ANA (Mouse Kidney) IFA Kit

Cat # 220708

PRINCIPLE OF THE ASSAY
The DAI IFA-ANA test system is a prestandardized kit designed to detect the presence of circulating ANA in human sera. The system employs mouse kidney 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. The first is the interaction of ANA in patients’ sera with the mouse nuclei.
2. The second is the interaction of FITC labeled anti-human immunoglobulin with nuclear antibodies attached to the mouse nuclei in a positive assay. (See Procedure section for details.)

The DAI IFA-ANA test system will detect all recognized systems of nuclear staining patterns. The DAI IFA ANA test 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).

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 refrigerated sera obtained by approved aseptic venipuncture
procedures should be used in this assay (28,29). 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 between 2° and 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 that 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. Moist chamber.
6. Cover Slips: 24 x 60mm, thickness No. 1.
7. Distilled water.
8. 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 darkfield assemblies:

<table>
<thead>
<tr>
<th>TRANSMITTED LIGHT</th>
<th>Light Source: Mercury vapor 200mW or 50 W</th>
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<tbody>
<tr>
<td><strong>Excitation filter</strong></td>
<td><strong>Barrier filter</strong></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>K530</td>
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<table>
<thead>
<tr>
<th>INCIDENT LIGHT</th>
<th>Light Source: Mercury vapor 200, 100, 50 W</th>
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<tbody>
<tr>
<td><strong>Excitation Filter</strong></td>
<td><strong>Dichroic Mirror</strong></td>
</tr>
<tr>
<td>KP500</td>
<td>TK510</td>
</tr>
<tr>
<td>FITC</td>
<td>TK510</td>
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</table>

<table>
<thead>
<tr>
<th>Light Source: Tungsten – Halogen 50 and 100 W</th>
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</thead>
<tbody>
<tr>
<td><strong>Excitation Filter</strong></td>
</tr>
<tr>
<td>KP500</td>
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<tr>
<td>FITC</td>
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</tbody>
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MATERIALS PROVIDED
Reactive Reagents:
1. Goat anti-human immunoglobulin (polyvalent) labeled with fluorescein isothiocyanate (FITC).
   Contains phosphate buffered saline (PBS) pH 7.2 ± 0.1 in 1.25% bovine albumin and counterstain.
2. Human ANA positive control serum (0.5mL, lyophilized). Composed of human serum producing 4+
homogeneous nuclear staining with mouse kidney substrate.
3. Human ANA negative control serum (0.5mL, lyophilized). Composed of normal human serum with no nuclear staining as determined by the IFA procedure.
4. Mouse Kidney substrate slides capable of detecting all recognized nuclear staining patterns.
5. Sample diluent formulated to reduce non-specific staining. Contains 0.1% sodium azide as a preservative.

Non-reactive Materials:
1. Phosphate buffered saline (PBS) sufficient to make 2 liters.
2. Buffered glycerol, 3mL.
*NOTE:* These reactive reagents contain preservative: thimerosal, mercury derivative 0.04%.

**STORAGE CONDITIONS**
1. Mouse Kidney substrate slides: Store at -20°C or 2-8°C.
2. Goat anti-human immunoglobulin labeled with FITC: Store at 2-8°C. Stable for 90 days after reconstitution when stored at 2-8°C. Alternately, aliquot in 0.5mL amounts and store at -20°C. Do not refreeze once aliquots have been thawed. Frozen aliquots are stable for 6 months.
3. Human ANA positive and negative control sera: Store at 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months.
4. Sample Diluent: Store at 2-8°C.
5. Phosphate buffered saline (PBS): Store packets at 2-25°C. Rehydrated PBS is stable for 30 days when stored at 2-8°C.
6. Buffered glycerol: Store at 2-8°C.

**NOTE:** 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. DO NOT freeze and thaw reagents 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.
   c. 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.

**PROCEDURE – STEPWISE**
Preparation of Reagents
1. Phosphate buffered saline (PBS) pH 7.2 ± 0.2: Empty contents of one buffer pack into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. Human ANA positive control serum: Reconstitute with 0.5mL distilled water. (Represents a 1:20 screening dilution). Use as reconstituted.
3. Human ANA negative control serum: Reconstitute with 0.5mL distilled water. Represents a 1:20 screening dilution. Use as reconstituted.
4. Goat anti-human immunoglobulin FITC labeled conjugate: Reconstitute with 3.0mL distilled water. Use as reconstituted.

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 DAIan 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 reach room temperature (20-25°C). Tear open the protective envelope and remove slides containing the Mouse Kidney sections. DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.
2. Prepare patient sera at a 1:20 dilution in PBS. (for example: 10µL of sample plus 190µL of PBS). (Alternatively, you may prepare 1:20 screening dilution in sample diluent).
3. Identify each well with the appropriate patient sera and controls.
4. If test samples are to be titrated, serial dilutions should be made in reconstituted PBS. DO NOT MAKE SERIAL DILUTIONS IN SAMPLE DILUENT.
5. With suitable dispenser (capillary, Pasteur, or automatic pipette), dispense one drop or approximately 0.020mL of patient sera and control sera on the tissue in the respective wells. Spread sera over entire area of the tissue section or well being careful not to touch tissue section with pipette tip.
6. Incubate slides in a moist chamber at room temperature (20-25°C) for 30 minutes.
7. 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.
8. Place slides in a staining dish and wash in PBS for two, 5-minute intervals with a change of PBS. Use a magnetic mixing setup or other means of gentle agitation.
9. 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.
10. Add 0.020mL of conjugate to each well.
11. Repeat steps 6 to 9.
12. Add 3-5 drops of mounting media to each slide (between the wells) and coverslip. Examine slides immediately with an appropriate fluorescence microscope assembly. 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 of testing.

CALCULATIONS/REPORTING RESULTS INTERPRETATION
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 endpoint 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

<table>
<thead>
<tr>
<th>PATTERN</th>
<th>DISEASE MOST FREQUENTLY FOUND IN</th>
<th>REFERENCES</th>
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<tbody>
<tr>
<td>1. Homogeneous</td>
<td>SLE</td>
<td>(3, 8, 9, 17)</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>
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<tr>
<td>2. Peripheral</td>
<td>SLE</td>
<td>(2, 8, 9, 17)</td>
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<tr>
<td>3. Speckled</td>
<td>Scleroderma</td>
<td>(12, 25, 26)</td>
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<tr>
<td></td>
<td>Raynaud’s Syndrome</td>
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<td></td>
<td>Sjögren’s Syndrome</td>
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<td></td>
<td>Mixed Connective Tissue Disease</td>
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<tr>
<td>4. Nucleolar</td>
<td>Scleroderma</td>
<td>(27)</td>
</tr>
</tbody>
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### 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. All human serum reagents have been tested for HBsAg by the RIA method and found to be negative. Care should still be exercised since it is now recognized that other hepatitis antigens may be present.
5. Remove only the amount of sample diluent needed to perform each test run to reduce the possibility of product contamination.
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 Mouse Kidney substrate may be observed with some human sera. Report nuclear staining results only and disregard non-nuclear staining.
9. Do not apply pressure to the slide envelope. This may damage the mouse tissue substrate.
10. The components of this kit are matched for optimum sensitivity and reproducibility. Reagents from other kits or sources should not be interchanged. Follow kit procedures 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. To determine whether components have been subjected to improper transit conditions, run IFA-ANA test as described under Procedure, using human positive ANA serum.
14. 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 (30).

15. 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 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.

REFERENCES
No. 12, Approved Guideline, 1990.

<table>
<thead>
<tr>
<th>Date Adopted</th>
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<tr>
<td>2004-09-15</td>
<td>DA-ANA Mouse Kidney-IFA-2010</td>
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