

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

## Indications for Ordering

Initial screen for autoimmune connective tissue diseases

## Test Description

Semiquantitative indirect fluorescent antibody (IFA)

- Detects IgG antinuclear antibodies (ANA) using HEp-2 substrate and IgG-specific conjugate

## Tests to Consider

### Typical testing strategy

- Initial testing
  - C-reactive protein (CRP)
  - Antinuclear antibody (ANA) with HEp-2 Substrate, IgG with Reflex by Pattern
  - Rheumatoid factor/cyclic citrullinated peptide
- Follow-up testing based on ANA pattern
  - Single test or reflex testing

### Primary test

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

- One or more reflexive tests may be added, depending on ANA pattern detected (see algorithm)

### Related primary tests

- [Antinuclear Antibody \(ANA\) with HEp-2 Substrate, IgG by IFA 3000082](#)
- [Antinuclear Antibodies \(ANA\), IgG by ELISA with Reflex to ANA, HEp-2 Substrate, IgG by IFA 0050080](#)

### Related secondary tests

Reflex testing

- [Lupus Comprehensive Reflexive Panel 0050119](#)
- [Double-Stranded DNA \(dsDNA\) Antibody, IgG by ELISA with Reflex to dsDNA Antibody, IgG by IFA 0050215](#)
- [Double-Stranded DNA \(dsDNA\) Antibody, IgG by IFA \(using \*Crithidia luciliae\*\) 2002693](#)
- [Rheumatoid Arthritis Panel with Reflex to Rheumatoid Factors, IgA, IgG, and IgM by ELISA 2003278](#)

Panel tests

- [Connective Tissue Diseases Profile 0051668](#)
- [Smith and RNP \(U1\) \(ENA\) Antibodies, IgG 3000460](#)
- [Extractable Nuclear Antigen Antibodies \(RNP, Smith, SSA 52, SSA 60, and SSB\) 0050652](#)
- [SSA 52 and 60 \(Ro\) \(ENA\) Antibodies, IgG 2012074](#)
- [Rheumatoid Arthritis Panel 2003277](#)
- [Interstitial Lung Disease Panel 2013993](#)
- [Myositis Extended Panel 2013961](#)
- [Polymyositis and Dermatomyositis Panel 2013992](#)
- [Polymyositis Panel 2013990](#)
- [Dermatomyositis Panel 2013991](#)
- Comprehensive [Systemic Sclerosis Panel 3000480](#)
- Criteria [Systemic Sclerosis Panel 3000479](#)

Single tests

- [Chromatin Antibody, IgG 2005287](#)
- [Smith \(ENA\) Antibody, IgG 0050085](#)
- [SSB \(La\) \(ENA\) Antibody, IgG 0050692](#)
- [RNP \(U1\) \(Ribonucleic Protein\) \(ENA\) Antibody, IgG 0050470](#)
- [Ribosomal P Protein Antibody 0099249](#)
- [Jo-1 Antibody, IgG 0099592](#)
- [Centromere Antibody, IgG 0050714](#)
- [Scleroderma \(Scl-70\) \(ENA\) Antibody, IgG 0050599](#)
- [RNA Polymerase III Antibody, IgG 2001601](#)
- [PM/Scl-100 Antibody, IgG by Immunoblot 2003040](#)
- [Fibrillarin \(U3 RNP\) Antibody, IgG 2012173](#)
- [3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase \(HMGCR\) Antibody, IgG 2013101](#)

## Disease Overview

**Incidence** – 15-50/100,000 (depending on disease)

**Age of onset** – 15-40 years, with peak onset in 20s

**Sex** – M<F, 1:6-10

## Symptoms

- Cardiopulmonary
  - Pleuritis, pericarditis, fibrosis, chest pain
- Constitutional
  - Fever, anorexia, weight loss
- Dermatologic
  - Skin rashes, Raynaud phenomenon, photosensitivity
- Gastrointestinal
  - Gastroesophageal reflux disease
- Hematologic
  - Cytopenias – neutrophils, erythrocytes, platelets
- Musculoskeletal
  - Arthralgias, arthritis, synovitis, myopathy
- Neurologic
  - Seizures, encephalopathy
- Otorhinolaryngologic
  - Sicca syndrome, oral ulcers
- Renal
  - Proteinuria, glomerulonephritis

## Diagnostic issues

- Autoimmune connective tissue diseases may present with similar features, making diagnosis difficult
  - May include
    - Inflammatory myopathies
    - Mixed connective tissue disease
    - Scleroderma
    - Sjögren syndrome
    - Systemic lupus erythematosus
    - Undifferentiated connective tissue disease
- ANA with reflex by IFA (based on ANA patterns) may help in distinguishing between diseases

## Pathophysiology

- Antigen/antibody complexes affect a variety of organs in connective tissue diseases
  - Frequently leads to a multisystem disease presentation
- ANA antibodies are the most common antibodies and may precede the onset of connective tissue disease
- 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)
  - Advanced age

## Test Interpretation

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### Results

Dual or mixed pattern may indicate disease overlap

### Limitations

- Only cytoplasmic, nuclear mitotic apparatus (NuMA), and/or nuclear dot pattern will be reported if observed
  - Titers are not performed
- 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 detected)

