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. 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). Hat 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) 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 fluorescein 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.
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. 1611 - 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 Hl&l/l0 ELISA (Cat# 1500)
Evans Blue Counterstain (Cat# 1600)
Moisture Chamber (Cat# 1716)

Note: All kit components are available separately. Please see the current SCIMEX 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. 1611. 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. Place slide in moisture chamber for 30 minutes at room temperature
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 moisture chamber for 30 minutes at room temperature (approximately 19° - 24°C).
4. Place 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 remove the fluorescence. A negative ANA test result will result in no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
5. Wash in PBS for five minutes. Repeat using fresh PBS.
6. Place a blotted on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue faces absorbent side of blotter. Line up well, blotter held. Place slide on top of blower. 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 positive to 3+ and negative to 0+ fluorescence, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the conjugate 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.

Storage & Stability of Components:
Evans Blue Counterstain (Cat# 1600)

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

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 positive to 3+ and negative to 0+ fluorescence, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the conjugate 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 by high dry objectives. The positive control (Cat.# 1202) demonstrates a homogenous 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 nuclei 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. Rheumatoid involvement, confirmed by anti-citrullinated peptide antibodies (SCLMED 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.

3. Various autoimmune processes induce positive ANA tests.

4. Further evidence for a diagnosis of SLE is provided by low complement levels, particularly C1, C3, and C4.

5. ANA tests may not agree with LE Prep tests or with latex tests.

6. Presence of antibodies to double stranded native DNA is diagnostic for SLE.

7. Management of therapy should be based not only on positive serologic tests for SLE, but should include the presence of active clinical disease.

8. Elderly patients with SLE have a better prognosis and their clinical symptoms differ substantially from those seen in younger patients.

9. Although the predominant class of antinuclear antibodies (ANA) is Immunoglobulin G, the presence of Immunoglobulin E may be of pathogenic importance in SLE.

**Table I**

**INCIDENCE OF ANA IN VARIOUS DISORDERS**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
<td>95-100</td>
</tr>
<tr>
<td>Lupoid hepatitis</td>
<td>95-100</td>
</tr>
<tr>
<td>Progressive systemic sclerosis</td>
<td>75-90</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>25-50</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>75-90</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>10-30</td>
</tr>
<tr>
<td>Polyarteritis Nodosa</td>
<td>15-25</td>
</tr>
<tr>
<td>Feilty's syndrome</td>
<td>95-100</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>45-75</td>
</tr>
<tr>
<td>Chronic dissectus lupus</td>
<td>15-50</td>
</tr>
</tbody>
</table>

**Table II**

**SLE INDUCING DRUG**

<table>
<thead>
<tr>
<th>GROUP I Induced by Pharmacological Action</th>
<th>GROUP II Induced by Allergic Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydralazine</td>
<td>Practolol</td>
</tr>
<tr>
<td>Procainamide</td>
<td>Pyrogallol</td>
</tr>
<tr>
<td>Anticonvulsant: D-penicillamine</td>
<td>Quinidine</td>
</tr>
<tr>
<td>Methysergide</td>
<td>Reserpine</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Antihistamines</td>
</tr>
<tr>
<td>Primidone</td>
<td>Anti-convulsant: D-penicillamine</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>Quinidine</td>
</tr>
<tr>
<td>Convulsinapine</td>
<td>Steroids</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Tolazamide</td>
</tr>
<tr>
<td>Valproate</td>
<td>Methysergide</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Chlorpromazine</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>Phenylbutazone</td>
</tr>
</tbody>
</table>

**BIBLIOGRAPHY**