ANA Rat Liver Tissue Test System

REF FA2001

IVD

CE

Rx Only

INTENDED USE

The ZEUS IFA ANA Rat Liver Tissue Test System is designed for the qualitative and semi-quantitative detection of antinuclear antibodies by the indirect fluorescent antibody IFA technique. It aids in determining SLE and differentiating clinically similar connective tissue disorders, and is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

It is generally agreed that the method of choice for ANA screening and quantitation is the indirect fluorescent antibody (IFA) technique. This method has been used extensively for detecting the presence of ANA in the sera of patients with systemic lupus erythematosus (SLE), and other clinically similar connective tissue disorders (1 - 5). In addition, ANA may be associated with numerous drug-induced lupus syndromes (6 - 7) which mimic the spontaneous form of SLE clinically. The IFA technique was adapted to ANA testing by several investigators (8 - 9) following the basic methods originally described by Coons (10). ANA are primarily composed of IgG antibodies; however, IgA and IgM ANA may also be detected (11). It is recognized that many sources of nuclear material may be employed as a substrate for ANA testing. However, most of the original ANA research work was performed using mouse or rat liver tissue sections. There are several different patterns of nuclear fluorescence (12 - 19). These various patterns and the basis for them are as follows:

Homogeneous (Solid diffuse) - Diffuse staining of the entire nucleus due to antibodies reactive with DNA-Nucleoprotein-Histone complexes (14 - 15).

Peripheral (Rim, Shaggy) - Staining of the nuclear membrane due primarily to antibodies directed against DNA (3 - 13, 16 - 17).

Speckled - Specks of staining dispersed throughout the nucleus due to antibodies directed against extractable nuclear antigens, RNP, or Sm (18, 19).

Nucleolar - Staining of the nucleolar membranes due to antibodies reactive with RNA - nucleoprotein complexes (13). Although the level of ANA may not correlate with the clinical course of a particular autoimmune disease state (6), the various patterns of nuclear staining may be associated with specific disease states (3, 17, 20 - 23).

NOTE: The rat liver tissue substrate will also detect mitochondrial and smooth muscle antibodies. Mitochondrial antibody staining will appear as a course granular pattern throughout the tissue section within the cytoplasm of the tubule cells. Such reactions should be confirmed by performing MA studies on the mouse kidney tissue substrate. SMA staining will occur in the smooth muscle cell cytoplasm within the blood vessel walls that are present in the mouse kidney tissue sections. Reticulin antibody will stain the sinusoidal lining cells. Heterophile antibodies will also stain in the sinusoidal areas.

PRINCIPLE OF THE ASSAY

The ZEUS IFA ANA Rat Liver Tissue Test System is a pre-standardized assay designed to detect the presence of circulating ANA in human sera. The system employs rat liver 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 ANA in patients' sera with the rat nuclei.
2. Step two is the interaction of FITC labeled anti-human immunoglobulin with nuclear antibodies attached to the mouse nuclei in a positive assay (see the Assay Procedure section for details).

The ZEUS IFA ANA Rat Liver Tissue Test System will detect all recognized systems of nuclear staining patterns and is a particularly useful laboratory diagnostic aid in the diagnosis of SLE since nearly 100% of untreated patients with active SLE will contain ANA in their serum (14). It should be noted that the incidence of positive ANA increases in apparently normal individuals, particularly in the 5th to 7th decade of life (24).

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. Rat Liver Substrate Slides: Ten, 8-well Slides with absorbent blotter and desiccant pouch.
CONJ	2. Conjugate: Goat anti-human immunoglobulin (polyvalent) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL +	3. Positive Control (Human Serum): Will produce positive apple-green staining of the nucleus. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL -	4. Negative Control (Human Serum): Will produce no detectable staining of the nucleus. One, 0.5mL, green-capped, vial. Ready to use.
DIL SPE	5. 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	6. 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	7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper 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 by 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 (28, 29). 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 (31).

STORAGE CONDITIONS

2°C – 8°C	Unopened Test System.
	Mounting Media, Conjugate, SAVe Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
2°C – 25°C	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 1:20 dilution (e.g.: 10µL of serum + 190µL 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. As an option, users may prepare initial sample dilutions using PBS, or Zorba-NS (Zorba-NS is available separately. Order Product Number FA025 – 2, 30mL bottles).
   b. 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 end-point titer for each lot of Positive Control.
   c. When titrating patient specimens, initial dilutions should be prepared in SAVe Diluent®, PBS, or Zorba-NS and all subsequent dilutions should be prepared in SAVe Diluent® or PBS only. Titrations should not be prepared in Zorba-NS.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash Slides for two, 5 minute intervals, changing PBS between washes.
7. 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**.
8. Add 20µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. 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, a Positive Control, 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 specific fluorescence and a red, or dull green, background staining of all cells due to counterstain.
   b. Positive Control - characterized by apple-green fluorescence. The homogeneous staining pattern is a diffused uniform staining of the entire nucleus.
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.

c. Non-nuclear staining of the Rat Liver substrate may be observed with some human sera. Report nuclear staining results only and disregard non-nuclear staining.

INTERPRETATION OF RESULTS

1. Titers less than 1:20 are considered negative.
2. Positive test: A positive reaction is the presence of any pattern of nuclear apple green staining observed at a 1:20 dilution, based on 1+ to 4+ scale of staining intensity. 1+ is considered a weak reaction, and a 4+ a strong reaction. All sera positive at 1:20 should be titered to end point dilution. This is accomplished by making a 1:20, 1:40, 1:80, etc., serial dilution of all positives. The end point is the highest dilution that produces a 1+ positive reaction.
3. Homogeneous patterns with peripheral accentuation are frequently found in sera from patients with SLE.

Interpretation According to Pattern of Nuclear Staining

Pattern	Disease Most Frequently Found In	References
Homogeneous High Titer Low Titer	 SLE Rheumatoid Arthritis and other diseases	 (3,8,9,17) (1)
Peripheral	SLE	(2,8,9,17)
Speckled	Scleroderma, Raynaud's Syndrome, Sjögren's Syndrome, Mixed Connective Tissue Disease	(12,25,26)
Nucleolar	Scleroderma	(27)

LIMITATIONS OF THE ASSAY

1. The ZEUS IFA ANA Rat Liver Tissue Test System is a laboratory diagnostic aid and by itself is not diagnostic. Positive ANA may be found in apparently healthy people. It is therefore imperative that ANA results be interpreted in light of the patient's clinical condition by a medical authority.
2. SLE patients undergoing steroid therapy may have negative test results.
3. Many commonly prescribed drugs may induce ANA (6, 7).
4. Some nuclear staining patterns may be masked at a 1:20 dilution. Serial dilution of these sera will unmask these patterns.
5. No definitive association between the pattern of nuclear fluorescence and any specific disease state is intended with this product.

EXPECTED RESULTS

The expected value in the normal population is negative. However, apparently healthy individuals may contain ANA in their sera (24). This percentage increases with aging, particularly in the 7th decade of life.

PERFORMANCE CHARACTERISTICS

The ZEUS IFA ANA Rat Liver Tissue Test System was tested in parallel with a reference procedure described in the literature. Routine ANA testing was performed by both procedures on 434 patient specimens. Of these 434 sera, 116 were positive by both procedures. The ZEUS IFA ANA Rat Liver Tissue Test System showed 97% agreement with respect to positive and negative results, and 100% with respect to staining pattern. Of the 29 discrepancies in titer, the ZEUS procedure was one dilution lower in 16 specimens, while the reference procedure was lower in 13 specimens. Of the 16 specimens with lower titers by the ZEUS procedure, all were one dilution discrepancies, and 13 of these 16 involved specimens that were negative by the ZEUS procedure and positive at 1:20 by the reference procedure.

Specificity: Although most ANA are of the IgG class, the goat anti-human immunoglobulin conjugate used in this test system produces precipitin reactions on immunoelectrophoretic analysis against IgG, IgA, and IgM immunoglobulins. This reagent is considered to be polyvalent.

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