NOVA Lite™ Slides

ANCA (Methanol Fixed Human Neutrophil, 8 wells) 508280
Islet Cell Antibody (Primate Pancreas, 8 wells) 508310
Islet Cell Antibody (Primate Pancreas, 4 wells) 508315
Skeletal Muscle Antibody (Primate, 4 wells) 508320
Endomysial (Primate Distal Esophagus, 4 wells) 508336
Endomysial (Primate Distal Esophagus, 8 wells) 508337
GBM (Primate Kidney, 4 wells) 508345
Heart (Primate, 4 wells) 508356
Cerebellum (Primate, 4 wells) 508357
Urinary Bladder (Primate, 4 wells) 508360
Cerebrum (Primate, 4 wells) 508362
Colon (Primate, 4 wells) 508363
Lip (Primate, 4 wells) 508364
Salivary Gland (Primate, 4 wells) 508367
Sciatic Nerve (Primate, 4 wells) 508368
Stomach (Primate, 4 wells) 508369
Adrenal Gland (Primate, 8 wells) 508370
Adrenal Gland (Primate, 4 wells) 508375
Testes (Primate, 4 wells) 508372
Liver (Primate, 4 wells) 508373

For Export Only. Not for sale in the United States.

Intended Use
NOVA Lite™ Slides are used in the INOVA indirect immunofluorescent assay (IFA) test system for the screening and semi-quantitative determination of anti-nuclear antibodies (ANA) in human serum.

Summary and Explanation of the test
The term "anti-nuclear antibodies" describes a variety of autoantibodies that react with constituents of cell nuclei including DNA, RNA and several proteins and ribonucleoproteins.1 These autoantibodies occur with high frequency in patients with connective tissue or rheumatic diseases, especially systemic lupus erythematosus. Virtually all SLE patients are ANA positive. This diagnostic sensitivity has led to the incorporation of ANA testing into the 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus by an American College of Rheumatology Subcommittee.2 While ANA testing is an excellent screening test for SLE (a negative result virtually rules out active SLE) it is by no means a specific test. Patients with other connective tissue diseases such as rheumatoid arthritis, scleroderma and dermatomyositis are frequently positive, and low ANA titers may be observed in other disease states and in the normal population. Positive ANA results can occur following severe burns or viral infection and have been reported in some normal, healthy people, especially in older populations. Because of this lack of specificity, it is recommended that all ANA positive samples be titered to endpoint and that more specific testing for autoantibodies to double stranded DNA (dsDNA) and extractable nuclear antigen (ENA) autoantibodies be performed.

Indirect immunofluorescence is the reference method for ANA testing. Common substrates are thin sections of organs or various types of cell lines. It is generally agreed that cell line substrates are preferable to organ sections since these rapidly dividing cells have higher levels of certain clinically relevant antigens, including centromere, SS-A(RO), Scl-70 and PCNA/Cyclin.

Besides the type of substrate, three other factors are critical to the performance of an ANA test: 1) the fixative used in preparing the slide, 2) the fluorescein to protein (F/P) ratio and 3) the immunoglobulin subclass specificity of the conjugate. Some fixatives or combinations thereof are known to destroy certain nuclear antigens and their use should be avoided. The sensitivity and non-specific background staining of a conjugate is determined by the F/P ratio while the disease specificity of a conjugate is determined by the immunoglobulin subclass reactivity. Virtually all clinically significant autoantibodies exhibit IgG subclass specificity even in the presence of IgM and IgA specific ANA. In contrast, ANA found in healthy blood donors are generally of the IgM and IgA subclass only. Because of this, conjugates specific for IgG are more disease specific.
Principles of the Procedure
In the indirect immunofluorescence technique, samples are incubated with antigen substrate and unreacted antibodies are washed off. The substrate is incubated with specific fluorescein labeled conjugate and then unbound conjugate is washed off. When viewed through a fluorescence microscope, autoantibody positive samples will exhibit an apple green fluorescence corresponding to areas of the cell or nuclei where autoantibody has bound.

Reagents
1. Substrate slide in foil pouch with desiccant.

Warnings
1. Use appropriate personal protective equipment while working with reagents.
2. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions
1. Substitution of components other than those used in the INOVA IFA system may lead to inconsistent results.
2. Incomplete or inefficient washing of IFA wells may cause high background.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the strength of the microscope bulb used, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.

Storage Conditions
1. Store all the slides at 2-8°C. Do not freeze. Slides are stable until the expiration date when stored and handled as directed.

Specimen Collection
This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Grossly hemolyzed or lipemic serum specimens should be avoided.
Following collection, the serum should be separated from the clot. NCCLS Document H18-A3 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Procedure
Material provided
1. Substrate Slide

Additional Materials Required But Not Provided
FITC Anti-Human IgG Conjugate
ANA Titratable Endpoint Pattern Control
IFA System Negative Control
PBS Concentrate (40x)
Mounting Medium
Coverslips
Micropipets to deliver 15-1000μL volume
Distilled or deionized water
Squeeze bottles or Pasteur pipets
Moist chamber
1L container (for diluting PBS)
Coplin jar
Fluorescence microscope with 495nm exciter and 515nm barrier filter

Method
Before you start
1. Bring all reagents and samples to room temperature (20 - 26°C).
2. Dilute PBS Concentrate: IMPORTANT: Dilute the PBS Concentrate 1:40 by adding the contents of the PBS Concentrate bottle to 975mL of distilled or deionized water and mix thoroughly. The PBS buffer is used for diluting patient samples and as a wash buffer. The diluted buffer can be stored for up to 4 weeks at 2-8°C.
3. **Dilute Patient Samples:**

<table>
<thead>
<tr>
<th>Slide Type</th>
<th>Starting Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANCA (Methanol Fixed Human Neutrophil)</td>
<td>1:20</td>
</tr>
<tr>
<td>Islet Cell Antibody (Primate Pancreas)</td>
<td>1:2</td>
</tr>
<tr>
<td>Skeletal Muscle Antibody (Primate)</td>
<td>1:10</td>
</tr>
<tr>
<td>Endomyosial (Primate Distal Esophagus)</td>
<td>1:5</td>
</tr>
<tr>
<td>Endomyosial (Primate Distal Esophagus)</td>
<td>1:5</td>
</tr>
<tr>
<td>GBM (Primate Kidney)</td>
<td>1:20</td>
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<tr>
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</tr>
<tr>
<td>Liver (Primate)</td>
<td>1:20</td>
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</tbody>
</table>

a. **Initial Screening:** Dilute patient samples according to the above table with diluted PBS buffer (i.e., add 100µL of serum to 0.40mL of PBS buffer for a 1:5, add 100µL of serum to 0.90mL of PBS buffer for a 1:10 or add 50µL of serum to 0.95mL of PBS buffer for a 1:20).

b. **Titration:** Make serial 2-fold dilutions from the initial screening dilution for all positive samples with diluted PBS buffer.

### Assay procedure

1. **Prepare Substrate Slides:** Allow the substrate slide to reach room temperature prior to removal from its pouch. Label it with pencil and place it in a suitable moist chamber. Add 1 drop of the undiluted positive and the negative control to wells 1 and 2 respectively. Add 1 drop of diluted patient sample to the remaining wells.

2. **Slide Incubation:** Incubate the slide for 30 + 5 minutes in a moist chamber (a dampened paper towel placed flat on the bottom of a closed plastic or glass container) that will maintain the proper humidity conditions. **Do not allow the substrate to dry out during the assay procedure.**

3. **Wash Slides:** After incubation, use a plastic squeeze bottle or pipet to gently wash off the serum with diluted PBS buffer. Orient the slide and stream of PBS buffer so as to minimize wash-over of samples between wells. Avoid directing the stream directly onto the wells to prevent substrate damage. If desired, place the slides in a Coplin jar of diluted PBS buffer for up to 5 minutes.

4. **Addition of Fluorescent Conjugate:** Shake off the excess PBS buffer. Place the slide back in the moist chamber and immediately cover each well with a drop of fluorescent conjugate. Incubate the slides for an additional 30 + 5 minutes.

5. **Wash Slides:** Repeat Step 3.

6. **Coverslip:** Coverslip procedures vary from lab to lab; however, the following procedure is recommended:
   a. Place a coverslip on a paper towel.
   b. Apply mounting medium in a continuous line to the bottom edge of the coverslip.
   c. Shake off the excess PBS buffer and touch the lower edge of the slide to the edge of the coverslip. Gently lower the slide onto the coverslip in such a way that the mounting medium flows to the top edge of the slide without air bubble formation or entrapment.

### Interpretation of Results

**Negative Reaction.** A sample is considered negative if specific staining is equal to or less than the IFA System Negative Control. Samples can exhibit various degrees of background staining due to heterophile antibodies or low-level autoantibodies to cytoplasmic constituents such as contractile proteins.

**Positive Reaction.** A sample is considered positive if specific staining is observed to be greater than the INOVA IFA System Negative Control. Determine the fluorescence grade or intensity using these criteria:

- 4+ Brilliant apple green fluorescence
- 3+ Bright apple green fluorescence
- 2+ Clearly distinguishable positive fluorescence
- 1+ Lowest specific fluorescence that enables the nuclear and/or cytoplasmic staining to be clearly differentiated from the background fluorescence.

### Limitations of the Procedure

1. **High-titered ANA is suggestive of connective tissue disease but should not be considered diagnostic. The ANA result should be considered in combination with other serological results as well as the overall clinical history of the patient.**
2. **ANA patterns often change as the sample is titered out to endpoint. This phenomenon is due to lower titer antibodies dropping below the sensitivity of the system as more dilute sample is tested.**
3. **A variety of external factors influence the test sensitivity including the type of fluorescence microscope used, the bulb strength and age, the magnification used, the filter system and the**
observer.
4. If a band pass filter is used instead of a 515 barrier filter, increased artifactual staining may be observed.
5. Only pencil should be used to label the slides. Use of any other writing material may cause artifactual staining.
6. All coplin jars used for slide washing should be free from all dye residues. Use of coplin jars containing dye residue may cause artifactual staining.
7. Results of this assay should be used in conjunction with clinical findings and other serological tests.
8. The assay performance characteristics have not been established for matrices other than serum.

References


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628280ENG July 2008 Revision 0