Serum Findings	Relevance of

Diseases	Direct Immunofluorescence	Indirect Immunofluorescence	Immunofluorescence Findings
Pemphigus, all forms	ICS deposits of IgG	ICS antibodies	DIF and/or IIF diagnostic
Pemphigus, all forms	BM linear IgG and/or C also sometimes other Ig and F	BM antibodies IgG about 70%	DIF or IIF diagnostic
Scarring pemphigoid and Brunsting-Perry variety	BM linear IgG and/or C; also other Ig-90%	BM antibodies IgG about 20%	DIF or IIF diagnostic
Herpes gestations	BM linear, mostly c; also Ig	BM antibodies IgG about 20%	DIF or IIF diagnostic
Hermatitis Herpetiformis typical	IgA also F and C granular or fibrillar in dermal papillae 85%		DIF diagnostic
Linear IgA dermatosis	IgA linear; 15% at BM; also F & C	No specific antibodies or BM antibodies IgA	DIF diagnostic

KEY TO ABBREVIATIONS:

ICS = Inter-Cellular Substance
BM = Basement Membrane
C = Complement
DIF = Direct Immunofluorescence
IIF = Indirect Immunofluorescence

Limitations of Procedure:

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.
2. Patients with Lydel's toxic neurolysis, extensive burns and myasthenia gravis may demonstrate intercellular substance staining.
3. Additional confirming tests for Bullous diseases are skin biopsy, for direct immunofluorescent analysis and electron microscopy study.

Precautions:

1. All human components have been tested by radioimmunoassay for (HB_sA_g) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_sA_g or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
3. Do not use components beyond their expiration date.

4. Follow the procedural instructions exactly as they appear in this insert to insure 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 artifacts.

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