Anti-Nuclear Antibody (ANA), IgG by IFA with Reflex by IFA 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 cell substrate

Tests to Consider

Typical testing strategy
  • Initial testing
    o C-reactive protein (CRP)
    o Anti-nuclear antibody (ANA), IgG with reflex by IFA pattern
    o Rheumatoid factor/cyclic citrullinated peptide
  • Follow-up testing based on ANA pattern
    o Single test or reflex testing

Primary test

Anti-Nuclear Antibody (ANA), IgG by IFA with Reflex by IFA Pattern 2008467
  • One or more reflexive tests may be added, depending on ANA pattern detected (see algorithm)

Related primary tests

• Nuclear Antibody (ANA) by IFA, IgG 0050639
• Anti-Nuclear Antibodies (ANA), IgG by ELISA with Reflex to ANA, 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
• Extractable Nuclear Antigen Antibodies (RNP, Smith, SSA 52, SSA 60, and SSB) 0050652
• SSA 52 and SSA 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
• Systemic Sclerosis Comprehensive Panel 2013325
• Systemic Sclerosis Panel 2012057

Single tests

• Chromatin Antibody, IgG 2005287
• Smith (ENA) Antibody, IgG 0050085
• SSA 52 and 60 (Ro) (ENA) Antibodies, IgG 2012074
• 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 (ScI-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**

**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
  - Titters 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)

Anti-Nuclear Antibody (ANA), IgG by IFA with Reflex by IFA Pattern 2008467

THE FOLLOWING REPORTED PATTERNS WILL NOT BE REFLECTED
- No pattern
- Centromere pattern
- Dual pattern
- Mixed pattern
- Rim/peripheral pattern
- Cytoplasmic pattern
- Nuclear mitotic apparatus protein (NuMA)
- Nuclear dot pattern

HOMOGENEOUS pattern detected

Reflexes to
- Double-Stranded DNA (dsDNA) Antibody, IgG by ELISA

AND
- Chromatin Antibody, IgG

dsDNA antibodies by ELISA detected

Confirmed by
- Double-Stranded DNA (dsDNA) Antibody, IgG by IFA (using Crithidia luciliae)

SPECKLED pattern detected

Reflexes to
- Extractable Nuclear Antigen Antibodies (RNP, Smith, SSA 52, SSA 60, and SSB)
  - RNP (U1) (Ribonucleic Protein) (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

NUCLEOLAR pattern detected

Reflexes to
- PM/SCL-100 Antibody, IgG by Immunoblot
- RNA Polymerase III Antibody, IgG
- Scleroderma (Scl-70) (ENA) Antibody, IgG

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