The Pathology Center is standardizing our reporting format of antinuclear antibodies (ANA) with that of national reference laboratories. The current routine testing algorithm starts with the ANA Enzyme Immunoassay (ANA screen). If positive/indeterminate, an Indirect Fluorescent Assay by Hep 2 (IFA Hep 2) with titer and pattern is automatically added. The IFA Hep2 with titer and pattern will now be separately orderable if that is desired as an alternative first line test.

Previously, if the IFA failed to demonstrate a clinically significant titer, the entire assay was reported as negative. **Beginning 4/15/15, the ANA screen and IFA Hep 2 will be reported separately, regardless of the IFA Hep 2 result.** Interpretive comments based on titer results will also be added. Examples of the new reporting format are as follows:

**Negative by EIA**
- **ANA screen:** Negative
- **Interpretation:** No clinically significant antinuclear antibody is detected by EIA. A negative result suggests an absence of connective tissue disease. False negatives may occur, especially in scleroderma, polymyositis/dermatomyositis, or inactive systemic lupus erythematosus. If suspicion for connective tissue disease is strong, consider testing by disease-specific antibodies.

**Positive or indeterminate by EIA, no titer detected by IFA**
- **ANA screen:** Positive/Indeterminate
- **IFA Hep 2:** < 1:80
- **Interpretation:** No clinically significant antinuclear antibody is detected by IFA. A negative result suggests an absence of connective tissue disease. False negatives may occur, especially in scleroderma, polymyositis/dermatomyositis, or inactive systemic lupus erythematosus. False positive ANA EIA results can occur with age, certain infections, cancers, and drugs. If suspicion for connective tissue disease is strong, consider testing by disease-specific antibodies.

**Positive or indeterminate by EIA, titer ≥1:80 detected by IFA**
- **ANA screen:** Positive/Indeterminate
- **IFA Hep 2:** ≥1:80 (titer reported up to 1:320), pattern (i.e. centromere, speckled, etc.)
- **Interpretation:** Antinuclear antibody detected by IFA (≥1:80). Further characterization by disease-specific antibodies based on pattern may be helpful, if clinically indicated.

**Negative by IFA Hep2 (EIA not performed)**
- **IFA Hep 2:** < 1:80
- **Interpretation:** No clinically significant antinuclear antibody is detected by IFA. A negative result suggests an absence of connective tissue disease. False negatives may occur, especially in scleroderma, polymyositis/dermatomyositis, or inactive systemic lupus erythematosus.
Positive by IFA Hep2 (EIA not performed)

- **IFA Hep 2:** \( \geq 1:80 \) (titer reported up to 1:320), pattern (i.e. centromere, speckled, etc.)
- **Interpretation:** Antinuclear antibody detected by IFA (\( \geq 1:80 \)). Further characterization by disease-specific antibodies based on pattern may be helpful, if clinically indicated.

**Summary of available testing:**

- **ANA screen with reflex to IFA (Hep 2) with titer and pattern:** recommended initial screen
- **ANA IFA (Hep 2) with titer and pattern:** alternative initial screen
- **Individual ENAs and dsDNA:** follow up testing for positive ANA screen (send out tests to Reference Lab)
  - U1 ribonucleoprotein (RNP)
  - Scleroderma (Scl-70)
  - Smith (Sm)
  - SSA (Ro)
  - SSB (La)
  - Double stranded DNA (ds-DNA)

**NOTE:**
Very high positive IFA (Hep 2) results are reported as > 1:320. Endpoint titers are not routinely reported, but are available upon request. If this is needed, please contact the client support services at 402-354-4541 or 1-888-432-8980. Please note that titers do not necessarily correlate with severity of disease or response to therapy.

**REFERENCES:**
1) CLSI ILA2-A2. Quality Assurance for the Indirect Immunofluorescence Test for Autoantibodies to Nuclear Antigen (IF-ANA); Approved Guideline-Second Edition

If you have any questions, please contact Dr. Tess Karre, Director of Microbiology (402)354-4762, or Jennifer Krifka MLS(ASCP)\textsuperscript{cm}, Microbiology Service Leader (402)354-3147.
INDICATIONS FOR TESTING
Patient with systemic symptoms
(arthritis, arthralgias, skin rashes, anemia, renal dysfunction, pleuritis, pericarditis)

Anti-Nuclear Antibodies (ANA), IgG by ELISA with Reflex to ANA, IgG by IFA*

Negative

Possible scenarios
- No connective tissue disease (CTD)
- False-negative result – consider SSc, PM/DM or inactive SLE
- If suspicion for CTD is strong, consider testing for disease-specific antibody tests or panels

Positive

Nuclear Antibody (ANA) by IFA, IgG

Centromere pattern
- IcSSc, CREST

Cytoplasmic pattern
- PM/DM, SLE, SSc

Peripheral/rim/homogenous pattern
- SLE, DIL

Nucleolar pattern
- SLE, SSc, PM/DM

Speckled pattern
- SLE, SjS, MCTD/UCTD, dcSSc

- False-positive results may be induced by age, certain infections, cancers, and drugs
- ANA may be positive in inflammatory diseases such as autoimmune liver diseases

Antibody Key
- RNP (U1) (Ribonucleic Protein) (ENA) Antibody, IgG
- ScI-70 Scleroderma (ScI-70) (ENA) Antibody, IgG
- Sm Smith (ENA) Antibody, IgG
- SS-A SSA (Ro) (ENA) Antibody, IgG
- SS-B SSB (La) (ENA) Antibody, IgG

Disease legend
- CREST CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia)
- DIL Drug-induced lupus erythematosus
- dcSSc Diffuse cutaneous scleroderma
- IcSSc Limited cutaneous scleroderma
- MCTD/UCTD Mixed connective tissue disease/Undifferentiated connective tissue disease
- PM/DM Polymyositis/Dermatomyositis
- SjS Sjögren syndrome
- SLE Systemic lupus erythematosus
- SSc Scleroderma (systemic sclerosis)