

Introduction:

Antinuclear Antibody (ANA) tests are commonly performed on sera from patients with various connective tissue diseases, particularly in systemic lupus erythematosus (SLE), for diagnostic evidence, prognostic significance, and management of therapy. The highest titers of ANA are found in active SLE and the presence of these antibodies is the second most common manifestations of SLE. Immunofluorescence is the test of choice for screening for the presence of ANA since it detects 95-100% of active SLE cases. The presence of ANA has been well documented in different disease states as well as in healthy relatives of SLE patients. The incidence of positive ANA varies with each disease (see Table 1). Rat or mouse liver is utilized for ANA detection in this test system.

Principles:

ANA antibodies are not organ or species specific. The primary test reaction involves circulating antinuclear antibodies present in the patient's serum, which attach to their homologous nuclear antigens. This occurs during the incubation period while the serum covers the antigen surface. A rinsing period is followed by a secondary reaction. The reagent used in the secondary reaction is a fluorescein labeled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence.

The clinical significance of the various nuclear immunofluorescent patterns is useful in evaluating patients for the presence of one of the connective tissue diseases. The homogeneous pattern is the most common pattern and is associated with SLE. The peripheral pattern confirms a clinical diagnosis of SLE. True speckled nuclear fluorescence is seen in Scleroderma, Raynaud's disease, Rheumatoid Arthritis, and Sjögren's syndrome. Nucleolar fluorescence is seen mainly in Scleroderma and Sjögren's syndrome.

Various drugs have been reported to induce or activate SLE. Patients on these drugs often demonstrate varying levels of ANA in their serum, see Table II.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1501L (3.0 ml)/1533L (5.0 ml) with Evans Blue Counterstain 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 IgG, IgM and IgA Immunoglobulin classes.
2. The antigen slides of rat/mouse liver sections must be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.
3. ANA positive control No. 1202L (1.0 ml) should be stored at 2-8°C upon receipt. Check label for specific expiration date. 1048L – page 1

4. 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.
5. Buffer Pack No. 1601 - Phosphate Buffered Saline is stored at room temperature storage. Check label for specific expiration date. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.
6. Mounting Medium No. 1610 is stable when stored at 2-8°C. Check label for specific expiration date.

Additional Components Available:

ANA Speckled Positive Control (Cat# 1203)

ANA Nucleolar Positive Control (Cat# 1204)

FITC IgG H&L w/o EB (Cat# 1500)

Evans Blue Counterstain (Cat# 1600)

Moisture Chamber (Cat# 1716)

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 H₂O

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 0.095% sodium azide is strongly recommended.

Test Instruction:

Screening: dilute test serums 1:20 (1 part patient sample to 19 part diluent) in PBS. Titration: set up doubling dilutions of serum starting at 1:20, (i.e. 1:20, 1:40, 1:80, 1:160, 1:320, 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 19 - 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:

The slide should be examined under 400X high dry or oil immersion objective at a final magnification of 1000X. A positive result is observed as one of four basic staining patterns seen individually or in various combinations. The characteristic patterns are best seen when viewed using high dry objectives. The positive control (Cat.# 1202) demonstrates a homogeneous ANA pattern.

1. Homogeneous (Diffuse)
An even, finely diffuse fluorescence of the entire nucleus is seen.
2. Peripheral (Rim, shaggy)
The nuclear membrane is more intensely fluorescent than the central area.
3. Speckled
The nuclei show numerous small "specks" of fluorescence throughout the nucleus.
4. Nucleolar
The nucleoli are uniformly stained and appear as 1 to 5 large spherical areas of fluorescence scattered throughout the nucleus.

Pattern Interpretation:

The nuclear immunofluorescent patterns found in SLE can be of prognostic significance.

Peripheral

Confirms clinical diagnosis of SLE. Renal involvement, confirmed by anti-DNA tests (SCIMEDX Cat.# 6050/ 6100), is associated with an intermediate prognosis.

Homogeneous

High titer anti-DNA antibodies suggest SLE with probable renal involvement and is associated with an intermediate prognosis.

Speckled

Large and small speckles seen in benign SLE and associated with good prognosis.

Nucleolar

High titers are associated with Sjögren's syndrome and Scleroderma.

Limitations of Procedure:

1. No diagnosis should be based upon a single ANA test result, since various host factors must be taken into consideration.
2. Among these host factors are age and sex. There is an increasing significance in positive ANA results in both males and females as age increases. Normal females between 20-60 have a 7% incidence of ANA: normal males, a 4% incidence. Normal males and females over 80 years of age have a 50% incidence of ANA.

Various medications including antibiotics, tranquilizers, aspirin and birth control pills can induce a lupus like condition resulting in high ANA titers, see Table 2. Drug-

Induced Lupus generally goes into a sustained clinical remission following removal of the triggering medication.

- Various autoimmune processes induce positive ANA tests.
- Further evidence for a diagnosis of SLE is provided by low complement levels, particularly C1, C3, and C4.
- ANA tests may not agree with LE Prep tests or with latex tests.
- Presence of antibodies to double stranded native DNA is diagnostic for SLE.
- Management of therapy should be based not only on positive serologic tests for SLE, but should include the presence of active clinical disease.
- Elderly patients with SLE have a better prognosis and their clinical symptoms differ substantially from those seen in younger patients.
- Although the predominant class of antinuclear antibodies (ANA) is Immunoglobulin G, the presence of Immunoglobulin E may be of pathogenic importance in SLE.

Precautions:

- All human components have been tested for (HB_sAg), HSV 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_sAg or HTLVIII/LAV. All human components should be handled with appropriate care.
- The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
- Do not use components beyond their expiration date.
- Follow the procedural instructions exactly as they appear in this insert to insure valid results.
- For in vitro diagnostic use.
- Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
- 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.

TABLE I

INCIDENCE OF ANA IN VARIOUS DISORDERS

Disease	% Incidence	Disease	% Incidence
Systemic lupus erythematosus	95-100	Dermatomyositis, polymyositis	10-30
Lupoid hepatitis	95-100	Polyarteritis Nodosa	15-25
Progressive systemic sclerosis (scleroderma)	75-80	Rheumatic fever	-5
Rheumatoid arthritis	25-60	Drug associated SLE-like syndrome (hydralazine; procainamide; isoniazid)	-50
Juvenile arthritis	15-30	Miscellaneous diseases	10-50
Feily's syndrome	95-100	Generally normal values	-5
Sjögren's syndrome	45-75	Normal old age	-40
Chronic discoid lupus	15-50	Healthy relative of SLE patient	-25

TABLE II - SLE INDUCING DRUG

GROUP I Induced by Pharmacological Action	GROUP II Induced by Allergic Action
hydralazine	Aminosalicylic Acid
Procainamide	Chlorthalidone
Anti-convulsant:	D-penicillamine
Mephentoin	Griseofulvin
Phenytoin	Isoquinazepone
Primidone	Levodopa
Trimethadione	Methyldopa
Ethosuximide	Methysergidie
Carvanmazepine	Methylthiouracil
Pheneturide	Oral Contraceptives
Isoniazid	Penicillin
Chlorpromazine	Phenylbutazone
	Practolol
	Propylthiouracil
	Quinidine
	Reserpine
	Streptomycin
	Sulfonamides
	Tetracycline
	Tolazamide
	Oxphenistan

BIBLIOGRAPHY:

- Feely RH: Systemic Lupus Erythematosus: A review. Rheum Rehab 17:79-82, 1978.
- Burnham TK: Antinuclear antibodies II. The prognostic significance of nuclear immunofluorescent patterns in Lupus Erythematosus. Arch Dermatol 11:203-7, 1975.
- Greenwald CA, Peebles CL and Nakamura RM: Laboratory tests for antinuclear antibody (ANA) in rheumatic disease. Lab Med 9:19-27, 1978.
- Nisengard RJ: Antinuclear antibodies: Significance of titers. In: Immunology of the Skin, by EH Beutne. TP Chorzelski and SE Bean (Eds), 2nd Edition, John Wiley and Sons, P. 387-98, 1979.
- Barnett EV: Immunofluorescence tests in immune technics and applications. Am J Clin Path 68:662-3, 1977.
- Lowenstein MB and Rothfield NF: Family study of Systemic Lupus Erythematosus. Arth Rheum 20:1293-1303, 1977.
- Cavallaro JJ, Palmer DF and Bigazzi PF: Immunofluorescence detect autoimmune diseases. Immunology Series No. USDHEWPHSCDC, 1977.
- Fritzner MR and Tan EM: Antibodies to histones in drug-induced and idiopathic Lupus Erythematosus. J Clin Invest 21:560, 1978.
- Schur PH: Complement in Lupus. In: Clinics of Rheumatic Diseases. Philadelphia, WB Saunders, 1978.
- Gladman DO, Urowitz MD and Keystone EC: Serologically active clinically quiescent Systemic Lupus Erythematosus. Am J Med 66:210-5, 1979.
- Baker SB, Rorura JR, Campion EW and Mills JA: Late onset Lupus Erythematosus. Amer J Med 66:727-32, 1978.
- Permin H and Wilik A: The prevalence of IgE antinuclear antibodies in the rheumatoid arthritis and Systemic Lupus Erythematosus. Acta Pathol Microbiol Scan Ser C 86:245-9, 1978.

Antinuclear Antibody Test System

For In - Vitro Diagnostic Use

CATALOG NO.:

- 1048L 48 Tests
- 1096L 96 Tests
- 10144L 144 Tests

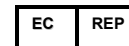


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Version D; 1048L 10/23/06