

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).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.

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](#) website² 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

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

Aids in initial diagnosis of connective tissue disease

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

ANA IFA Reflex Testing Algorithms

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. PubMed
2. International Consensus on Antinuclear Antibody Patterns. [Nuclear patterns](#). International Consensus on ANA Patterns. Gainesville, FL. [Updated: 2019; Accessed: Jun 2020]

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: Qualitative 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 2003278](#)

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

[Connective Tissue Diseases Profile 0051668](#)

Method: Semi-Quantitative Multiplex Bead Assay

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

Method: Semi-Quantitative Multiplex Bead Assay

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

Method: Semi-Quantitative Multiplex Bead Assay

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

Method: Semi-Quantitative Multiplex Bead Assay

[Rheumatoid Arthritis Panel 2003277](#)

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

ARUP Laboratories is a nonprofit enterprise of the University of Utah and its Department of Pathology, 500 Chipeta Way, Salt Lake City, UT 84108
(800) 522-2787 | (801) 583-2787 | aruplab.com | arupconsult.com
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