Antinuclear Antibody (ANA) With HEp-2 Substrate by IFA

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Antinuclear antibody (ANA) testing is used in the diagnostic evaluation of various autoimmune diseases, including connective tissue diseases such as systemic lupus erythematosus (SLE), Sjögren syndrome, and systemic sclerosis (SSc).[1] Initial testing for autoimmune connective tissue diseases (also referred to as systemic autoimmune rheumatic diseases, or SARDs) should include tests for C-reactive protein (CRP), ANAs, rheumatoid factor, and cyclic citrullinated peptide antibodies. If ANA results are positive, follow-up or confirmatory testing may be guided by the pattern(s) observed and/or the patient's clinical presentation.

Featured ARUP Testing

Antinuclear Antibody (ANA) with HEp-2 Substrate, IgG by IFA with Reflex by Pattern 3000601

Method: Semi-Quantitative Indirect Fluorescent Antibody/Qualitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Multiplex Bead Assay/Qualitative Immunoblot

- Initial screening test for connective tissue diseases (SARDs).
- One or more reflexive tests may be added, depending on ANA pattern detected (see ANA IFA Reflex Testing Algorithm).

Antinuclear Antibody (ANA) with HEp-2 Substrate, IgG by IFA 3000082

Method: Semi-Quantitative Indirect Fluorescent Antibody (IFA)

- Preferred screening test for SARD.
- Reported patterns may help guide differential diagnosis, but may not be specific for individual antibodies or diseases.
- Negative results do not necessarily rule out SARD.

Disease Overview

Diagnostic Issues

Autoimmune connective tissue diseases may present with similar features, making diagnosis difficult. Possible diagnoses may include:

- Inflammatory myopathies
- Mixed connective tissue disease
- SSc
- Sjögren syndrome
- SLE
- Undifferentiated connective tissue disease

ANA with reflex by immunofluorescent assay (IFA) (based on ANA patterns) may help guide differential diagnosis but may not be specific for individual diseases.

Pathophysiology

Antigen/antibody complexes affect a variety of organs in connective tissue diseases, which frequently leads to a multisystem disease presentation. ANA antibodies are the most common antibodies and may precede the onset of connective tissue disease. Although certain antibodies may show specificity for certain diseases (e.g., SSA 52, SSA 60, and SSB antibodies for Sjögren syndrome), ANA antibodies are not specific for connective tissue disease, and may also be associated with infectious diseases, cancers, other autoimmune disorders (e.g., autoimmune liver disease), and advanced age, and may even be present in healthy patients.

Test Interpretation

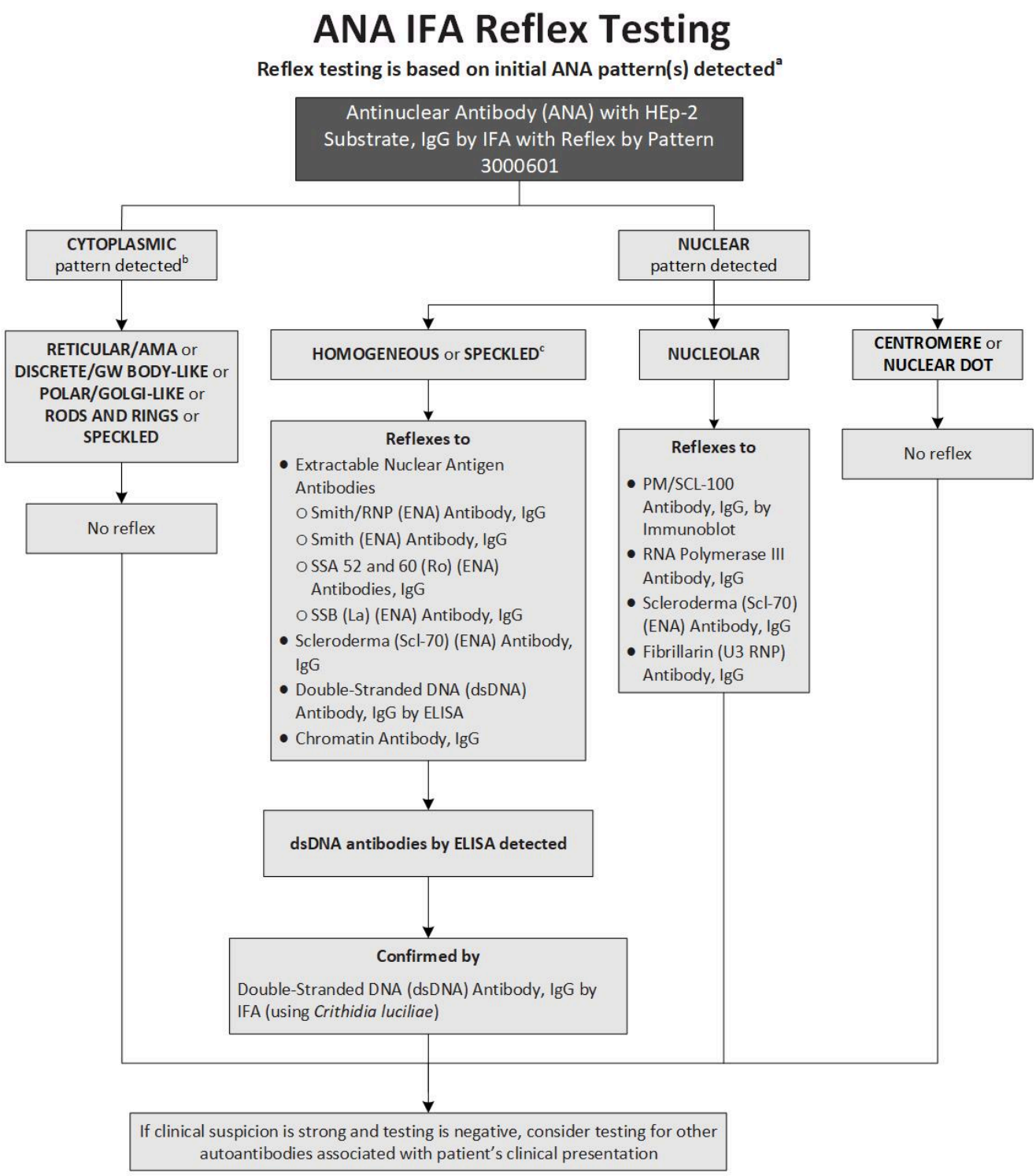
Results

A dual or mixed pattern may indicate disease overlap. Visit the International Consensus on Antinuclear Antibody Patterns website[2] for additional information about pattern and disease associations.

Limitations

- Dual or mixed patterns will not be reflexed; additional testing for dual or mixed patterns should be determined by the ordering physician.
- A negative ANA by IFA test does not rule out the presence of connective tissue disease.

ANA IFA Reflex Testing Algorithms



[a]If more than one pattern is observed (homogenous or speckled and nucleolar), reflex testing will be performed for both patterns; however, no duplicate testing will be performed.

[b]If a cytoplasmic pattern is reported, consider ordering the Ribosomal P Protein Antibody test (for systemic lupus erythematosus), the Polymyositis Panel (for myositis), and/or the Mitochondrial M2 Antibody, IgG (ELISA) test (for primary biliary cholangitis).

[c]If the speckled pattern is detected and reflex tests are negative, consider ordering the Dermatomyositis Autoantibody Panel, Extended Myositis Panel, or RNA Polymerase III Antibody, IgG, if clinically indicated.

See the following ARUP Consult algorithms for ANA testing and expanded information on cytoplasmic and nuclear patterns:

Antinuclear Antibody Disease Testing Algorithm

Antinuclear Antibody Disease Testing - Nuclear Patterns

Antinuclear Antibody Disease Testing - Cytoplasmic Patterns

References

1. Tebo AE. Recent approaches to optimize laboratory assessment of antinuclear antibodies. *Clin Vaccine Immunol.* 2017;24(12):e00270-17.

2. International Consensus on Antinuclear Antibody Patterns. Nuclear patterns. International Consensus on ANA Patterns. Accessed Jan 2026.

Related Information

Connective Tissue Diseases - Systemic Autoimmune Rheumatic Diseases
Extended Myositis Panel
Inflammatory Myopathies
Mixed Connective Tissue Disease - MCTD
Primary Biliary Cholangitis - PBC
Systemic Sclerosis - Scleroderma
Sjögren Syndrome
Systemic Lupus Erythematosus - SLE

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