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

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)

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

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

HOMOGENOUS 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