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TECHNICAL BULLETIN

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ANA – Antinuclear Antibodies

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: ≥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.

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)

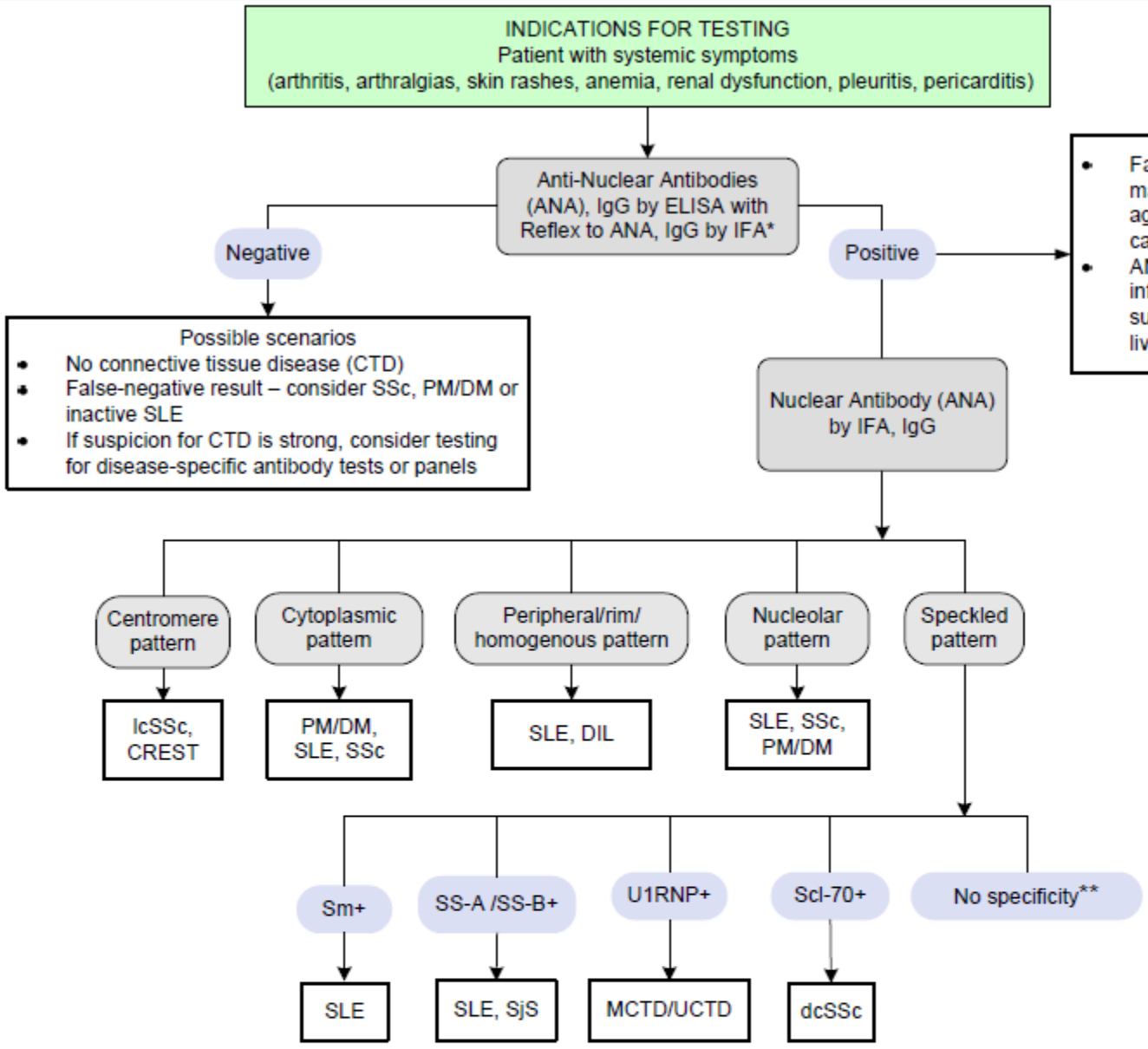
NOTE:

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
2) Kavanaugh et al. Guidelines for Clinical Use of the Antinuclear Antibody Test and Tests for Specific Autoantibodies to Nuclear Antigens. Arch Pathol Lab Med. Vol 124, January 2000

If you have any questions, please contact Dr. Tess Karre, Director of Microbiology (402)354-4762, or Jennifer Krifka MLS(ASCP)^{cm}, Microbiology Service Leader (402)354-3147.



Antibody Key

RNP	RNP (U1) (Ribonucleic Protein) (ENA) Antibody, IgG
Scl-70	Scleroderma (Scl-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
lcSSc	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)