INTENDED USE
The Diagnostic Automation, Inc. ANA/HEp-2 Test System is a prestandardized kit designed for the qualitative and semi-quantitative detection of antinuclear antibodies. The test is intended to aid 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 clinically mimic the spontaneous form of SLE. 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 now recognized that many sources of nuclear material may be employed as a substrate for ANA testing. Although most of the original ANA research was performed using rat or mouse liver or kidney tissue section substrate, the use of human or animal embryonic tissue cell culture substrates has provided a reliable and easy to interpret alternative substrate for ANA testing. The HEp-2 cell line is a recommended substrate for detecting centromere
antibody, which is highly indicative of the CREST variant of progressive systemic immunofluorescence. These various patterns and the basis for them are as follow:

**Homogeneous** – Homogeneous or diffuse staining patterns of the nucleus is consistent with autoantibodies to native DNA (nDNA) histones and/or deoxyribonucleoprotein (DNP) (12, 13). The chromosomes of the mitotic cells (dividing cells) are important indicators of a homogenous pattern because they will stain as irregularly shaped masses with marc Intensely stained outer.

**Speckled Patterns** – The speckled patterns is the most commonly observed ANA pattern. A uniform ‘true speckled’ pattern may be seen with centromere antibodies in cells not in division. A clumpy speckled patterns may be seen with antibodies to n-RNP, Sm, and SSB/La.

1. **Fine speckled pattern, chromosome-negative:**
   Numerous small uniform points of fluorescence uniformly scattered throughout the nucleus. The nucleoli will generally appear unstained. The mitotic cells may demonstrate a few speckles in their cytoplasm, but the chromosomes will be negative.

2. **Course speckled pattern, chromosome-negative:**
   Medium-sized points of fluorescence will be scattered throughout the nuclei with distinct nuclear margins. Larger-sized points of fluorescence may also be observed; however, they are too numerous and variable in size to be identified as a nucleolar pattern. The chromosomes in the mitotic cell will be negative.

3. **Discrete speckled, chromosome (centromere specificity) positive:**
   The chromosomes will be positive in mitotic cells; in fact, the discrete speckles will only be clustered in the chromosome mass clearly demonstrating the various stages of mitosis. The centromere pattern has been recognized to be associated with the CREST syndrome, which is a milder variant of progressive systemic sclerosis (PSS).

   The centromere pattern will demonstrate discrete and uniform points of fluorescent speckles scattered throughout the nucleus. Mitotic cells will be positive, demonstrating a clustering of the centromeres in the chromosomes in different arrangements according to the mitotic stage. Harmon and co-workers (7) demonstrated that serum samples containing highly monospecific anti-SSA/Ro gave an IF-ANA test pattern of discrete nuclear speckles on a wide variety of human cells and tumor nuclei. Such serum samples with monospecific anti-SSA/Ro produced very little cytoplasmic staining of substrate cells.

   A distinct, large, variable speckled pattern of 3 to 10 large speckles in the nucleus has been described. These patients with large, variable speckles have undifferentiated rheumatic disease syndromes with IgM antihistone H-3 antibody (18).

**Nucleolar Pattern** – The nucleolar pattern demonstrates a homogeneous speckled staining of the nucleolus. This pattern is often associated with a dull, homogeneous fluorescence in the rest of the nucleus. The chromosomes in the mitotic cells will be negative. The nucleolar pattern suggests autoantibodies to 4-6S RNA. The nucleolar fluorescence will appear as homogeneous, clumped, or speckled, depending on the antigen to which the autoantibody reacts.

   Antinucleolar antibodies occur primarily in the sera of patients with scleroderma, systemic lupus erythematosus, Sjögren’s syndrome, or Raynaud’s phenomenon (19).

**Peripheral (Rim)** – The nuclei stain predominantly at their periphery. The chromosomes of the mitotic cells stain as irregularly shaped masses with more intensely stained outer edges. This pattern is often seen with autoantibodies to nDNA (3, 14-16). If the chromosomes of the mitotic cells are negative, then the pattern would be suggestive of autoantibodies to the nuclear membrane and not to nDNA, and not reported as a peripheral pattern. (See nuclear membrane interpretation below).

**ADDITIONAL PATTERNS**

1. **Spindle fiber pattern, chromosome-positive:**
   The spindle fiber pattern is unique to cells undergoing mitosis where only the spindle apparatus fluoresces. This pattern has “spider web” appearance extending from the centriole to the centromeres. The pattern is suggestive of autoantibodies to the microtubules and its significance is unclear; however, an association
between the spindle fiber pattern and carpal tunnel syndrome has been suggested.

2. **Midbody pattern:**
The midbody pattern is a densely staining region near the cleavage furrow of telophase cells, that is, in the area where the two daughter cells separate. The clinical significance of the pattern is unknown; however, the pattern has been recognized in selected patients with systemic sclerosis.

3. **Centriole pattern:**
The centriole pattern is characterized by two distinct points of fluorescence in the nucleus of the mitotic cells or one distinct point of fluorescence in the resting cell. The significance of this pattern in not known; however, it has been observed in PSs.

4. **Proliferating cell nuclear antigen (PCNA) pattern:**
The proliferating cell nuclear antigen pattern is observed as a fine to coarse nuclear speckling in 30-60% of the cells in interphase, and a negative staining of the chromosome region of mitotic cells. The PCNA is very specific for patients with SLE but not detected in other connective tissue disease disorders. It has been reported that SLE patients with the PCNA pattern have a higher incidence of diffuse glomerulonephritis.

5. **Antinuclear membrane (nuclear laminae):**
The antinuclear membrane pattern appears as a rim around the nucleus and resembles a rim pattern; however, it is distinguished from the rim pattern by the fact that the metaphase chromosome stage is negative. This autoantibody is important to report because it was recently recognized to be associated with autoimmune liver disease.

**CYTOPLASMIC PATTERNS**

1. **Mitochondrial (AMA) pattern:**
The pattern will characteristically have numerous cytoplasmic speckles with the highest concentration in the peri-nuclear area. The pattern can be observed in interphase and mitotic cells. The clinical significance of AMA is most frequently an association with primary biliary cirrhosis, especially when the AMA is a high titer.

2. **Golgi apparatus pattern:**
The golgi apparatus pattern is characterized by positive cytoplasmic staining that is concentrated on only one side of the perinuclear region. The clinical significance is uncertain, but reports in the literature have suggested an association with SLE and Sjögren’s Syndrome.

3. **Lysosomal pattern:**
The lysosomal pattern is observed as a few discrete speckles sparsely spaced throughout the cytoplasm. The pattern is observed in the cytoplasm of interphase and mitotic cells. The clinical significance is unknown.

4. **Ribosomal pattern:**
The ribosomal pattern is characterized by numerous cytoplasmic speckles with the highest concentration around the nucleus. It is distinguished from the mitochondrial pattern because of the smaller specks and higher density. The significance of the pattern is unknown.

5. **Cytoskeletal pattern:**
The cytoskeletal pattern is characterized by a distinct “spider web” or fibrous appearance throughout the cell. It has been reported to be associated with autoimmune liver disease (anti-smooth muscle).

**ANA Negative**
Autoantibody to SSA/Ro is present in high frequency in a clinical subset of lupus called subacute cutaneous lupus erythematosus (SCLE). Many patients with SCLE have been falsely labeled as having “ANA-negative” lupus. We now know that many of these so-called “ANA-negative” LE patients will demonstrate a positive IF-ANA on substrate of HEp-2 cells containing the SS/Ro antigen (20).

Anti-SSA/Ro antibodies may be present in the absence of traditional ANAs, with SLE seen in persons genetically deficient in C4 and occasionally other complement components (21, 22). This combination may be more common in black persons (23, 24). In addition, C4 deficiency may be associated with increased
susceptibility to development of SLE upon treatment with hydralazine (25). These patients, if female, are likely to deliver infants with congenital heart block or lupus dermatitis (26).

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, 16, 28-31).

The following table summarizes the various autoantibodies noted above with respect to disease association:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Disease State</th>
<th>Relative Frequency of antibody Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Jo-1</td>
<td>Myositis</td>
<td>25-44% (18)</td>
</tr>
<tr>
<td>Anti-SM</td>
<td>SLE</td>
<td>30*</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>MCTD, SLE</td>
<td>100** and &gt; 40, respectively</td>
</tr>
<tr>
<td>Anti-SSA/Ro</td>
<td>SLE, Sjögren’s</td>
<td>15 and 30-40, respectively</td>
</tr>
<tr>
<td>Anti-SSBLa</td>
<td>SLE, Sjögren’s</td>
<td>15 and 60-70, respectively</td>
</tr>
<tr>
<td>Anti-Scl-70</td>
<td>Systemic sclerosis</td>
<td>20-28*</td>
</tr>
</tbody>
</table>

* Highly Specific
** Highly Specific when present alone at high titer

**PRINCIPLE OF THE ASSAY**

The Diagnostic Automation, Inc. ANA/HEp-2 Test System is a prestandardized kit designed to detect the presence of circulating ANA in human sera. The system employs tissue cell culture substrate and goat anti-human immunoglobulin adjusted for optimum use and free of nonspecific background staining. The reaction occurs in two steps:

1. The first is the interaction of ANA in patient sera with the cell culture substrate.
2. The second is the interaction of FITC labeled anti-human immunoglobulin with antibodies attached to the cell substrate in step one.

**SPECIMEN COLLECTION**

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. 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.
3. Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (32,33). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10°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.

**EQUIPMENT AND MATERIALS**

**MATERIALS REQUIRED BUT NOT PROVIDED:**

1. Small serological, Pasteur, capillary or automatic pipettes.
2. Small test tubes, 13 x 100mm or comparable.
3. Test tube racks.
4. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
5. Cover slips, 24 x 60mm, thickness No. 1 for 5-well and 12-well test system. (NOTE: Cover slips for the 16- and 96-well test systems are provided with the kit).
6. Moist chamber.
7. Distilled water.
8. Pipette (fixed or variable) capable of delivering 5mL when using the 96-well or 16-well test system.
9. Adjustable multi-channel pipettor capable of delivering 5, 10, 15, and 195 ml when using the 96-well or 16-well test system.
10. Disposable reagent reservoirs (2) for PBS and conjugate when using the 96-well or 16-well test system.
11. Microtiter sample dilution plate for preparing sample dilutions when using the 96-well or 16-well test system.
12. Microscope stage capable of handling a 3-1/2” x 5” glass slide when using the 96-well test system.
13. Properly equipped fluorescence microscope assembly.

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

<table>
<thead>
<tr>
<th>TRANSMITTED LIGHT</th>
<th>Light Source: Mercury vapor 200W or 50W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Filter</td>
<td>Barrier Filter</td>
</tr>
<tr>
<td>KP490</td>
<td>K510 or K530</td>
</tr>
<tr>
<td>BG12</td>
<td>K510 or K530</td>
</tr>
<tr>
<td>FITC</td>
<td>K520</td>
</tr>
<tr>
<td>Light Source: Tungsten – Halogen 100W</td>
<td></td>
</tr>
<tr>
<td>KP490</td>
<td>K510 or K530</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INCIDENT LIGHT</th>
<th>Light Source: Mercury Vapor 200, 100, 50 W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Filter</td>
<td>Dichroic Mirror</td>
</tr>
<tr>
<td>KP500</td>
<td>TK510</td>
</tr>
<tr>
<td>FITC</td>
<td>TK510</td>
</tr>
<tr>
<td>Light Source: Tungsten – Halogen 50 and 100 W</td>
<td></td>
</tr>
<tr>
<td>KP500</td>
<td>TK510</td>
</tr>
<tr>
<td>FITC</td>
<td>TK510</td>
</tr>
</tbody>
</table>

**MATERIALS PROVIDED**

**Reactive Reagents:**
1. Goat anti-human immunoglobulin (polyvalent) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer in 1.25% bovine albumin and counter stain. 2.5mL (Product #:230512)*, or 15 mL, for use with test system 231005 (Product #:230512)*. **NOTE:** When using test system 231005, conjugate (Product #: 230512 * DOES NOT contain counterstain.
2. Human ANA-positive control serum, 0.7mL. Consists of human serum producing 4+ homogeneous nuclear staining with the substrate cells (Product #: 230512)*.
3. Human ANA-negative control serum, 0.7mL. Composed of normal human serum with no nuclear staining as determined by the IFA procedure (Product #:& 231005)*.
4. Evans blue counterstain, 3.0mL (Product #:230512)*. For use with test system 231005 ONLY.
5. ANA HEp-2 cell culture substrate slides (Product #:230512, containing 10 wells plus 2 control wells); (Product #:231005, 12 wells); (Product #:230512, 96-wells); (Product #:231005, 5-wells); or (Product #:230512 16-wells).
6. Sample diluent formulated to reduce nonspecific staining. (Product #:231005, 25mL) or (Product #:230512, 125mL). Contains 0.1% sodium azide as a preservative.

**Non-reactive Reagents:**
1. Phosphate-buffered-saline (PBS), pH 7.2 ± 0.2. (Product #:230512).
2. Phosphate buffered glycerol (mounting media), 3mL. (Product #:231005)*.
3. Cover Slips (Product #: 230512) for use with 16-well kit, or (Product #:231005) for use with 96-well kit.
4. Sample Dilution Plate for preparing sample dilutions (Product #: 230512), for use with the 96-well kit.
5. Blotters.

*NOTE: These reactive reagents contain preservative: thimerosal, mercury derivative 0.04%.

**STORAGE CONDITIONS**
1. Tissue cell culture substrate slides: Store at -20ºC to 8ºC.
2. Goat anti-human immunoglobulin labeled with FITC: Store at 2-8ºC.
3. Human ANA positive and negative control sera: Store at 2-8ºC.
4. Sample diluent Store at 2-8ºC.
5. Phosphate-buffered-saline (PBS): Store packets at 2-25ºC. Rehydrated PBS is stable for 30 days when stored at 2-8ºC.
6. Phosphate buffered glycerol (mounting media): Store at 2-8ºC.

**NOTE:**
1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. DO NOT use beyond expiration date.
2. Do not freeze and thaw reagents or patient samples more than once. Repeated freezing and thawing destroys antibody activity. Do not store in self-defrosting freezers.

**QUALITY CONTROL**
1. Every time the assay is run, a positive control (homogeneous), a negative control, and a buffer must be included.
2. It is recommended that positive and negative controls be read prior to evaluating the test samples. If the controls do not appear as described, results are invalid.
   a. Negative Control - Characterized by the absence of specific fluorescence and a red background staining of all cells due to counterstain.
   b. The homogeneous positive control is 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.

**NOTE:**
1. Non-specific reagent trapping may exist. It is important to adequately wash slides to eliminate false positive results.
2. The intensity of the observed fluorescence may vary with the microscope and filter system used. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

**PROCEDURE – STEPWISE**
**Preparation of Reagents:**
1. Phosphate-buffered-saline (PBS). Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. Human ANA positive control serum. Use as packaged. Do not dilute other than to make the 1+ dilution.
3. Human ANA negative control serum. Use as packaged. Do not dilute other than to make the 1+ dilution.

**Note:** The controls are intended to be used undiluted. As an option, users may titrate the positive control(s) to endpoint. In such cases, the control(s) should be diluted two-fold in PBS. When evaluated by DAI, 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 mean titer for each lot of control.

**TEST PROCEDURE**
1. Remove slides from storage and allow them to warm to room temperature (20-25ºC). Tear open the
protective envelope and remove slides containing the HEp-2 cell culture substrate. DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.

2. Prepare patient sera at 1:40 dilution in PBS (for example: 10µL of sample plus 390µL of PBS). (Alternatively, you may prepare 1:40 screening dilution in sample diluent). If additional serial dilutions are to be tested, prepare subsequent dilutions in reconstituted PBS.

3. Identify each well with the appropriate patient sera and controls.

4. If test samples are to be titered, serial dilutions should be made in reconstituted PBS. DO NOT MAKE SERIAL DILUTIONS IN SAMPLE DILUENT.
   a. 5-WELL, OR 12-WELL TEST SYSTEM:
      Using suitable dispenser (capillary, Pasteur, or automatic pipette), dispense one drop or approximately 0.020 mL of patient sera and control sera on the cells in the respective wells. Spread sera over entire area of the wells being careful not to touch substrate with pipette tip.
   b. 16-WELL, OR 96-WELL TEST SYSTEM:
      After adding patient sample to PBS or sample diluent in microtiter dilution plate, mix the sample well by withdrawing and expelling the specimen several times. Transfer 0.020mL of the diluted specimen to the appropriate wells of the substrate slide. This step can be accomplished using single channel or multichannel pipetting equipment. Use a new pipette tip for each specimen.

5. Incubate slides in a moist chamber at room temperature (20-25ºC) for 30 minutes.*
   * The incubation time in step #6 may be reduced to as little as 20 minutes with no loss of sensitivity or specificity.

6. Remove slides from the moist chamber one at a time and gently rinse with a stream of PBS. DO NOT DIRECT THE STREAM OF PBS INTO THE TEST WELLS. NOTE: To avoid cross-contamination when using the 96-well test system, place slide in palm of hand and grasp edges with fingertips. Quickly invert the slide and, using a “snap” motion, expel excess sera.

7. Place slides in a staining dish and wash in PBS for two, 5 minute intervals with one change of PBS. Use a magnetic mixing setup or other means of gentle agitation.

8. 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 substrate. DO NOT ALLOW THE SLIDES TO DRY DURING THE TEST PROCEDURE.
   a. 5-WELL, OR 12-WELL TEST SYSTEM:
      Add one drop (approximately 0.020 ml) of conjugate to each well.
   b. 16-WELL TEST SYSTEM:
      Transfer one drop (approximately 0.020 ml) of conjugate to each substrate well. When using multichannel pipetting equipment, the conjugate should be transferred to a reagent reservoir prior to adding to the substrate wells.

The following table may be helpful in determining the amount of conjugate required:

<table>
<thead>
<tr>
<th>No. of Slides</th>
<th>No. of Wells</th>
<th>Quantity Required*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>0.24mL</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>0.48mL</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>0.72mL</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>0.96mL</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>1.20mL</td>
</tr>
<tr>
<td>6</td>
<td>96</td>
<td>1.44mL</td>
</tr>
</tbody>
</table>

c. 96-WELL TEST SYSTEM:
   Transfer one drop (approximately 0.020 ml) of conjugate to each substrate well. When using multichannel pipetting equipment, the conjugate should be transferred to a reagent reservoir prior to adding to the substrate wells. The following tables may be helpful in determining the amount of conjugate required:
<table>
<thead>
<tr>
<th>8 CHANNEL PIPETTOR</th>
<th>12 CHANNEL PIPETTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Wells</td>
<td>Quantity Required</td>
</tr>
<tr>
<td>8</td>
<td>0.12mL</td>
</tr>
<tr>
<td>16</td>
<td>0.24mL</td>
</tr>
<tr>
<td>24</td>
<td>0.36mL</td>
</tr>
<tr>
<td>32</td>
<td>0.48mL</td>
</tr>
<tr>
<td>70</td>
<td>0.60mL</td>
</tr>
<tr>
<td>48</td>
<td>0.72mL</td>
</tr>
<tr>
<td>56</td>
<td>0.84mL</td>
</tr>
<tr>
<td>64</td>
<td>0.96mL</td>
</tr>
<tr>
<td>72</td>
<td>1.08mL</td>
</tr>
<tr>
<td>80</td>
<td>1.20mL</td>
</tr>
<tr>
<td>88</td>
<td>1.32mL</td>
</tr>
<tr>
<td>96</td>
<td>1.44mL</td>
</tr>
</tbody>
</table>

9. Repeat steps 6 through 9. **NOTE:** If using test system 24012 or 24052, 5 to 10 drops of Evans blue counterstain (#2406) is to be added to the staining dish at the time of the second 5 minute PBS rinse.
   a. 5-WELL, 12 WELL OR 16-WELL TEST SYSTEM:
      Apply 3 to 7 drops of mounting media (according to the number of wells per slide) to each slide (between the wells) and coverslip.
   b. 96-WELL TEST SYSTEM:
      Apply 10 to 15 drops of mounting media randomly to the mask of each slide (between the wells) and coverslip.
      **NOTE:** Be sure each well has mounting media coverage.

10. Examine slides immediately with an appropriate fluorescence microscope assembly. **NOTE:** If delay in examining slides is anticipated, seal coverslip with nail polish and store in refrigerator. It is recommended that slides be examined on the same day of testing.

**CALCULATIONS/REPORTING RESULTS**

**INTERPRETATION:**

1. The interpretation of the results depends on the pattern observed, the titer of the autoantibody, and the age of the patient. The elderly, especially women, are prone to develop low-titered autoantibodies (<1:80) in the absence of clinical autoimmune disease. In contrast, a 1:20 titer of a significant pattern of autoantibody(s) in a young person may suggest that overt disease may occur later. Experience suggests that a 1:40 dilution is a good dilution to screen for ANA. Low-titer positive results may occur in apparently healthy persons; therefore, the ANA results must always be interpreted in light of the patient’s total clinical presentation.

2. Titers less than 1:40 are considered negative.

3. Positive test: A positive reaction is the presence of any pattern of nuclear apple-green staining observed at a 1:40 dilution based on a 1+ to 4+ scale of staining intensity. 1+ is considered a weak reaction and 4+ a strong reaction. All sera positive at 1:40 should be titered to endpoint dilution. This is accomplished by making 1:40, 1:80, 1:160, etc. serial dilutions of all positives. The endpoint titer is the highest dilution that produces a 1+ positive reaction.

4. Homogeneous patterns with peripheral accentuation are frequently found in sera from patients with SLE.
### Table

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Disease Most Frequently Found In</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous</td>
<td>SLE</td>
<td>(3, 8, 9, 16)</td>
</tr>
<tr>
<td>High Titer</td>
<td>Rheumatoid Arthritis and other diseases</td>
<td>(1)</td>
</tr>
<tr>
<td>Low Titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centromere</td>
<td>CREST Syndrome variant of PSS</td>
<td>(27)</td>
</tr>
<tr>
<td>Speckled</td>
<td>Scleroderma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raynaud’s Syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sjögren’s Syndrome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed connective tissue disease</td>
<td>(34-36)</td>
</tr>
<tr>
<td>Nucleolar</td>
<td>Scleroderma</td>
<td>(37)</td>
</tr>
<tr>
<td>Peripheral</td>
<td>SLE</td>
<td>(2, 8, 9, 16)</td>
</tr>
</tbody>
</table>

### Procedure Notes

1. For *in vitro* diagnostic use.
2. The thimerosal and sodium azide preservative may be toxic if ingested.
3. Sample diluent contains sodium azide as a preservative. 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.
4. Remove only the amount of sample diluent needed to perform each test run to reduce the possibility of product contamination.
5. Use sample diluent for screening dilutions only. DO NOT PREPARE SERIAL DILUTIONS FOR ENDPOINT TITERS IN sample diluent.
6. Sample diluent should be used only as a diluent for patient specimens:
   a. DO NOT use sample diluent to reconstitute the controls or conjugate.
   b. DO NOT use sample diluent in any of the wash steps.
7. The volume of sample diluent supplied has been calculated to provide sufficient material for all the individual test wells included in this kit when used according to the instructions herein. The use of larger volumes for sample preparation will result in insufficient sample diluent to allow each test well to be utilized.
8. Non-nuclear staining of the cell substrate may be observed with some human sera.
9. Do not apply pressure to slide envelope. This may damage the substrate.
10. Reagents from other sources or manufacturers should not be used. Follow test procedure carefully.
11. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.
12. DO NOT freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity.
13. The human serum controls are POTENTIALLY BIOHAZARDOUS 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 Biosafety 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 (39).
14. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.

### Limitations of the Procedure

1. The ANA test is a laboratory diagnostic aid and by itself is not diagnostic. Positive ANA may be found in apparently healthy individuals. 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. One autoantibody pattern may partially or completely obscure the diagnostic features of the other. In such instances, it is necessary to titrate the serum.
5. No definitive association between the pattern of nuclear fluorescence and any specific disease state is
EXPECTED VALUES
The expected value in the normal population is negative. However, apparently healthy individuals may contain ANA in their sera (38). This percentage increases with aging, particularly in the 7th decade of life.

PERFORMANCE CHARACTERISTICS
A. The Diagnostic Automation, Inc. ANA/HEp-2 test system was tested in parallel with a reference procedure employing rat liver substrate. Routine ANA testing was performed by both procedures on 434 patient specimens. Of these 434 sera, 116 were positive by both procedures. The DAI ANA/HEp-2 test system showed 97% agreement with respect to positive and negative results, and 100% with respect to staining pattern. Of the 21 discrepancies in titer, the DAI ANA/HEp-2 procedure was one dilution lower in 18 specimens. Five of these 18 specimens that were negative using the ANA/HEp-2 procedure were positive at 1:20 by rat liver reference procedure.

B. A study was performed using 206 samples obtained from a plasma donor center and a reference laboratory. The DAI ANA/HEp-2 test system with sample diluent was compared to a commercial ANA/HEp-2 test system (PBS sample diluent) and the DAI ANA HEp-2 test system (PBS sample diluent). With respect to positive and negative results, there was 96% agreement (198/206) between the DAI ANA/HEp-2 with sample diluent and the DAI ANA/HEp-2 test system with PBS.

The discrepant results involved 15 different samples. All 15 were borderline positive when screened using PBS as the sample diluent and negative when screened using sample diluent. One sample was positive on both tests used PBS as the sample diluent. Seven samples were positive using the DAI ANA/HEp-2 with PBS diluent. The other seven samples were positive on the commercial ANA/HEp-2 with PBS diluent. The discrepant samples were retested at 1:40 and titrated in the DAI ANA/HEp-2 (PBS) samples were again borderline reactive at 1:40, but negative at 1:80.

Seventy-nine samples were positive by all three assays with no discrepancies in staining patterns. Twenty-five of the positive samples were used for an endpoint titer analysis. The endpoint titer results are as follows:

<table>
<thead>
<tr>
<th>DAI ANA/HEp-2 with sample diluent</th>
<th>DAI ANA Hep-2 (PBS)</th>
<th>Commercial ANA HEp-2</th>
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</thead>
<tbody>
<tr>
<td>Identical Titer</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>+ one, two-fold dilution</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>+ two, two-fold dilutions</td>
<td>0</td>
<td>7</td>
</tr>
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<td></td>
<td>25</td>
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REFERENCES

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<tr>
<th>Date Adopted</th>
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<tr>
<td>2004-04-27</td>
<td>DA-ANA HEP-2-Liquid Format-IFA-2010</td>
</tr>
</tbody>
</table>

DIAGNOSTIC AUTOMATION, INC.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
ISO 13485-2003

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