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## Rheumatic Disease Laboratory

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## ANTINUCLEAR ANTIBODY TESTING IN THE SYSTEMIC RHEUMATIC DISEASES

Autoimmune disease is characterized by the development of autoantibodies against specific self-antigens. While these autoantibodies may or may not be involved in pathology, they are often useful diagnostic markers for a given disorder. **Antinuclear antibodies (ANA)** are characteristic of the systemic rheumatic diseases and testing for this family of antibodies is indicated for the evaluation of patients with suspected systemic rheumatic disease and for monitoring patients with evolving or established conditions. An elevated ANA titer is a sensitive (*though not specific*) indicator for systemic rheumatic disease and the observed pattern of nuclear/cytoplasmic staining can provide additional clinical information. At FBR, we measure ANA by indirect immunofluorescence using Hep2 cells and define an elevated ANA titer as  $\geq 1:256$  in adults ( $>20$  years) and  $\geq 1:128$  in children and adolescents; these values represent the 98<sup>th</sup> centile in a normal population (Craig et al., 1999).

Although an abnormal ANA result may be due to autoantibodies against a wide variety of antigens, certain reactivities have specific clinical associations. **Anti-double stranded DNA (dsDNA)** is a specific finding in systemic lupus erythematosus (SLE), associated with more severe disease and nephritis, and can be useful in monitoring disease activity. Autoantibodies against **extractable nuclear antigens (ENA)** provide important diagnostic information for several diseases (von Mühlen and Tan, 1995; Hoffman *et al.*, 2004, Hasegawa *et al.*, 1998). While most frequently observed in sera that demonstrate a speckled ANA pattern, ENA reactivity may also be identified in sera with low ANA titers or with cytoplasmic staining, depending on the subcellular distribution and concentration of the antigen target. ENA reactivity observed in an individual with systemic rheumatic disease may change over time, as the condition evolves. At FBR, ENA are assayed by immunoblotting (Ritchie et al., 1990).

**Indications for testing\*:** ANA testing is recommended to evaluate patients suspected of having a systemic rheumatic disease. Periodic testing may be indicated, even when ANA is negative, due to the evolution of disease expression over time. Laboratory results must be interpreted in the context of clinical findings; the following conditions are associated with positive ANA results:

*Systemic lupus erythematosus*  
*Drug-associated lupus*  
*Autoimmune liver disease*

*Systemic sclerosis*  
*Sjögren's syndrome*  
*Raynaud's phenomenon*

*Polymyositis –dermatomyositis*  
*Mixed connective tissue disease*  
*Juvenile chronic arthritis*

ENA testing is recommended for the further evaluation of patients with a positive ANA result and with clinical findings suggestive of a specific systemic rheumatic disease. dsDNA testing is recommended when SLE is a clinical concern.

**Specimen requirements:** Serum (2 mL, adults; 0.2 mL, child); room temperature or 4°C

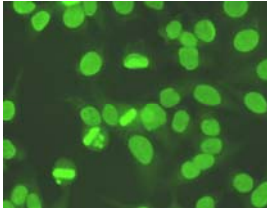
**Analytic time:** 48 hours

**CPT codes:** ANA screen, 86038; ANA titer\*\*, 86039; dsDNA screen, 86225; dsDNA titer\*\*, 86256; ENA 86235 (1x per antigen tested).

\* Kavanaugh *et al.*, 2000; Solomon *et al.*, 2002 (Guidelines developed by the College of American Pathologists in collaboration with the American College of Rheumatology and the National Institutes of Health)

\*\*Titering is performed on all samples that are ANA screen positive (titer  $>1:32$ )

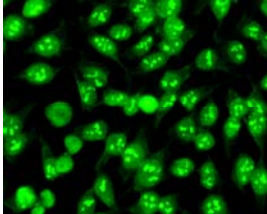
## ANA PATTERNS: CLINICAL ASSOCIATIONS



### Homogeneous ANA pattern

Antigens: dsDNA, ssDNA, histones, and chromatin.

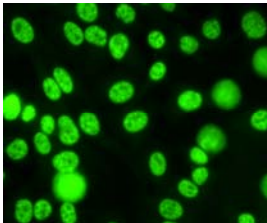
**Clinical associations:** systemic lupus erythematosus (SLE); drug-induced lupus; drug-induced, patient asymptomatic



### Nucleolar ANA pattern

Antigens: Scl-70, NOR-90, RNA polymerase, fibrillarin

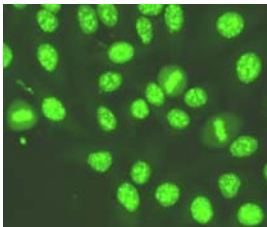
**Clinical associations:** SLE, scleroderma, systemic sclerosis



### Speckled ANA pattern

Antigens: Sm, RNP, SSA, SSB, other

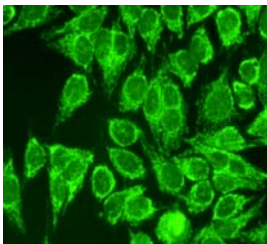
**Clinical associations:** SLE, systemic sclerosis, Sjogren's syndrome, rheumatoid arthritis, other connective tissue diseases



### Centromere ANA pattern

Antigens: mitotic cell kinetochore, CENP-A, CENP-B, CENP-B

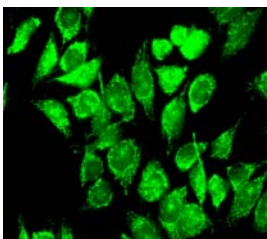
**Clinical association:** 50-70% of patients with CREST syndrome (calcinosis, Raynaud's phenomenon, sclerodactyly, telangiectasis); 25% of patients with Raynaud's disease. Also found in patients with thoracic complaints of unknown etiology



### Cytoplasmic pattern

Antigens: multiple cytoplasmic structures or proteins

**Clinical associations:** SLE; autoimmune hepatitis (anti-mitochondrial antibodies, see left); polymyositis (anti-Jo-1); systemic sclerosis/Sjogren's syndrome (anti-SSA); rheumatoid arthritis



### Nuclear dot pattern

Antigens: p80 coilin, M2-mitochondrial protein

**Clinical associations:** primary biliary cirrhosis; chronic active hepatitis; has been reported in SLE

## ENA: CLINICAL ASSOCIATIONS

### Sm

The Smith antigen (Sm) includes the U series of small nuclear ribonucleoproteins, which are involved in the conversion of hnRNA to mRNA by splicing.

- highly specific for SLE
- observed in 40% of SLE patients
- associated with a high frequency of anti-dsDNA antibodies and nephritis
- causes speckled ANA pattern
- levels fluctuate with disease activity

### RNP

The RNP antigen includes a series of proteins comprising U1-RNP (ribonucleoprotein).

- SLE ( $\leq 40\%$ ) (associated with Raynaud's phenomenon)
- scleroderma (15%)
- rheumatoid arthritis or Sjögren's syndrome (5%).
- high ANA titers due to RNP suggest mixed connective tissue disease
- causes speckled ANA pattern

### SSA

The SSA antigen (also referred to as Ro) is a small ribonucleoprotein associated with hY RNA.

- Sjögren's syndrome (70%) (associated with vasculitis)
- SLE (30-40%) (associated with xerostomia, increased incidence of anti-dsDNA and nephritis)
- associated with fetal heart block and neonatal lupus (85%) when present in maternal blood
- cytoplasmic, speckled nuclear pattern, or *negative* in the ANA assay
- levels fluctuate with disease activity

### SSB

The SSB antigen (also referred to as La) is a 47 kDa peptide that acts as a termination factor for the RNA polymerase III complex.

- Sjögren's syndrome (50-60%)
- SLE (15%) (associated with pericarditis and xerostomia)
- may indicate a milder form of SLE
- associated with later onset SLE (>50 years) when present with SSA

### Sci-70

The Sci-70 antigen is DNA topoisomerase I, which is associated with chromatin and relaxes supercoiled DNA.

- highly specific for scleroderma/systemic sclerosis
- observed in 15-20% of scleroderma/systemic sclerosis patients
- observed in 25% of SLE patients
- prevalence higher in more severe diffuse or progressive systemic disease than in the milder CREST variant
- fine speckled or nucleolar staining in the ANA assay

### Jo-1

The Jo-1 antigen is histidyl tRNA synthetase.

- specific for autoimmune myositis, particularly polymyositis
- observed in 30-40% of polymyositis patients
- observed in 5% of dermatomyositis patients
- may be associated with pulmonary fibrosis
- cytoplasmic and nuclear staining in the ANA assay, often at low titer

### Histones

Histones are the structural protein components of chromatin; H2A, H2B, H3, and H4, together with DNA, form the nucleosome unit.

- SLE (30%) (associated with arthritis)
- drug-induced lupus (95%)
- drug induced serologic response, but patient asymptomatic
- reported in rheumatoid arthritis and in systemic sclerosis, where H1 is associated with milder and H2B with more severe clinical features
- homogeneous ANA pattern

## References

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### CURRENT RDL RESEARCH

#### Serologic Associations of Anti-Cytoplasmic Antibodies in ANA-negative sera.

**Purpose:** To examine the frequency of specific autoantibodies among subjects with ANA-negative sera that demonstrate an undefined pattern of cytoplasmic staining.

**Method:** We collected 200 sera with an ANA titer  $\leq 1:32$  and a cytoplasmic (undefined pattern) titer of  $\geq 1:64$ , identified sequentially among 12,740 sera received for routine ANA testing. These sera were assayed for ENA: (Sm, RNP, SSA, SSB, Jo-1, Scl-70, histones), anti-neutrophil cytoplasmic antibodies (ANCA), anti-mitochondrial antibodies (AMA), anti-thyroid microsomal antibodies (AMSA), anti-thyroglobulin antibodies (ATGA), anti-parietal cell antibodies (APCA), and anti-smooth muscle antibodies (ASMA).

**Results:** Eighty five sera (42.5%) were positive in one ( $n=57$ ) or more ( $n=28$ ) of the serologic tests performed. Autoantibodies identified were AMA (15%), AMSA (13%), ANCA (10%), ASMA (6%), APCA (4%), and ENA (8.5%, including histones, SSA, SSB, Sm, Jo-1 and Scl-70). A positive result in one or more of these assays was more frequent at anti-cytoplasmic titers  $\geq 1:1024$  (77.8%) than at titers of 1:64-1:128 (7%) ( $\chi^2 = 25.3$ ,  $p < 0.001$ ).

**Discussion:** There are currently no guidelines concerning additional testing among ANA-negative sera with undefined anti-cytoplasmic antibodies, identified in the context of routine ANA testing.

- The present data demonstrate that undefined anti-cytoplasmic staining in ANA-negative sera is associated with a high frequency of specific autoantibodies.
- When routine ANA testing identifies an ANA-negative serum with undefined cytoplasmic staining, decisions on further laboratory testing must rely primarily on clinical judgment.
- Further work is needed to examine the clinical implications of identifying specific autoantibodies (particularly ANCA, AMSA, and AMA) in this laboratory context.