Fluorescent Patterns
Associated with Antinuclear Antibody Detection

Suggested Reading


References


Systemic Rheumatic Disease

Identification of the antinuclear antibody (ANA) pattern remains a crucial step in the process of diagnosing the systemic rheumatic diseases. ANA patterns often give the clinician insight into which autoantibodies are present and indications of disease likelihood.

It has now been over 55 years since the LE cell was first described by Hargraves. (1) During this time, substantial improvements in physician awareness, diagnostics and treatments have increased the survival rate for lupus patients from a 5 year rate of only 50% to a 10 year rate of >90%. (2)

There is growing evidence that the appearance of autoantibodies may precede the onset of the disease, often by many years. Early detection of these autoantibodies may offer the opportunity for earlier diagnosis and treatment, improving the length and quality of life for the patient.

A recent study in the New England Journal of Medicine reported that, on average, at least one antinuclear antibody was present 3 years prior to the clinical diagnosis of SLE being made and at the time of diagnosis there were often 3 autoantibodies present. (3) The earliest antibodies detected were ANA’s, anti-SSA/SS-A and anti-SSB/La antibodies. These antibodies are readily detected and reported using the HEp-2000® slide based assay.

The HEp-2000® substrate is Immuno Concepts’ patented ANA substrate that has been consistently proven in independent studies to be superior to standard HEp-2 for the detection and identification of ANA’s. (4-7). When the unique SSA/Ro pattern is present on the HEp-2000® substrate the laboratory can immediately report the presence of anti-SSA/Ro antibodies to the clinician, potentially accelerating the correct diagnosis for the patient.

Flow Chart
ANA Pattern and Confirmatory Testing

If the ANA is negative, test is completed and no specific autoantibody is detected.
If the ANA is positive, proceed with testing for specific autoantibodies.

Immuno Concepts has taken care that the information and recommendations contained herein are accurate and compatible with current standards. Nevertheless, it is difficult to ensure that all of the information given is entirely accurate in all circumstances. Immuno Concepts disclaims any liability, loss or damage incurred as a consequence, directly or indirectly, of the use and application of any of the contents of this chart.
### Antigen Chart
Associated with ANA Patterns

<table>
<thead>
<tr>
<th>Pattern observed by indirect immunofluorescence</th>
<th>Type of antibody</th>
<th>Disease in which antibodies are seen</th>
<th>Characteristics of antigenic determinants</th>
<th>Tests used to confirm specific antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous or peripheral</td>
<td>Double-stranded DNA, DNA, RNA, dsDNA</td>
<td>Characteristic of SLE, lower levels in other rheumatic diseases</td>
<td>IF using Cardiolipin, RIA, ELISA</td>
<td>None</td>
</tr>
<tr>
<td>Demyelinating protein, soluble form</td>
<td>SLE, Drug-induced LE</td>
<td>DNA-histone complex</td>
<td>LE cell prep, RIA, ID, HA</td>
<td>None</td>
</tr>
<tr>
<td>Histone</td>
<td>Drug-induced LE, SLE, others</td>
<td>Different classes of histone</td>
<td>ELISA, RIA</td>
<td>None</td>
</tr>
<tr>
<td>Unusual Homogeneous</td>
<td>Nuclear Envelope or Nuclear Membrane</td>
<td>Lupoid hepatitis</td>
<td>Nuclear Lamin A, B, C</td>
<td>None</td>
</tr>
<tr>
<td>Speckled</td>
<td>Sm</td>
<td>Marker antibody for SLE</td>
<td>Core proteins of the U1, U2, U4, U6, U11, U12, and U12 snRNPs</td>
<td>ID, ELISA, HA, CF</td>
</tr>
<tr>
<td>U1-RNP</td>
<td>High levels in MCTD and SLE, low levels in other diseases</td>
<td>A, C, and 70kDa proteins complexed with U1 RNA</td>
<td>ID, ELISA, HA, CF</td>
<td>None</td>
</tr>
<tr>
<td>SSA/Ro</td>
<td>High prevalence in Sjogren’s syndrome, include complex, lower prevalence in other rheumatic diseases</td>
<td>52 and 80 kDa proteins complexed with Y1-15 RNA’s</td>
<td>Confirmatory with HEp-2000B staining pattern; rule out other ENAs with ID, ELISA, CIEP (IP, western blot)</td>
<td>None</td>
</tr>
<tr>
<td>SSB/La</td>
<td>High prevalence in Sjogren’s syndrome, include complex, lower prevalence in other rheumatic diseases</td>
<td>48 kDa protein complexed with RNA polymerase I</td>
<td>ID, ELISA, CIEP (IP, western blot)</td>
<td>None</td>
</tr>
<tr>
<td>Sci-70</td>
<td>Marker antibody for Scleroderma</td>
<td>70 kDa protein of topoisomerase</td>
<td>ID, ELISA</td>
<td>None</td>
</tr>
<tr>
<td>PCNA</td>
<td>Marker antibody for SLE</td>
<td>Auxiliary protein to DNA polymerase</td>
<td>ID, CIEP</td>
<td>None</td>
</tr>
<tr>
<td>Matrix</td>
<td>Seen in some patients with evolving rheumatic disease</td>
<td>Heterogeneous nuclear RNP (nRNP), others</td>
<td>Confirmed by staining pattern</td>
<td>None</td>
</tr>
<tr>
<td>Unusual Speckled</td>
<td>NsP I, sp, 100, MND, PBC 95</td>
<td>Some association with Primary Bilayer Synthesis</td>
<td>95-100 kDa protein</td>
<td>None</td>
</tr>
<tr>
<td>NsP II, CENP F</td>
<td>unknown</td>
<td>387 kDa protein</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Scl-70</td>
<td>Low percentage of patients with scleroderma</td>
<td>Undetermined proteins of Scl-70</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>p 80 (coeliac)</td>
<td>unknown</td>
<td>80 kDa proteins in the coiled body</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Centromere</td>
<td>Centromere</td>
<td>Seen in 57 - 82% of patients with CREST variant of scleroderma</td>
<td>CENP A, CENP B, CENP C</td>
<td>Confirmed by staining pattern</td>
</tr>
<tr>
<td>Nuclear</td>
<td>Clumped Nuclear</td>
<td>Scleroderma</td>
<td>Fibroblasts, others?</td>
<td>None</td>
</tr>
<tr>
<td>Specified Nuclear</td>
<td>Scleroderma</td>
<td>Scleroderma</td>
<td>Fibrillar, others?</td>
<td>None</td>
</tr>
<tr>
<td>Smooth Nuclear</td>
<td>Polymyositis/Scleroderma</td>
<td>Polymyositis/Scleroderma</td>
<td>Fibrillar, others?</td>
<td>None</td>
</tr>
</tbody>
</table>

### Fluorescent Patterns

**Negative**
1. Report as: ANA Negative
2. Suspected antigen specificity: None
3. Clinical significance: None
4. Follow-up testing: None

**Speckled**
5. Report as: Speckled
6. Suspected antigen specificity: Sm, U1-RNP, others?
7. Clinical significance: Sm positive marker antibody seen in 4-10% of SLE patients. U1-RNP positive seen in high titers in patients with MCTD and SLE, low titers in other diseases.
8. Follow-up testing: Confirm by ENA testing

**Homogeneous**
9. Report as: Homogeneous
10. Suspected antigen specificity: nDNA, DNP. Histone, DNA-binding proteins, others?
11. Clinical significance: nDNA positive marker antibody seen in 80% of SLE patients
12. Follow-up testing: Confirm by DNA antibodies

**Nucleolar (clumpsy)**
13. Report as: Nucleolar
14. Suspected antigen specificity: Fibrillar, others?
15. Clinical significance: May be seen in patients with systemic sclerosis.
16. Follow-up testing: None required

**Centromere**
17. Report as: Centromere
18. Suspected antigen specificity: Centromere protein A, B, or C
19. Clinical significance: Seen in 57 - 86% of patients with the limited form of scleroderma (CREST).
20. Follow-up testing: None required, pattern confirmed by staining pattern

**Bi-70**
21. Report as: Homogenous, speckled, and nucleolar
22. Suspected antigen specificity: Topoisomerase I
23. Clinical significance: Topoisomerase I positive marker antibody seen in 15 - 25% of patients with scleroderma.
24. Follow-up testing: Confirm by ENA testing

**PCNA**
25. Report as: Speckled, possible PCNA
26. Suspected antigen specificity: DNA polymerase B (3’-5’)
27. Clinical significance: PCNA positive marker antibody seen in 2 - 10% of SLE patients
28. Follow-up testing: Confirm by ENA testing

**Mixed ANA pattern**
29. Report as: Homogenous and Specified
30. Mixed ANA patterns can be caused by autoantibodies to several different antigens in one antibody to all antigens located in several different areas of the cell nucleus. Testing and appropriate confirmatory testing for each pattern is recommended.

**Mixed ANA pattern on HEp-2000B**
31. Report as: Homogenous and SSA/Ro
32. Suspected antigen specificity: SSA/Ro-confirmed by pattern
33. Homogeneous—nDNA, Histone or others?

**Mixed ANA pattern on HEp-2000B**
34. Report as: Specified and SSA/Ro
35. Suspected antigen specificity: SSA/Ro—confirmed by pattern
36. SSA/Ro—confirmed by pattern
37. Follow-up testing: None required, pattern confirmed by staining pattern

**Mixed ANA pattern on HEp-2000B**
38. Report as: Specified and SSA/Ro
39. Suspected antigen specificity: SSA/Ro—confirmed by pattern
40. SSA/Ro—confirmed by pattern
41. Follow-up testing: None required, pattern confirmed by staining pattern