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DynaLIFE MEDICAL LABS

Laboratory Bulletin

DATE:	28 November 2022
TO:	North Zone, Edmonton Zone, and Central Zone Physicians, Nurses and Healthcare Practitioners, and all Laboratory Services Staff
FROM:	Chemistry, Alberta Precision Laboratories (APL) and DynaLIFE Medical Labs (DL)
RE:	**Anti-Nuclear Antibodies (ANA) Test Harmonization and Improvements to Extractable Nuclear Antigen Antibodies (ENA) Test Order and Report**

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Key Message

- Effective December 5, 2022, ANA testing will be performed by the APL Calgary Diagnostic and Scientific Centre (DSC) Immunochemistry Laboratory. In addition, changes will be made to ANA and ENA testing to support provincial harmonization, international recommendations, and appropriate test utilization.
    1. Standardization of ANA and ENA test order
    2. Standardization of ANA specimen collection
    3. ANA results reported with titre, and interpretive comment
    4. Improvements to ENA reporting

Background

- A provincial ANA ENA working group was formed between laboratories and multidisciplinary clinical stakeholders to inform Alberta standard of care.
- Positive ANA results will be reported with titre in accordance with ICAP. For more information, see ANA Patterns. Interpretive comments assist with evaluating patients with high clinical suspicion for systemic autoimmune rheumatic disease and improve laboratory follow-up testing. See Appendix A for a table of standardized ANA IFA reporting comments.

How this will impact you

- Test order will determine method rather then collection site (DynaLIFE or APL)
    - ANA order = indirect immunofluorescence assay (IFA)
    - ENA order = multiplex bead immunoassay (MBIA)
- ENA and anti-dsDNA orders will continue to route to APL Edmonton UAH Special Chemistry Lab.
- For ANA, collect blood into a gold SST not red.

Table: ANA test order and method.

	Pre-Transition test referred to Edmonton	Post-Transition test referred to Calgary
Test Name	Anti-Nuclear Antibody (ANA) Panel	Anti-Nuclear Antibodies (ANA)
Performing Location	DynaLIFE Edmonton Base Lab/ APL Edmonton UAH Lab	APL Calgary DSC Immunochemistry Lab
Method	IFA/MBIA	IFA
Cells	HEp-2 cells	HEp-20-10 cells
Screen Dilution	1 in 80 all ages	1 in 40 pediatric, 1 in 80 adults

- ANA titre and twenty-two nuclear, cytoplasmic, and mitotic ICAP patterns with interpretive report comments for positive ANA tests are now available throughout the province (previously only in Calgary and South Zones).
- Expected ANA report turnaround time is within 1 week.
- Antibodies against Jo1, Scl-70, dsDNA, histone, and centromere B will be added to the ENA test.
- Individual test codes for Sjögrens Syndrome A (SS-A/RO) and B (SS-B/LA), Anti-Jo-1, Anti-Scl-70, Anti-Ribonucleoprotein, Anti-Smith, and Anti-Centromere B will be inactivated in Epic Beaker.
- Semi-quantitative ENA results (both normal and abnormal) will be reported.

Action Required

- Be aware of ANA and ENA test changes; see Test Directory for APL.
- Be aware of Rheumatology Choosing Wisely Canada guidelines: "*Don't order ANA as a screening test in patients without specific signs or symptoms of Systemic Lupus Erythematosus or another connective tissue disease".*

Questions/Concerns

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Reference

1. Agmon-Levin N, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis 2014;73:17-23.

Appendix A

ANA Pattern	ICAP Name	Interpretive Comment
Homogeneous	**AC-01**	Antibodies may be reactive with DNA, nucleosomes, or histones. These autoantibodies are associated with systemic lupus erythematosus (SLE), drug-induced lupus, and juvenile idiopathic arthritis.
Dense Fine Speckled (DFS)	**AC-02**	Potential antigen may be DFS70. Autoantibodies against DFS70 have been found in patients with atopic dermatitis, asthma, and interstitial cystitis, and in healthy individuals. They are rare in Sjogren's syndrome, systemic sclerosis, and SLE.
Centromere	**AC-03**	Antibodies against centromere antigens are characteristic for the limited form of progressive systemic sclerosis (CREST) and may be also found in patients with primary biliary cirrhosis.

Speckled	**AC-04/-05**	Antibodies may be reactive with SS-A/Ro, SS-B/La, Mi-2, TIF1alpha, TIF1beta, Ku, nuclear, ribonucleoproteins or RNA polymerase III. These autoantibodies are associated with Sjogren's syndrome, SLE, systemic sclerosis, mixed connective tissue disease (CTD), and dermatomyositis.
Multiple nuclear dots	**AC-06**	Antibodies may be reactive against Sp-100, PML proteins, or MJ/NXP-2. These autoantibodies are associated with primary biliary cirrhosis, systemic autoimmune diseases, and dermatomyositis.
Few nuclear dots	**AC-07**	Antibodies may be reactive against p80-coilin or Cajal bodies. These autoantibodies are associated with Sjogren's syndrome, SLE, systemic sclerosis, polymyositis, and asymptomatic individuals.
Nucleolar	**AC-08/-09**	Antibodies may be reactive against PM/Scl, Th/To, B23/nucleophosmin, nucleolin, No55/SC65, U3-snoRNP/fibrillarin, Scl-70. These autoantibodies are associated with systemic sclerosis and systemic sclerosis/polymyositis overlap syndrome.
Punctate nucleolar or nucleolus organizer region	**AC-10**	Antibodies may be reactive against RNA polymerase I and hUBF/NOR-90. The diagnostic significance of these autoantibodies remains unclear, but they have been seen in patients with systemic sclerosis, Raynaud's phenomenon, and Sjogren's syndrome.
Nuclear envelope (previously known as peripheral rim)	**AC-11/-12**	Antibodies may be reactive against lamins A, B, C, or lamin-associated proteins, nuclear pore complex proteins (i.e., gp210). These autoantibodies are associated with SLE, Sjogren's syndrome, seronegative arthritis, and primary biliary cirrhosis.
Proliferating cell nuclear antigen (PCNA)	**AC-13**	Antibodies may be reactive against proliferating cell nuclear antigen or other cell cycle-related proteins. These autoantibodies are associated with SLE and other autoimmune conditions.
Centromere-related protein (CENP-F)	**AC-14**	Antibodies may be reactive against the centromere-related protein CENP-F or Nsp II. These autoantibodies are associated with malignancy and other conditions.
Cytoskeletal	**AC-15/-16/-17**	Cytoplasmic fibrillar linear, filamentous, or segmental pattern. Antibodies may be reactive against a variety of antigens such as actin, non-muscle myosin, intermediate filaments (vimentin, cytokeratin), alpha-actinin, vinculin, or tropomyosin. These autoantibodies are associated with autoimmune hepatitis, chronic active hepatitis, liver cirrhosis, primary biliary cirrhosis, hepatitis C, mixed CTD, myasthenia gravis, Crohn`s disease, and ulcerative colitis.
Cytoplasmic dots	**AC-18**	Antibodies may be reactive against GW bodies. These autoantibodies are associated with Sjogren's syndrome, ataxia, and other neuropathies, SLE, primary biliary cirrhosis and other autoimmune conditions.

Cytoplasmic speckled	**AC-19/-20**	Antibodies may be reactive against ribosomal P or other related proteins (PL-7, PL-12, SRP), or Jo-1/histidyl-tRNA synthetase. These autoantibodies are associated with SLE, polymyositis and dermatomyositis, anti-synthetase syndrome, systemic sclerosis, and interstitial lung disease.
Cytoplasmic mitochondrial or reticular	**AC-21**	Antibodies may be reactive against mitochondria or endoplasmic reticulum. These autoantibodies are associated with primary biliary cirrhosis and overlap syndrome with autoimmune hepatitis.
Golgi or polar	**AC-22**	The diagnostic significance of autoantibodies reactive against the Golgi apparatus remains unclear, but they have low disease specificity to a variety of disease conditions including SLE, Sjogren's syndrome, rheumatoid arthritis, mixed CTD, systemic sclerosis, polymyositis, vasculitis, and viral infections.
Cytoplasmic rods and rings	**AC-23**	Antibodies may be reactive against IMPDH2 and other uncharacterized antigens. These autoantibodies are associated mainly in patients with hepatitis infections, particularly after treatment with interferon-alpha or ribavirin.
Centrosome	**AC-24**	The diagnostic significance of autoantibodies reactive against centrosomes remains unclear, but they have been seen in patients with systemic sclerosis, Raynaud's phenomenon, and infections.
Spindle fibers	**AC-25**	The diagnostic significance of autoantibodies reactive against mitotic spindle apparatus antigens remains unclear, but they have been seen in patients with Sjogren's syndrome, SLE, and other connective tissue diseases.
Nuclear mitotic apparatus (NuMA)	**AC-26**	The diagnostic significance of autoantibodies reactive against the nuclear mitotic apparatus or centrophilin antigens remains unclear, but they have been seen in patients with Sjogren's syndrome, SLE, CREST syndrome, other connective tissue diseases, primary biliary cirrhosis, infections, and malignancy.
Midbody	**AC-27**	The diagnostic significance of autoantibodies reactive against the midbody or stem body remains unclear, but they have been seen in patients with systemic sclerosis, Raynaud's phenomenon, and malignancies.
Chromosomal coat protein	**AC-28**	The diagnostic significance of autoantibodies reactive against the mitotic chromosomal coat remains unclear, but they have been seen in patients with discoid lupus erythematosus, chronic lymphocytic leukemia, Sjogren's syndrome, and polymyalgia rheumatica.

Abbreviations: ICAP – International Consensus on Antinuclear Antibody Pattern; CREST – Calcinosis, Raynaud Phenomenon, Esophageal Dysmotility, Sclerodactyly, and Telangiectasia; CTD – Connective Tissue Disease; SLE – Systemic Lupus Erythematosus