

INTENDED USE

The ZEUS IFA Anti-Skin Antibody (ASA) Test System is designed for the qualitative and semi-quantitative detection of antibodies associated with Pemphigus and Bullous Pemphigoid by the indirect fluorescent antibody (IFA) technique, and is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Most authorities agree that serum antibody studies of all cases of suspected Pemphigus Vulgaris (PV) and Bullous Pemphigoid (BP) are advisable (1 - 4). Specific antibodies are demonstrable in virtually all active cases, particularly if repeated tests are performed during different stages of the disease (1). The most frequently recommended substrate for these serum antibody studies is monkey esophagus (6). Both PV and BP specific antibodies can be detected in a single assay using this substrate. PV antibodies react with the intercellular substances between the squamous epithelium of monkey esophagus, and BP antibodies react with the basement membrane of the squamous epithelial cell layer (1, 4, and 6). Antinuclear antibody staining of the squamous epithelial cell nuclei may occur if serum is obtained from patients with systemic lupus or other connective tissue disorders (7). Distinct intercellular staining may be detected in cases of pemphigus vegetans, pemphigus foliaceus (including the Brazilian form), and pemphigus erythematosus (1). Although a single serological test is not sufficient for determining the dosage of drug therapy in PV, a two-fold drop in titer is an indication of effective control of the disease process. Although some correlation in titer and disease activity is found in BP, the prognostic value is less than in PV due to fluctuations in titer. Serum studies may be diagnostic of PV or BP; however, it is frequently advisable to perform direct immunohistochemical studies on biopsies of skin lesions (1, 2). Direct immunofluorescent studies of skin biopsies may be facilitated with ZEUS Tissue Fixative and Wash Solution, prepared according to Michel (5). This new fixative eliminates the necessity of immediate freezing of the biopsy specimen and allows transport of fresh tissue biopsies of skin and other tissues up to 5 days at ambient temperatures.

PRINCIPLE OF THE ASSAY

The ZEUS IFA ASA Test System is designed to detect the presence of circulating pemphigus and bullous pemphigoid antibodies in human sera. The assay employs monkey esophagus tissue substrate and goat anti-human immunoglobulin adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps:

1. Step one is the interaction of anti-skin antibodies in patient's sera with the monkey esophagus.
2. Step two is the interaction of FITC-labeled anti-human immunoglobulin with antibodies attached to the intercellular cement of the epithelial cells in pemphigus, or the basement membrane zone in bullous pemphigoid in a positive assay (see Assay Procedure section for details).

The ZEUS IFA ASA Test System will detect both PV and BP antibodies. The assay is a particularly useful laboratory aid in the diagnosis of PV and BP since the majority of untreated patients with active disease will contain anti-skin antibodies in their serum.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAVe Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

• • •	1. Monkey Esophagus Substrate Slides: Ten, 8-well Slides with absorbent blotter and desiccant pouch.
CONJ	2. Conjugate: Goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL + 1	3. PV Positive Control (Human Serum): Will produce staining of the squamous epithelial cells of the substrate. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL + 2	4. BP Positive Control (Human Serum): Will produce staining of the basement membrane of the substrate. One, 0.5mL, blue-capped, vial. Ready to use.
CONTROL -	5. Negative Control (Human Serum): Will produce no substrate staining. One, 0.5mL, green-capped, vial. Ready to use.
DIL SPE	6. SAVe Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. NOTE: The SAVe Diluent® will change color when combined with serum.
BUF PBS	7. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
MNTMED	8. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dropper tipped vials.

NOTES:

1. The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAVe Diluent® (Product #: FA005CC), Mounting Media (Product #: FA0009S), and PBS (Product #: 0008S).
2. Test System also contains a Component Label containing lot specific information inside the Test System box.

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety 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": current edition; and OSHA's Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay.** Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.

7. The SAVE Diluent®, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may be toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

The following filter systems, or their equivalent, have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

Transmitted Light			
Light Source: Mercury Vapor 200W or 50W			
Excitation Filter	Barrier Filter	Red Suppression Filter	
KP490	K510 or K530	BG38	
BG12	K510 or K530	BG38	
FITC	K520	BG38	
Light Source: Tungsten – Halogen 100W			
KP490	K510 or K530	BG38	
Incident Light			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (8). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (11).

STORAGE CONDITIONS

	Unopened Test System.
	Mounting Media, Conjugate, SAVE Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
	Phosphate-buffered-saline (PBS) Packets.

ASSAY PROCEDURE

1. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
 2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted.** Prepare a 1:10 dilution (e.g.: 10µL of serum + 90µL of SAVe Diluent® or PBS) of each patient serum. **The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.**
Dilution Options:
 - a. Users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVe Diluent® or PBS. When evaluated by ZEUS Scientific, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected endpoint titer for each lot of Positive Control.
 - b. When titrating patient specimens, initial and all subsequent dilutions should be prepared in SAVe Diluent® or PBS only.
 3. Wash Slides for 3 - 5 minutes in PBS.
 4. Remove Slides from PBS and blot dry with six-well blotting paper. It is suggested that blotting paper be placed on a flat surface. Then place substrate Slide in an inverted position over the blotter. Press firmly on back of Slide. Do not allow tissue substrate to dry throughout the test procedure.
 5. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
 6. Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
 7. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
 8. Wash Slides for two, 5 minute intervals, changing PBS between washes.
 9. Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe. **CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. Do not allow the Slides to dry during the test procedure.**
 10. Add 20µL of Conjugate to each well.
 11. Repeat steps 6 through 9.
 12. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.
- NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.**

QUALITY CONTROL

1. Every time the assay is run, the Positive Controls, a Negative Control and a Buffer Control must be included.
 2. It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid.
 - a. Negative Control - characterized by the absence of fluorescent staining of the squamous epithelial cells, and/or basement membrane zone.
 - b. Positive Controls - PV Positive control is characterized by any apple-green fluorescent staining between the squamous epithelial cells. The BP Positive Control is characterized by specific staining of the basement membrane zone.
 3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
- NOTES:**
- a. **The intensity of the observed fluorescence may vary with the microscope and filter system used.**
 - b. **Non-specific reagent trapping may exist. It is important to adequately wash slides to eliminate false positive results.**

INTERPRETATION OF RESULTS

1. Any apple-green staining of the specific structures noted above on a scale of 1+ to 4+ is considered positive. A 1+ is considered a weak reaction, and 4+ a strong reaction. All sera positive at a 1:10 dilution should be titered to endpoint dilution. This is accomplished by making 1:20, 1:40, 1:80, etc., serial dilutions of all positives. The endpoint is the highest dilution that produces a discernible positive reaction. Specific nuclear staining of the epithelial cell nuclei is considered a positive test for antinuclear antibodies which may be associated with SLE and other connective tissue diseases.
2. Titers less than 1:10 are considered negative.
3. Positive Test:
 - a. Specific intercellular staining between the squamous epithelial cells is considered a positive test for pemphigus antibodies.
 - b. Specific basement membrane zone staining is considered a positive test for bullous pemphigoid.

LIMITATIONS OF THE ASSAY

1. The ZEUS IFA ASA Test System is a laboratory aid and by itself is not diagnostic.
2. The results should be interpreted in light of the patient's clinical condition.
3. No definitive association between the pattern of fluorescence and any specific disease state is intended with this product.
4. No U.S. standard of potency.

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ZEUS IFA ASA Test System

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