CLINICAL PATHOLOGY LABORATORIES
A Sonic Healthcare Clinical Laboratory

August 2022

Client Communication

New Anti-Nuclear Antibody (ANA) Test System

Effective August 8, 2022:

Clinical Pathology Laboratories is pleased to announce the implementation of the new Inova Diagnostics NOVA View indirect immunofluorescence (IFA) platform for the detection and titer of anti-nuclear antibodies (ANA) to assist in the diagnosis of systemic autoimmune rheumatic diseases (SARD). The NOVA View system includes an automated IFA microscopy technology to assess patient reactivity to the HEp-2 human epidermoid carcinoma cultured cell line. The platform applies digital image capture with FDA-approved proprietary mathematical algorithms that stores individual pixels of intranuclear light intensity. The system generates preliminary screen result interpretation, a digital estimate of end-point titer and a computer-assisted IFA pattern interpretation. *The American College of Rheumatology supports the immunofluorescence antinuclear antibody (ANA) test using Human Epithelial type 2 (HEp-2) substrate, as the gold standard for ANA testing. ACR notes that alternate bead-based or solid phase methods should demonstrate comparable sensitivity to IFA.* **The NOVA View platform includes changes to initial screen dilution, cellular substrate and reported patterns as given below.**

Notes on ANA testing:

- Up to 100-150 autoantigens are present in HEp-2 cell lines while multiplex or solid-phase immunoassays detect only auto-antibodies directed against the limited number (typically 8-10) of antigens incorporated in the assay.
- The individual antibodies directed to nuclear autoantigens may be more or less frequently associated with specific SARDs. Those disease associations are given in Table 1 below.
- Individual specific autoantibodies may be qualitatively or semi-quantitatively identified using multiplex bead-based immunoassays, solid-phase immunoassays, complementary IFA (e.g. Crithidia lucida) or radio-immunoassay methods. CPL offers a variety of ANA panels with reflex or non-reflex nuclear antibodies as given in the test menu at the end of this announcement.
- Specific ANA patterns by IFA may be associated with individual antibodies to specific nuclear autoantigens as given in Table 2 below.
- The International Consensus on ANA Patterns (ICAP; 12th International Workshop 2014) established a framework for consensus on nomenclature and classification of prevalent HEp-2 IFA patterns (See www.anapatterns.org).

Table 1: List of most frequent clinically relevant autoantigen targets and prevalence in autoimmune disease:

Marker	Disease	Prevalence	Reference
dsDNA	SLE	70-100%	2,3,4
U1RNP	SLE, MCTD	30-40%, 100%	5
Smith (Sm)	SLE	20-30%	5
SS-A/Ro	SLE, Sjogren Syndrome, neonatal SLE	40-50%, 60-75%, 86-96%	6,7

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SS-B	SLE, Sjogren Syndrome, neonatal SLE	~7%, ~30%, 78%	2,6,7
Scl-70	PSS	20-40%	8
Centromere B	Limited PSS, CREST	~48%	9
Jo-1	PM, DM	~24%	10

Notes: SLE (systemic lupus erythematosus), MCTD (mixed connective tissue disease), PSS (progressive systemic sclerosis), CREST (calcinosis, Raynaud, esophageal dysmotility, sclerodactyly, telangiectasia syndrome), PM (polymyositis), DM (dermatomyositis)

Table 2: Associations of HEp-2 IFA patterns with antibodies to specific nuclear antigens

IFA Pattern (ICAP nomenclature)	Representative autoantibodies to	Disease Associations
Nuclear homogeneous (AC-1)	dsDNA, ssDNA, chromatin, histones	Suggestive of SLE, or other connective tissue diseases
Nuclear dense fine speckled (DFS, AC-2)	DFS70	Suggestive of SLE, often nonspecific, particularly at low titers
Centromere (AC-3)	CENP-A, CENP-B	Highly suggestive of CREST
Nuclear coarse speckled (AC-5)	Sm, U1-RNP	Suggestive of SLE
Discrete nuclear dots (AC-6)	P80-coilin, Sp-100, PML, p80-coilin	Highly suggestive of primary biliary cirrhosis
Nucleolar (clumped, AC-9)	Fibrillarin, U3-RNP (RNA polymerase), PM/Scl, Th/To	High titers are prevalent in PSS
Cytoplasmic reticular (mitochondrial AC-21)**	Anti-mitochondrial	Primary biliary cirrhosis

** Cytoplasmic, non-nuclear fluorescence

Change in Screening Dilution:

- The NOVA View platform is FDA approved for an initial screen dilution of 1:80. ANA detection at 1:40 is prevalent in healthy populations (19.5%) and non-autoimmune mimics of SARDs (26.4%; using the Immuno Concepts Hep-2000 method).[11] Screen thresholds at 1:80 reduce the number of false-positive tests, opportunity for misdiagnosis of connective tissue disorders and consumption of resources without substantially decreasing sensitivity (97.3% vs. 96.4% sensitivity).[11]
- EULAR/ACR classification criteria use ANA positive at >=1:80 an IFA method or equivalent as an obligatory entry criterion for the diagnosis of SLE.

Change in Substrate and Detection System:

- The NOVA View platform uses an optimally fixed HEp-2 cell line. The HEp-2 substrate replaces the prior HEp-2000 cell line which is genetically transfected with multiple copies of the SS-A DNA sequences. Optimal fixation preserves immunoreactivity for SS-A antigen, avoiding the need for transfection with 60-kDa SSA/Ro, which "caused difficulty in reading patterns."[12]

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- The NOVA View reagent employs IgG conjugate rather than polyspecific conjugate in accordance with Clinical Laboratory Standard Institute guidelines to eliminate false-positive results due to nonspecific low titer IgM autoantibodies. These may be common in older healthy adults.

Change in Staining Pattern Reporting:

- The change in substrate eliminates the previously reported SS-A staining pattern.
- Reported patterns will include homogenous, dense fine speckled, centromere, coarse speckled, discrete nuclear dots, nucleolar, nuclear envelope, and cytoplasmic reticular.

Order Code	Profile Name	Notes
3550	ANA (ANTI-NUCLEAR AB) WITH REFLEX TITER	Includes ANA with reflex testing of pattern and titer for up to 3 separate patterns
3551	ANA CONFIRMATION	Includes ANA with reflex pattern and titer for up to 3 separate patterns; should only be used for positive prior screen.
4521	ANA AUTOIMMUNE PROFILE	Includes ANA with reflex pattern and titer, non-reflex (always performed) specific nuclear antibody panel, TPO Ab, complement, RF
3549	ANA SCREEN ONLY	Non-reflexed to pattern and titer or specific nuclear antibody panel
4278	ANA REFLEX AUTOIMMUNE AB PROFILE	Includes ANA with reflex pattern and titer, reflexed (conditionally performed) specific nuclear antibody panel, TPO Ab, complement, RF
4296	ANA REFLEX NUCLEAR AB PROFILE	Includes ANA with reflex pattern and titer, reflexed (conditionally performed) specific nuclear antibody panel
2677	ANA WITH REFLEX AUTOIMMUNE AB CASCADE	Includes ANA with reflex pattern and titer, reflexed (conditionally performed) specific nuclear antibody panel performed in stepwise cascade of nuclear antibody groups
391	ARTHRITIS PROFILE	Includes ANA with reflex pattern and titer, RF, uric acid, ESR
3597	POST-COVID RHEUMATOLOGIC PANEL	Includes ANA with reflex pattern and titer, CCP IgG, cardiolipin IgG/IgM/IgA, CPK, RF
9190	MISCARRIAGE/ THROMBOPHILIA PROFILE	Includes ANA with reflex pattern and titer, cardiolipin IgG/IgM/IgA, lupus anticoagulant panel, beta-2-glycoprotein 1 IgG/IgM, AT-III activity, protein C activity, protein S activity, APC resistance, homocysteine, phosphadidylserine/PT antibodies, prothrombin gene mutation

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References:

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9. Russo K, et al. Circulating anticentromere CENP-A and CENP-B antibodies in patients with diffuse and limited systemic sclerosis, systemic lupus erythematosus, and rheumatoid arthritis. J Rheumatol. 2000 Jan;27(1):142-8.

10. Maddison PJ. Aminoacyl-tRNA histidyl (Jo-1) synthetase autoantibodies. In Peter JB, Shoenfeld Y, eds. Autoantibodies. Amsterdam: Elsevier Science BV. 1996:31-35.

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12. Susan S. Copple, et al, Screening for IgG Antinuclear Autoantibodies by HEp-2 Indirect Fluorescent Antibody Assays and the Need for Standardization, American Journal of Clinical Pathology, Volume 137, Issue 5, May 2012, Pages 825–830

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Order Code and Test Name:	**3550 ANA (ANTI-NUCLEAR AB) WITH REFLEX TITER** **
Specimen Requirements:	*2 ML SERUM. ALLOW SST TO CLOT IN AN UPRIGHT POSITION FOR AT LEAST 30 MINUTES, THEN CENTRIFUGE SAMPLE WITHIN 2 HOURS OF COLLECTION. REFRIGERATE.*
Transport Temperature:	REFRIGERATED
Stability (collection to initiation of testing):	Ambient, 15-25°C: 1 Day Refrigerated, 2-8°C: 1 Week Frozen, (-15)-(-25)°C: 1 Month
Performed:	Monday through Friday / PM Shift
Analytic Time:	2 Days
CPT Code:	86038

**This order code is included in the following order codes: 4521, 3549, 4278, 4296, 2677, 2304, 2306, 391, 3597, and 9190

Order Code and Test Name:	**3551 ANA CONFIRMATION**
Specimen Requirements:	*2 ML SERUM. ALLOW SST TO CLOT IN AN UPRIGHT POSITION FOR AT LEAST 30 MINUTES, THEN CENTRIFUGE SAMPLE WITHIN 2 HOURS OF COLLECTION. REFRIGERATE.*
Transport Temperature:	REFRIGERATED
Stability (collection to initiation of testing):	Ambient, 15-25°C: 1 Day Refrigerated, 2-8°C: 1 Week Frozen, (-15)-(-25)°C: 1 Month
Performed:	Monday through Friday / PM Shift
Analytic Time:	2 Days
CPT Code:	86039

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