

# Antinuclear Antibody (ANA) With HEp-2 Substrate

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).<sup>1</sup> 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.

## 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 (eg, 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 (eg, 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<sup>2</sup> 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

### Tests to Consider

#### [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

- 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.

#### [Antinuclear Antibodies \(ANA\), IgG by ELISA with Reflex to ANA, HEp-2 Substrate, IgG by IFA 0050080](#)

**Method:** Qualitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Indirect Fluorescent Antibody

Aids in initial diagnosis of connective tissue disease.

#### [Antinuclear Antibodies \(ANA\), IgG by ELISA with Reflex to ANA HEp-2 Substrate, IgG by IFA and ENA Confirmation 0050317](#)

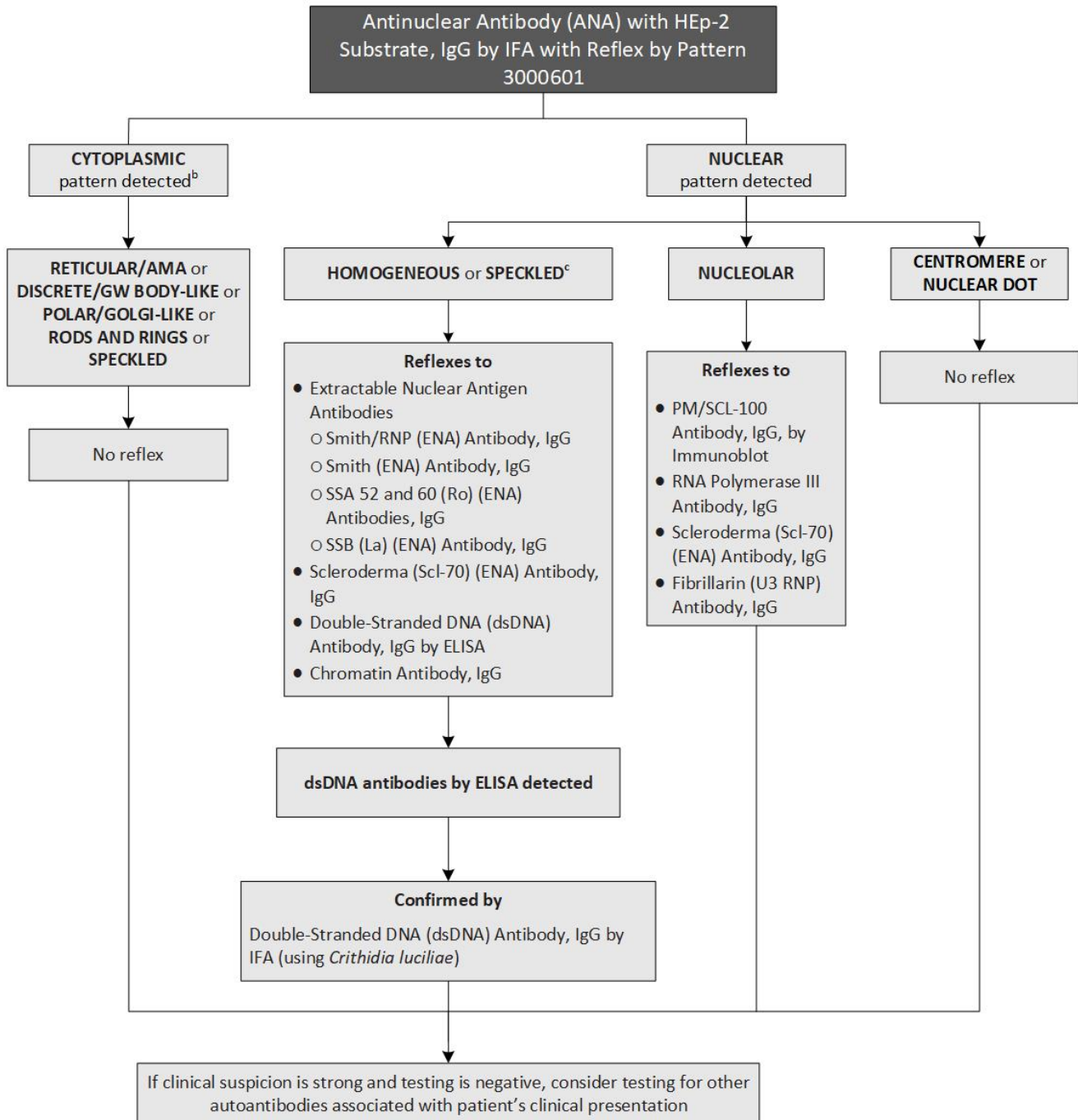
**Method:** Qualitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Indirect Fluorescent Antibody/Semi-Quantitative Multiplex Bead Assay/Semi-Quantitative Enzyme-Linked Immunosorbent Assay

Aids in initial diagnosis of connective tissue disease.

For other reflex tests, panel tests, and single tests, see [Related Tests](#).

# ANA IFA Reflex Testing

Reflex testing is based on initial ANA pattern(s) detected<sup>a</sup>



<sup>a</sup>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.

<sup>b</sup>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).

<sup>c</sup>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](#)

## 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. [Updated: 2019; Accessed: Mar 2021]

## Related Information

[Connective Tissue Diseases - Systemic Autoimmune Rheumatic Diseases](#)  
[Inflammatory Myopathies](#)  
[Mixed Connective Tissue Disease - MCTD](#)  
[Primary Biliary Cholangitis - PBC](#)  
[Systemic Sclerosis - Scleroderma](#)  
[Sjögren Syndrome](#)  
[Systemic Lupus Erythematosus - SLE](#)

## Related Tests

### [Lupus Comprehensive Reflexive Panel 0050119](#)

**Method:** Quantitative Immunoturbidimetry/Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Indirect Fluorescent Antibody/Quantitative Chemiluminescent Immunoassay/Semi-Quantitative Multiplex Bead Assay

### [Double-Stranded DNA \(dsDNA\) Antibody, IgG by ELISA with Reflex to dsDNA Antibody, IgG by IFA 0050215](#)

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Indirect Fluorescent Antibody

### [Double-Stranded DNA \(dsDNA\) Antibody, IgG by IFA \(using Crithidia luciliae\) 2002693](#)

**Method:** Semi-Quantitative Indirect Fluorescent Antibody

### [Rheumatoid Arthritis Panel with Reflex to Rheumatoid Factors, IgA, IgG, and IgM by ELISA 3004056](#)

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Quantitative Immunoturbidimetry

### [Connective Tissue Diseases Profile 0051668](#)

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Multiplex Bead Assay

### [Smith and Smith/RNP \(ENA\) Antibodies, IgG 3000460](#)

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Semi-Quantitative Multiplex Bead Assay

### [Extractable Nuclear Antigen Antibodies \(Smith/RNP, Smith, SSA 52, SSA 60, and SSB\) 0050652](#)

**Method:** Quantitative Enzyme-Linked Immunosorbent Assay/Quantitative Multiplex Bead Assay

### [SSA 52 and 60 \(Ro\) \(ENA\) Antibodies, IgG 2012074](#)

**Method:** Semi-Quantitative Multiplex Bead Assay

### [Rheumatoid Arthritis Panel 3004055](#)

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Quantitative Immunoturbidimetry

### [Interstitial Lung Disease Autoantibody Panel 3001784](#)

**Method:** Qualitative Immunoprecipitation/Semi-Quantitative Multiplex Bead Assay/Qualitative Immunoblot/Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Quantitative Immunoturbidimetry

### [Extended Myositis Panel 3001781](#)

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay/Qualitative Immunoprecipitation/Semi-Quantitative Multiplex Bead Assay/Qualitative Immunoblot

### [Dermatomyositis and Polymyositis Panel 3001783](#)

**Method:** Qualitative Immunoprecipitation/Semi-Quantitative Multiplex Bead Assay/Qualitative Immunoblot

### [Polymyositis Panel 2013990](#)

**Method:** Qualitative Immunoprecipitation/Semi-Quantitative Multiplex Bead Assay

### [Dermatomyositis Autoantibody Panel 3001782](#)

**Method:** Qualitative Immunoprecipitation/Qualitative Immunoblot

Comprehensive Systemic Sclerosis Panel 3000480

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

Criteria Systemic Sclerosis Panel 3000479

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

Chromatin Antibody, IgG 2005287

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay

Smith (ENA) Antibody, IgG 0050085

**Method:** Semi-Quantitative Multiplex Bead Assay

SSB (La) (ENA) Antibody, IgG 0050692

**Method:** Semi-Quantitative Multiplex Bead Assay

Smith/RNP (ENA) Antibody, IgG 0050470

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay

Ribosomal P Protein Antibody 0099249

**Method:** Semi-Quantitative Multiplex Bead Assay

Jo-1 Antibody, IgG 0099592

**Method:** Semi-Quantitative Multiplex Bead Assay

Centromere Antibody, IgG 0050714

**Method:** Semi-Quantitative Multiplex Bead Assay

Scleroderma (Scl-70) (ENA) Antibody, IgG 0050599

**Method:** Semi-Quantitative Multiplex Bead Assay

RNA Polymerase III Antibody, IgG 2001601

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay

PM/Scl-100 Antibody, IgG by Immunoblot 2003040

**Method:** Qualitative Immunoblot

Fibrillarin (U3 RNP) Antibody, IgG 2012173

**Method:** Qualitative Immunoblot

3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (HMGCR) Antibody, IgG 2013101

**Method:** Semi-Quantitative Enzyme-Linked Immunosorbent Assay

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