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). 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 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

Reflex testing is based on initial ANA pattern(s) detected

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

- CYTOPLASMIC pattern detected
  - RETICULAR/AMA or DISCRETE/GW BODY-LIKE or POLAR/GOLGI-LIKE or RODS AND RINGS or SPECKLED
    - No reflex

- NUCLEAR pattern detected
  - HOMOGENOUS or SPECKLED
    - Reflexes to
      - Extractable Nuclear Antigen Antibodies
        - Smith/RNP (ENA) Antibody, IgG
        - Smith (ENA) Antibody, IgG
        - SSA 52 and 60 (Ro) (ENA) Antibodies, IgG
        - SSB (La) (ENA) Antibody, IgG
        - Scleroderma (Scl-70) (ENA) Antibody, IgG
        - Double-Stranded DNA (dsDNA) Antibody, IgG by ELISA
        - Chromatin Antibody, IgG
    - dsDNA antibodies by ELISA detected
      - Confirmed by
        - Double-Stranded DNA (dsDNA) Antibody, IgG by IFA (using Crithidia luciliae)

- NUCLEOLAR
  - Reflexes to
    - PM/SCL-100 Antibody, IgG, by Immunoblot
    - RNA Polymerase III Antibody, IgG
    - Scleroderma (Scl-70) (ENA) Antibody, IgG
    - Fibrillarin (U3 RNP) Antibody, IgG
  - CENTROMERE or NUCLEAR DOT
    - No reflex

If clinical suspicion is strong and testing is negative, consider testing for other autoantibodies associated with patient's clinical presentation

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.

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).

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


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

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

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

Criteria Systemic Sclerosis Panel 3000479

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