Anti-Nuclear Antibody (ANA)

1. Screening Test
   A. Refers to auto-antibodies to nuclear components of the cell
   B. Seen in a wide variety of systemic autoimmune diseases
   C. Useful as diagnostic markers and to monitor the course of autoimmune diseases
   D. Can be found in low titers in healthy individuals

   E. High titers (>1:320) is highly suggestive of an autoimmune disease
   F. Low or negative result does not rule out autoimmune disease
   G. Many patients with different kinds of autoimmune diseases will have generic ANA’s
   H. Specific types of ANA’s will help to identify a specific autoimmune diseases:

Anti-Nuclear Antibody (ANA)

I. ANA Screening Tests
   1) Indirect Immunofluorescence
      a. Hep-2 cell substrate
         1. Human cancer cell line
      b. Incubate patient serum on cell substrate
      c. Wash
      d. Incubate substrate with fluorescent-conjugated anti-human antibody
      e. Observe staining pattern under fluorescent microscope

   2. ANA’s stain different nuclear components
      a. ds-DNA
      b. Histones
      c. Non-histone proteins
      d. Nucleolus

   3. There are 5 major staining patterns
      a. Homogeneous
      b. Rim
      c. Speckled
      d. Nucleolar
      e. Centromere

   Incidence of ANA in Various Diseases of Conditions
   (See Table to Right)

   15-30% Juvenile Arthritis
   100% Felty’s Syndrome
   40-70% Sjogren’s Syndrome
   25-30% Rheumatoid Arthritis
   60-90% Progressive Systemic Sclerosis (Scleroderma)
   Over 95% Systemic Lupus Erythematosus (SLE)

Homogeneous Pattern

Rim Pattern
Antigen Nuclear Antibody (ANA) Speckled Pattern

The centromere pattern has the following characteristics:

- The resting cell nuclei have numerous discrete speckles. If it were possible to count these nuclei, the number of centromeres present in human cells is
- The chromosomes of the dividing cells have speckled staining.

Resting Cell Dividing Cell

- Discrete Speckled Staining
- Speckled Staining of the Chromatin

Anti-Nuclear Antibody (ANA) Nucleolar Pattern

An especially fine speckling with little background staining. This is anti-centromere, the marker for CREST/Pulmonary hypertension

Centered Pattern

- anti-Th or anti-fibrillarin / anti-U3RNP or anti-U17RNP
- Think of scleroderma, though most scleroderma patients don’t show the nucleolar pattern

Anti-Nuclear Antibody (ANA) Summary

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rim Pattern</td>
<td>Probably anti-dsDNA. Your patient probably has systemic lupus</td>
</tr>
<tr>
<td>Homogeneous Pattern</td>
<td>Probably anti-histones. Your patient probably has drug-induced lupus</td>
</tr>
<tr>
<td>Speckled Pattern</td>
<td>Could be anti-Th and/or anti-fibrillarin and/or anti-U3RNP and/or anti-U17RNP and/or any of several others. You’ll certainly want to continue your workup</td>
</tr>
<tr>
<td>Centromere Pattern</td>
<td>An especially fine speckling with little background staining. This's auto-centromeres, the marker for CREST/Pulmonary hypertension</td>
</tr>
<tr>
<td>Nucleolar Pattern</td>
<td>anti-Th or anti-fibrillarin / anti-U3RNP or anti-U17RNP or anti-CREST/Pulmonary hypertension</td>
</tr>
</tbody>
</table>

Anti-Nuclear Antibody (ANA) Anti-dsDNA Antibodies

J. Follow-up Tests for a Positive ANA Screen
1) Anti-double stranded DNA (dsDNA) antibody
2) Anti-Smith (Sm) antibody
3) Anti-histone antibody
4) Anti-ribonucleoprotein (RNP) antibody
5) Anti-scl70 antibody
6) Anti-extractable nuclear antigen (ENA) antibody
7) Jo-1 (Histidyl tRNA Synthetase)

K. Specific Information About Follow-up Tests for a Positive ANA Screen
1) Anti-dsDNA Antibodies
   a) Peripheral or homogeneous pattern on routine IFA
   b) Definitive detection and quantitation is performed on more specific substrates
      1. IFA using the hemoflagellate Crithidia luciliae as substrate
      2. Very specific for SLE
      3. 40-60% of patients with SLE have anti-dsDNA antibodies
      4. Tends to be a Rim Pattern
   c) Positive results support a diagnosis for SLE and can be used to monitor disease activity and response to therapy
      1. Found in 40-60% of patients with SLE during the active phase of their disease
Anti-Nuclear Antibody (ANA)

**Crithidia Organisms (dsDNA)**

- Anti-Nuclear Antibody (ANA)
  - Anti-Smith (Sm) antibodies
    - Speckled pattern on IFA
    - Specific for SLE
    - Only 30% of patients with SLE have high titers
    - Used in follow-up to collagen vascular disorders
  - Anti-Histone antibodies
    - Homogeneous pattern on IFA
    - Most commonly seen in drug-induced lupus
    - In drug-induced lupus there is not normally an association with other autoantibodies
  - Anti-RNP antibodies (Speckled or Nucleolar Pattern)
    - Specifically for the differential diagnosis of:
      1. Systemic rheumatic diseases
      2. Follow-up analysis of collagen vascular disorders
  - Anti-SSA and Anti-SSB (Speckled Pattern)
    - Valuable when a patient is anti-nuclear antibody negative but thought to have SLE
  - Anti-Centromere antibody appears in 90% of patients with CREST syndrome which is a variant of scleroderma
  - Anti-Centromere antibody appears in 90% of patients with SLE overlap syndromes
  - Jo-1 (Histidyl tRNA Synthetase)
    - Polymyositis/Dermatomyositis

**Anti-Nuclear Antibody (ANA)**

- Autoantibodies in Rheumatic Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Antibody to</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>dsDNA and/or Sm</td>
</tr>
<tr>
<td>Drug-induced SLE</td>
<td>Histone</td>
</tr>
<tr>
<td>SLE</td>
<td>Anti Ro (SS-A)</td>
</tr>
<tr>
<td>MCTD</td>
<td>RNP</td>
</tr>
<tr>
<td>Scleroderma (Diffuse)</td>
<td>Anti-SSA</td>
</tr>
<tr>
<td>CREST</td>
<td>Anti-SSB</td>
</tr>
<tr>
<td>Polymyositis/Dermatomyositis</td>
<td>Anti-SSA</td>
</tr>
<tr>
<td>SLE</td>
<td>Anti Ro (SS-A), SS-B</td>
</tr>
<tr>
<td>Wegener’s Granulomatosis</td>
<td>cANCA (Proteinase 3)</td>
</tr>
</tbody>
</table>

**Autoantibodies in Rheumatic Diseases**

- Low titre ANAs (ie 1:40 or 1:100) are usually not clinically relevant whereas high titre ANA (particularly 1:1600) are infrequent amongst healthy individuals and so therefore carry a greater clinical significance. The table above is of common ANA patterns and their clinical association. Possibly the most important use is to exclude SLE.