EDUCATIONAL COMMENTARY – AUTOIMMUNE DISORDERS

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Learning Outcomes

Upon completion of this exercise, the participant will be able to:

- State the ANA pattern(s) associated with common autoimmune disorders.
- Discuss the follow-up testing to be performed after a positive pattern is detected.

Laboratory diagnosis of many autoimmune diseases is accomplished through the use of antinuclear antibody testing. Antinuclear antibodies are commonly detected using latex agglutination, indirect immunoflorescence (IFA), or enzyme immunoassay (EIA) techniques.

The Systemic Lupus Erythematosus (SLE) Latex Agglutination test is a screening test that detects autoantibodies to deoxyribonucleoprotein (anti-DNP) in patient serum. The technique is qualitative, yielding either a positive or a negative result. A positive test (agglutination) indicates that the sample has anti-DNP at a concentration commonly found in patients with SLE. All positive specimens should be referred for quantitative testing.

Quantitative testing using indirect immunofluorescence assay (IFA) may be helpful in differentiating several autoimmune disorders. Autoantibodies may be produced against many components of the cell nucleus, such as DNA, histone, or nuclear proteins. The substrate most commonly utilized in the IFA procedure is composed of human epithelial cells. Patient serum is added to a slide that bears the substrate, then is washed and incubated with antihuman globulin (AHG) tagged with an immunofluorescent dye. The slide is then viewed under a fluorescence microscope and the pattern of immunofluorescence is evaluated.

If antinuclear antibodies are present in the patient specimen, the ANAs will attach to the nuclei of the epithelial cells on the slide and the tagged AHG will subsequently attach to the ANA. The test pattern produced is read as homogeneous (diffuse), peripheral rim, speckled, or nucleolar. Using human epithelial cells as a substrate, peripheral rim patterns will appear as homogeneous patterns. Serial dilution (1:40, 1:80, 1:160, etc.) should be performed on positive samples and an ANA titer determined by reading the highest dilution which gives a positive result. The higher the titer, the more likely a clinically significant disease is present. Healthy individuals may produce low titers of ANA.

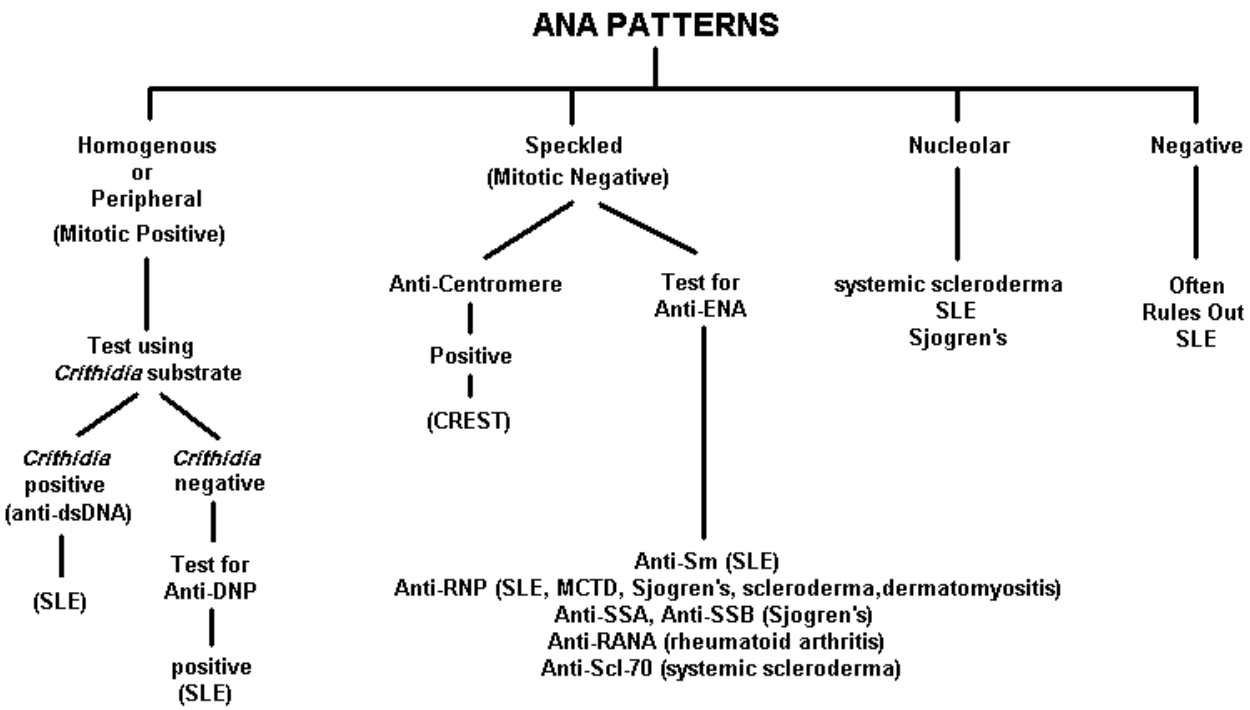
Positive ANAs occur in rheumatic diseases, inflammatory diseases, and infectious diseases. Autoimmune disorders are associated with each of the patterns described below. More than one pattern may be present in a patient's sample. Patients with systemic lupus erythematosus often produce multiple antinuclear antibodies that may be positive in any of the following ANA patterns. Please see the attached chart for assistance in associating autoimmune disorders with ANA patterns and determining appropriate follow-up testing.

1. **Homogeneous Pattern.** High titers of homogeneous pattern ANAs are suggestive of SLE. Low titers may also be found in SLE, Sjogren's syndrome, mixed connective tissues diseases (MCTD), and rheumatoid arthritis. Mitotic cells are positive for fluorescence in homogeneous patterns and their presence is helpful in differentiating a homogeneous pattern from a finely speckled pattern. Using the organism *Crithidia luciliae* as a substrate, positive staining of its kinetoplast demonstrates antinuclear antibodies to double- stranded DNA (ds-DNA). High titers occur in patients with severe SLE.

2. **Speckled Pattern.** This pattern occurs in CREST syndrome (milder form of scleroderma), SLE, mixed connective tissue disease, Sjogren's syndrome, rheumatoid arthritis, and scleroderma. Mitotic cells are negative for fluorescence in speckled patterns. To differentiate among the autoimmune disorders that produce speckled ANA patterns further testing should be performed. Substrates for saline extractable nuclear antigens (ENA) are utilized. Several techniques are available for extractable nuclear antibody testing including IFA, enzyme immunoassay, radioimmunoassay, and radial immunodiffusion. Extractable nuclear antigens include Smith (Sm), ribonucleoprotein (RNP), SSA, SSB, rheumatoid arthritis nuclear antigen (RANA), and Scl-70. Anti-Sm is highly suggestive of SLE. Anti-RNP is found in many rheumatic diseases such as SLE, RA, Sjogren's, scleroderma, MCTD, and dermatomyositis. Anti-SSA and anti-SSB indicate Sjogren's syndrome. Anti-RANA is found in patients with rheumatoid arthritis, and anti-Scl-70 is found in 15-20% of scleroderma patients.

3. **Nucleolar pattern.** This pattern is found in patients with scleroderma, SLE, and Sjogren's syndrome.

Indirect immunofluorescent antibody assay and its interpretation are complex and should only be performed by laboratory personnel who have had extensive training in evaluating the staining patterns produced by this procedure. Careful attention to manufacturer's protocol for performing testing is essential.


ANA PATTERNS
Homogenous or Peripheral (Mitotic Positive)
Test using *Crithidia* substrate
Crithidia positive (anti-dsDNA)
(SLE)
Crithidia negative
Test for Anti-DNP
positive (SLE)
Speckled (Mitotic Negative)
Anti-Centromere
Positive
(CREST)
Test for Anti-ENA
Anti-Sm (SLE)
Anti-RNP (SLE, MCTD, Sjogren's, scleroderma,dermatomyositis)
Anti-SSA, Anti-SSB (Sjogren's)
Anti-RANA (rheumatoid arthritis)
Anti-Scl-70 (systemic scleroderma)
Nucleolar
systemic scleroderma
SLE
Sjogren's
Negative
Often Rules Out SLE