homogeneous fluorescence of the condensed chromosomal region may be autocrine or drug induced (see Table I). Drug induced lupus is differentiated from classic lupus by the absence of anti-DNA antibodies and the presence of anti-histone antibodies.

**TABLE I**

**SLE INDUCING DRUGS:**

<table>
<thead>
<tr>
<th>Group I: Induced by Pharmacological Action</th>
<th>Group II: Induced by Allergic Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyzine</td>
<td>Aminosalicly Acid</td>
</tr>
<tr>
<td>Procainamide</td>
<td>Chlorothalidone</td>
</tr>
<tr>
<td>Anti-convulsants:</td>
<td>D-Penicillamine</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Griseofulvin</td>
</tr>
<tr>
<td>Trimethadione</td>
<td>Levodopa</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>Methyldopa</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Methysulfonamide</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>Methtylphenothiazine</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Oral Contraceptives</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Oxazepam</td>
</tr>
</tbody>
</table>

The detection of positive antinuclear antibodies depends in part on the antigenic substrate and the fixation method used in the preparation of the substrate. ANA testing can utilize many substrate sources such as liver or kidney tissue sections which can be derived from rats or mice. The use of human tissue culture cells (specifically HEp-2) has provided an alternative substrate to tissue sections.

HEP-2 substrate allows for differentiation of pattern recognition on cells in all phases of mitosis which is not necessarily possible using tissue sections as substrate. The high level of mitotic cells in tissue culture HEp-2 cells allows for the definitive differentiation of "centromere" antigen staining of the condensed chromosomal region of metaphasic cells. Centromere positive sera are generally reported as "speckled" staining patterns when tissue sections are used.

The high level of mitotic cells found in HEP-2 cells also aids in the determination of mixed homogeneous and speckled antigenic patterns. Most speckled patterns stain the nucleolus surrounding the mitotic cell but do not stain the condensed chromosomal region of the metaphasic HEP-2 cells. Homogenous patterns will stain the condensed chromosomal region with an intense homogeneous or peripheral stain. Therefore, staining in the same mitotic HEP-2 cell of both homogenous condensed chromatin of metaphasic cells and a speckled surrounding nucleolus of metaphasic cells indicates the presence of two types of nuclear specificities in the same sample. Mixed homogeneous/speckled specificities using tissue sections are generally observed upon titration of the sample which reveals patterns present in conjuction with the homogeneous pattern.

SS-A antibodies are specifically fixed in the HEP-2 substrate preparation to assure its reproducible abundance in testing for this clinically important antigenic determinant. Tissue sections may vary in the amount of SS-A present from manufacturer to manufacturer. Anti-SS-A is found in neonatal lupus syndrome, Sjogren's Syndrome and SLE. Anti-SS-A antibodies bind to skin and heart tissue. Study of expectant mothers with rheumatic disease symptoms for the presence of anti-SS-A is recommended to prevent untoward neonatal death.

Postpartum clinical monitoring of mothers of children with heart block and rashes for the presence of heart tissue. Study of expectant mothers with rheumatic disease symptoms for the presence of anti-SS-A may be beneficial to prevent untoward neonatal death.

**TABLE II**

**CLASSIFICATION OF CLINICALLY SIGNIFICANT SINGLE PATTERN ANA REACTIONS USING HEP-2 CELLS:**

1. **HOMOGENEOUS PATTERN:**
   - Fluorescence is uniform and diffuse in the nucleus of the interphasic cells.
   - Metaphasic cells demonstrate homogeneous fluorescence of the condensed chromosomal region.
   - Specificity: The homogeneous pattern is obtained with antibodies to DNA, DNP, and histone.

2. **PERIPHERAL PATTERN:**
   - Fluorescence is uniform and diffuse with staining of a greater intensity at the outer region of the nucleus.
   - Metaphasic cells demonstrate strong staining of the condensed chromosomal submembranual region.
   - Specificity: The peripheral pattern is obtained with antibodies to DNA and histone.

3. **COARSE SPECKLED WITHOUT NUCLEOLUS PATTERN:**
   - Fluorescence is uniform with coarse specks in the nuclear matrix with no nuclear staining.
   - Metaphasic cells demonstrate no staining of the condensed chromosomal region.
   - Specificity: The coarse speckled pattern without nucleolar staining is obtained with anti-SM and anti-RNP antibodies.

4. **COARSE SPECKLED WITH OCCASIONAL NUCLEOLUS PATTERN:**
   - Fluorescence is uniform with coarse flat specks in the nuclear matrix with occasional nuclear staining.
   - Metaphasic cells demonstrate no staining of the condensed chromosomal region.
   - Specificity: The coarse speckled pattern with occasional nuclear staining is obtained with anti-SS-B antibodies.

5. **FINE SPECKLED WITHOUT NUCLEOLUS PATTERN:**
   - Fluorescence is uniform with fine specks in the nuclear matrix without nuclear staining.
   - Metaphasic cells demonstrate no staining of the condensed chromosomal region.
   - Specificity: The fine speckled pattern without nucleolar staining can be found with several antibodies including SS-A, Ms-1, M2, and SLE.

6. **FINE SPECKLED WITH NUCLEOLUS PATTERN:**
   - Fluorescence is uniform with fine specks in the nuclear matrix with nucleolar staining.
   - Metaphasic cells demonstrate staining of the condensed chromosomal region.
   - Specificity: The fine speckled with nucleolar pattern is demonstrated by antibodies to Sm-70. This pattern is considered a marker antibody specific for protein kinases (70K) and is clinically diagnostic of scleroderma.

7. **DIFFUSE SPECKLED (CENTROMERE) WITHOUT NUCLEOLUS PATTERN:**
   - Fluorescence is uniform with spherical specks approximately 40-60 per cell nucleus.
   - Metaphasic cells demonstrate intense speckled staining of the condensed chromosomal region corresponding to the centromere region.
   - Telophase cells do not demonstrate speckles.
   - Specificity: Diffuse speckled with centromeric staining of metaphasic cells is demonstrated by antibodies to the kinetocore antigens of the centromere. This pattern is considered a marker antibody and is diagnostically specific for the CREST syndrome.

8. **ATYPICAL DISCRETE SPECKLED (PSEUDOCENTROMERE, NUCLEAR DOTS):**
   - Occasional speckled fluorescence of less than 10 bright dots in the nuclear of interphasic cells only.
   - Metaphasic cells demonstrate no speckling of condensed chromosomal region.
   - Specificity: The chemical nature of the pseudocentromeric pattern is not yet known. The antibody has been defined as an anti Nsp-1. This antibody is associated with PBC and Chronic Hepatic Disease.

9. **PLEOMORPHIC SPECKLED OF VARIOUS INTENSITIES:**
   - Varying fluorescence of fine to coarse speckling in approximately 30-60% of the cells.
   - Both the number of cells staining and the intensity of the speckles vary.
   - Metaphasic cells may demonstrate positive or negative staining of the condensed chromosomal regions.
   - Specificity: The antibody responsible for pleomorphic speckled fluorescence is proliferating cell nuclear antibody (PCNA) and is active against cyclin.

10. **NUCLEOLAR ONLY:**
    - The staining of the nucleolus may be either homogeneous, clumpy, speckled or perinuclear. Metaphasic cells generally do not show staining of the condensed chromosomal regions.
    - Positive staining can be seen in the nucleolus of cells at the end of anaphase in telophase.
    - Specificity: The antibodies responsible for staining of the nucleolus include: Anti-4-6 RNA, Anti-RNA and RNP, and Anti-DNA Nuclear Organizer.
The primary reaction occurs during the first incubation period while the patient's serum covers the substrate.

The secondary reaction follows a PBS rinsing to remove any unbound human antibody. The reagent used in the secondary reaction is a fluorescein labeled anti-human conjugate which has been affinity purified for use with HeP-2 cell culture substrates and has been adjusted for the optimum use dilution and is free of most non-specific staining of the HeP-2 cell.

After a second PBS rinse to remove any unbound anti-human globulin conjugate, the specific type of nuclear fluorescence observed and its fluorescence intensity is reported visually using a fluorescence microscope at a magnification of 400X. (See Table II).

The phosphate buffer saline and mounting medium found in this kit are irritating to the eyes, respiratory system and skin.

Some components in this kit contain 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitisation by skin contact.

The conjugate and controls in this kit contain sodium azide at a concentration of less than 0.1 %. Sodium azide is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.

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.

All reagents must be brought to 20 to 25°C before performing the test procedure.

Once the procedure has been started do not allow antigens in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

Xi - Irritant
R34: May cause sensitization by skin contact
S23-37: After contact with skin, wash immediately with plenty of water and soap. Wear suitable gloves

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
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<tbody>
<tr>
<td>Description</td>
</tr>
<tr>
<td>10-1012</td>
</tr>
<tr>
<td>10-1202</td>
</tr>
<tr>
<td>10-1206</td>
</tr>
<tr>
<td>10-1201</td>
</tr>
<tr>
<td>10-1513</td>
</tr>
<tr>
<td>90-1612</td>
</tr>
<tr>
<td>90-1607</td>
</tr>
<tr>
<td>90-1700</td>
</tr>
<tr>
<td>90-1712</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test tubes, test tube rack, pipettes.</td>
</tr>
<tr>
<td>Volumetric flask (1 liter)</td>
</tr>
<tr>
<td>Staining dish.</td>
</tr>
<tr>
<td>Epifluorescence microscope</td>
</tr>
<tr>
<td>Microscope Slide Roller</td>
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<tr>
<td>Humid Chamber</td>
</tr>
</tbody>
</table>

**STORAGE AND STABILITY**

1. Antigen slides Prod# 10-1012 should be stored at +2 to +8°C. Slides are stable until their expiration date on the product label.

2. Positive control Prod# 10-1202 and Prod# 10-1206 should be stored at +2 to +8°C. Refer to expiration date on label.

3. Negative control Prod# 10-1201 should be stored at +2 to +8°C. Refer to expiration date on label.

4. FITC labeled anti-human conjugate Prod# 10-1513 should be stored at +2 to +8°C. Refer to expiration date on label.

5. Mounting Medium Prod# 90-1612 should be stored at +2 to +8°C. Refer to the expiration date on label.

6. Phosphate buffered saline pH 7.5 Prod# 90-1607 are stable at room temperature. Reconstitute each vial of PBS buffer salts with 1.0L of distilled water. The PBS contains no preservative and should be stored at +2 to +8°C. Discard if turbidity develops.
**TEST PROCEDURE**

Dilute test sera 1:40 in PBS if testing is being performed for screening purposes. For titrations set up doubling dilutions of serum starting at 1:40, i.e. 1:40, 1:160, 1:320, etc.) The slide, controls, and conjugate are ready to use.

1. Tear 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 (15 to 20 µl) over the antigen wells.
3. Place slide with serum and controls in a moist chamber for 30 minutes at room temperature. (approximately 20°C)
4. Remove slide from moisture chamber. Using a wash bottle, gently rinse remaining sera from slides being careful not to aim the stream directly on the well.
5. Wash in PBS for two separate five minute changes.
6. Remove the slides from PBS and place slide antigen side facing up on a dry paper towel. Carefully place the blotter over the slide so that the blotter is indexed to the surface of the microscope slide. Hold one edge of the blotter with one hand to keep the blotter in place and apply sufficient gentle pressure with the microscope slide roller to remove the moisture between the antigen wells. DO NOT ALLOW THE ANTIGEN WELLS TO DRY.
7. Using dispenser provided, deliver 1 drop (25 µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a humid chamber placed in a dark refrigerator.

* The conjugate dispenser is provided with a calibrated tip and allows quantitative delivery of reagents from the storage battle. To use, wipe the tip with a paper towel, invert the bottle and squeeze gently to release one drop. If the tip contains an air bubble, tap the bottle gently to remove air bubble which will ensure precise drop delivery.

**SPECIMEN COLLECTION AND STORAGE**

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°C to 8°C if it is to be analyzed within 4-7 days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lysic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, additions of a preservative such as 0.01% thimerosal (merthiolate) or 0.1% sodium azide is strongly recommended. The CLSI provides recommendations for storing blood specimens (Approved Standard Procedure for the Handling and Processing of Blood Specimens, H15-A2 2005).

**TITER INTERPRETATION**

The titer is the highest dilution of patient's serum showing weak (1+) fluorescence. Less than 1:40 Normal; virtually rules out active SLE provided patient is not on immunosuppressive therapy or in remission.

1:40-1:80 Positive; test often in RA and other connective tissue diseases. A fresh sample should be tested in two weeks. If the titer increases active SLE is suggested. No change in titer indicates other possible autoimmune disease in a static condition or a treated controlled SLE patient. A decrease in titer indicates an SLE case in remission or a treated controlled SLE case or another autoimmune process.

1:160 or greater Strongly suggests SLE although other autoimmune diseases and drugs may induce these high titer.

**PATTERN INTERPRETATION**

ANA patterns are generally reported as: Homogeneous, Peripheral, Speckled, Centromere, Nuclear and in multiple combinations. The nuclear ANA patterns found in IFA may be of diagnostic and/or prognostic significance. (SEE TABLE II & TABLE III).

**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 sex and age. There is an increasing incidence in positive ANA results in both males females as age increases (10). Normal females between 20-60 have 7% incidence of ANA; normal males, a 3% incidence. Normal males and females over 80 years of age have a 50% incidence of ANA.
3. Various medications including antibiotics, tranquilizers, aspirin and birth control pills can induce a Lupus-like condition resulting in high ANA titers (11). (See table I) Drug-induced Lupus generally goes into sustained clinical remission following removal of the triggering medication.
4. Various autoimmune processes induce positive ANA tests.
5. Further evidence for a diagnosis of SLE is provided by low complement levels, particularly C3 and C4 (1).
6. ANA tests may not agree with LE Prep tests or with latex tests.
7. Management of therapy should be based not only on positive serologic tests for SLE, but should include the presence of active clinical disease. (13)
8. Early patients with SLE have a better prognosis and their clinical symptoms differ substantially from those seen in younger patients. (14)
9. Although the predominant class of antinuclear antibodies (ANA) is immunoglobulin G, the presence of immunoglobulin M is suggestive of SLE (15).
10. Staining patterns often change with titration of the sera revealing multiple patterns not seen in the lower dilutions.
11. Identification of antibodies based only upon patterns could be misleading and should be confirmed using other serologic tests such as DNA double gel diffusion tests, specific nDNA tests and histone tests.

**QUALITY CONTROL**

1. Positive 4+, 1+ 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, 1:160 or greater should be included to ensure that no fluorescence of the nuclei of this control shows bright fluorescence either the control or the antigen may be at fault.
3. The positive 4+ serum control should result in 3+ to 4+ fluorescence of the type specified on the label. If this control shows little or no fluorescence either the control, antigen, conjugate or technique may be at fault.
4. The positive 1+ serum control should result in 1+ fluorescence of the type specified on the label. If this control shows little or no fluorescence either the control, antigen, conjugate or technique may be at fault.
5. 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 the antigen may be at fault.

**REFERENCES**

Consult Instructions for Use

Product Number
Lot Number
In Vitro Diagnostic Medical Device

Authorized Representative in the European Community

Use By

Caution, consult accompanying documents

Temperature Limitation

Manufacturer

Irritant - Precaution

Negative Control

ANA 4+ Homogeneous Positive Control

ANA 1+ Homogeneous Positive Control

Conjugate

Phosphate Buffered Saline

Mounting Medium

Coverslips

Blotters, 12 Well

Slide, 12 Well

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Fax: 760-920-0124
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