# ANA's and Beyond: Introduction of Nova View Digital IFA Reader

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The Immunology Laboratory is proud to introduce the NOVA View Automated Fluorescence Microscope (Inova Diagnostics, Inc.) which will go live on May 09, 2017. The NOVA View is an automated system consisting of a fluorescence microscope and software that acquires, analyzes, stores, and displays digital images of stained anti-nuclear antibody (ANA) indirect immunofluorescent slides. It is intended as an aid in the detection and classification of certain antibodies by indirect immunofluorescence technology. The device can only be used with cleared or approved in vitro diagnostic assays that are indicated for use with the device. A trained operator must confirm results generated with the device.

Introduction of the NOVA View will result in a number of changes including the screening dilution used, endpoint titer reported, single-well titer technology, pattern nomenclature, and the reporting of additional patterns. Currently we screen at a 1:40 dilution, however, in a recent U.S. Study it was reported that more than 32 million persons in the U.S. have ANAs while only 0.5 – 1 million persons in the U.S. have a connective tissue disease (CTD). Only 3% (max) of ANA positive patients have CTD. The NOVA View starts at a 1:80 screening dilution using the NOVA Lite DAPI ANA kit which resulted in a 59% reduction of ANA positives detected in 150 apparently healthy subjects from 27% at a 1:40 screening dilution to 11% at a 1:80 screening dilution. In addition to the change in screening dilution, we will be reporting titers up to $\geq$1:5,120, currently we only report up to $\geq$1:1280. The NOVA View also takes advantage of a single well titer application that estimates the endpoint titer (highest dilution that would give a positive result) for wells containing a positive reaction, based on the nuclear intensity (LIU) and pattern.

The change in pattern nomenclature and the reporting of additional patterns stems from the International Consensus on ANA Patterns (ICAP) workshop. The goal of ICAP is to promote harmonization and understanding of autoantibody test nomenclature, as well as interpretation guidelines for ANA testing, thereby optimizing usage in patient care. The ICAP broke HEp-2 cell (substrate used for ANA) patterns down into nuclear, cytoplasmic, and mitotic patterns and further categorized each pattern as competent-level vs. expert-level patterns (refer to figure 1). We will be reporting all competent-level patterns as well as some of the more easily distinguished expert-level patterns, specifically centrosome and mitotic spindle fibers. The other expert-level patterns will be reported as nuclear pattern noted, NOS; cytoplasmic pattern noted, NOS; and mitotic pattern noted, NOS.

The classification tree in Figure 1 shows 11 competent-level reportable patterns. The six competent-level reportable nuclear patterns include homogeneous, speckled, dense fine speckled, centromere, (discrete) nuclear dots and nucleolar (refer to Figure 2 for synonyms, antigen associations, and disease associations). We currently report homogeneous as diffuse but will be changing our nomenclature for standardization purposes. The speckled, centromere, and nucleolar

patterns are currently reported patterns, however, the dense fine speckled and nuclear dots are new patterns we will be reporting.

The dense fine speckled (DFS) pattern is a relatively newly recognized pattern whose antigen associations include DFS70 and lens epithelium derived growth factor (LEDGF). The clinical significance of this pattern is that it is **rarely** associated with CTD such as systemic lupus erythematosus (SLE), sjogren's syndrome, or systemic scleroderma. The nuclear dot pattern antigen associations and disease associations can be seen in Figure 2.

At this time there is still no consensus as to whether cytoplasmic and mitotic patterns should be reported as ANA positive or negative although it is agreed that these patterns should be noted and differentiated where possible. For this reason we will continue to report these as ANA negative with a comment added indicating the cytoplasmic or mitotic pattern observed or not otherwise specified, as appropriate. The five competent-level reportable cytoplasmic patterns are fibrillar, speckled, reticular/mitochondrion-like, polar/Golgi-like, and rods and rings (RR). These patterns will be differentiated and reported as a comment or otherwise reported as cytoplasmic pattern noted, NOS. Refer to figure 3 for synonyms, antigen associations, and disease associations. Although mitotic patterns are deemed as expert-level patterns we will differentiate centrosome and mitotic spindle fibers due to the ease of which they are identified. The mitotic pattern synonyms, antigen associations and disease associations can be seen in figure 4.

So in summary, currently we report titers of 1:40, 1:80, 1:160, 1:320, 1:640, and $\geq$ 1:1280 and patterns we report are Diffuse, Speckled, Centromere, and Nucleolar and any combination (mixed patterns) of these. We have two comment codes: CPN (Greater than or equal to 2 + Cytoplasmic Pattern Noted) and CPMN (Greater than or equal to 2+ Cytoplasmic Pattern Noted suspicious of Mitochondrial Pattern. Suggest follow-up testing of Anti-Mitochondrial Antibodies if clinically indicated).

### FOR MORE INFORMATION

Contact Laboratory Customer Service or email the Lab Ambassadors for clinical questions.

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Beginning 05/09/2017 we will be change reporting for ANA Titers.

| Current reported titers | Current Reported ANA Patterns |
|---|---|
| 1:40 | Diffuse |
| 1:80 | Speckled |
| 1:160 | Centromere |
| 1:320 | Nucleolar |
| 1:640 | Mixed patterns |
| ≥1:1280 | |
| We currently have two comment codes:<br>Greater than or equal to 2 + Cytoplasmic Pattern Noted and<br>Greater than or equal to 2+ Cytoplasmic Pattern Noted suspicious of Mitochondrial Pattern.<br>Suggest follow-up testing of Anti-Mitochondrial Antibodies if clinically indicated. | |

| New Reported Titers 5/9/2017 | New Reported ANA Patterns 5/9/2017 |
|---|---|
| 1:80 | Diffuse (will change to Homogeneous 5/9/2017) |
| 1:160 | Speckled |
| 1:320 | Centromere |
| 1:640 | Nucleolar |
| 1:1280 | Mixed patterns |
| 1:2560 (New reported titer 5/9/2017) | Nuclear Dots (New pattern 5/9/2017) |
| ≥1:5120 (New reported titer 5/9/2017) | Dense Fine Speckled (New pattern 5/9/2017) |
| Additional comment codes 5/9/2017<br>Cytoplasmic Pattern Noted, NOS<br>Cytoplasmic Pattern Noted, Fibrillar<br>Cytoplasmic Pattern Noted, Speckeld<br>Cytoplasmic Pattern Noted, Reticular/ Anti-Mitochondrial<br>Cytoplasmic Pattern Noted, Polar/Golgi-like<br>Cytoplasmic Pattern Noted, Rods and rings<br>Mitotic Pattern Noted, NOS<br>Mitotic Pattern Noted, Centrosome<br>Mitotic Pattern Noted, Spindle Fibers<br>Mitotic Pattern Noted, Intercellular Bridge | |

The Nova View can identify five patterns: Homogeneous, Speckled (includes Dense Fine Speckled), Centromere, Nucleolar, and Nuclear Dots and will give us an endpoint titer off the 1:80 dilution based on the light intensity units. Other patterns and mixed patterns will have to be manually titered. Cytoplasmic and Mitotic Patterns will not be titered.

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Figure 1: **ICAP HEp-2 Nomenclature and Classification Tree**

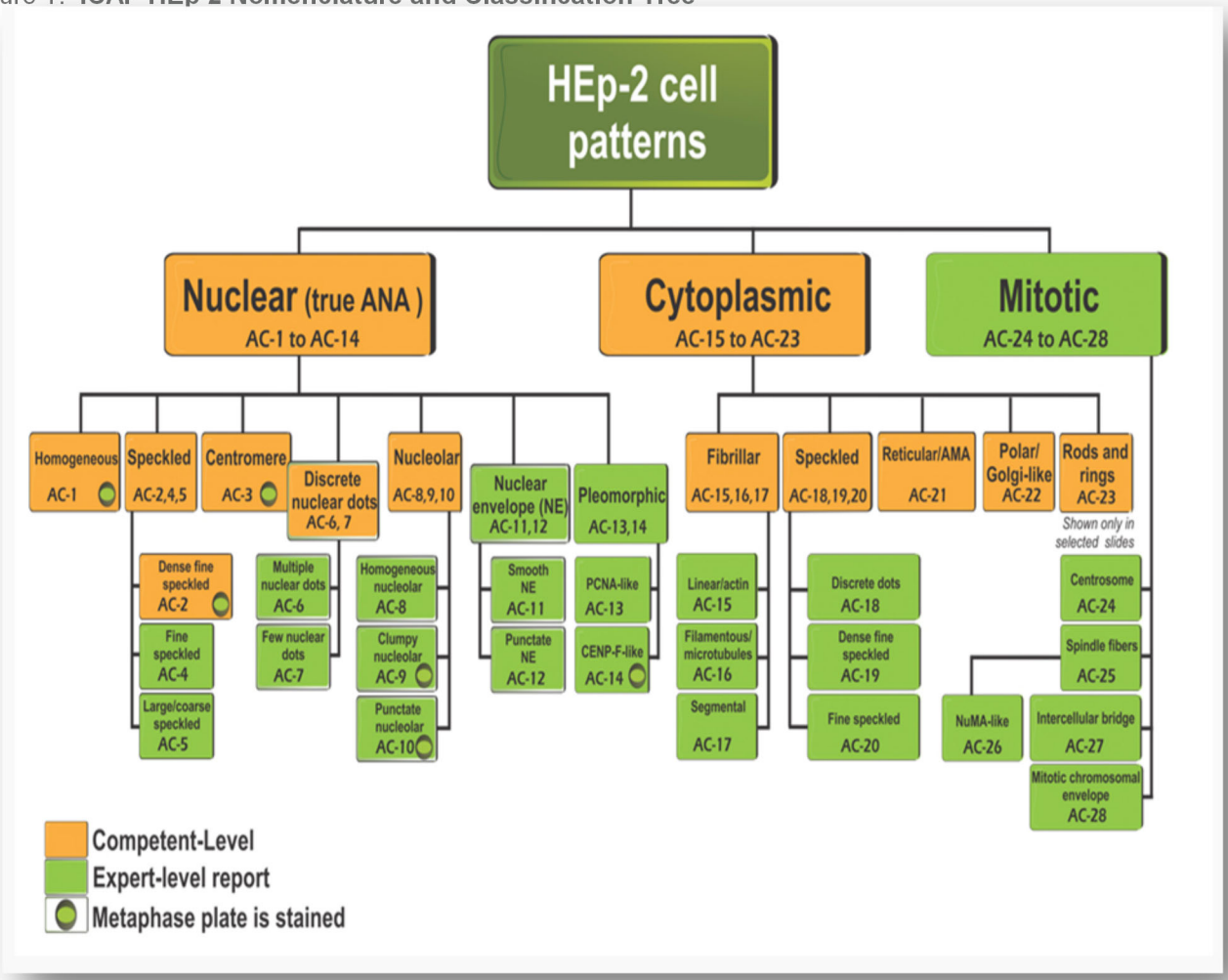


Figure 2: **Synonyms for Nuclear Patterns and Association with Specific Antigens and Diseases**

| | | Synonyms | Antigen associations | Disease association |
|---|---|---|---|---|
| *Nuclear patterns* | **Homogeneous** (AC-1) | Diffuse | dsDNA, nucleosomes, histones | SLE, drug-induced lupus, juvenile idiopathic arthritis |
| | **Speckled** (AC-2,4,5) | Granular | hnRNP, U1RNP, Sm, SS-A/Ro (Ro60), SS-B/La, RNA polymerase III, Mi-2, Ku | MCTD, SLE, SjS, DM, SSc/PM overlap |
| | ***Dense fine speckled*** (AC-2) | None | DFS70/LEDGF | Rare in SLE, SjS, SSc |
| | Fine speckled (AC-4) | Fine granular | SS-A/Ro (Ro60), SS-B/La, Mi-2, TIF1γ, TIF1β, Ku, RNA helicase A, Replication protein A | SjS, SLE, DM, SSc/PM overlap |
| | Large/coarse speckled (AC-5) | Spliceosome/nuclear matrix | hnRNP, U1RNP, Sm, RNA polymerase III | MCTD, SLE, SSc |
| | **Discrete nuclear dots** | | | |
| | Centromere (AC-3) | Kinetochore | CENP-A/B (C) | Limited cutaneous SSc, PBC |
| | Multiple nuclear dots (AC-6) | 6–20 nuclear dots, NSpl, PML bodies | Sp100, PML proteins, MJ/NXP-2 | PBC, SARD, PM/DM |
| | Few nuclear dots (AC-7) | 1–6 nuclear dots, Cajal bodies (coiled body) | p80-coilin, SMN | SjS, SLE, SSc, PM, asymptomatic individuals |
| | **Nucleolar** (AC-8,9,10) | | | |
| | Homogeneous (AC-8) | None | PM/Scl-75, PM/Scl-100, Th/To, B23/nucleophosmin, nucleolin, No55/SC65 | SSc, SSc/PM overlap |
| | Clumpy (AC-9) | None | U3-snoRNP/fibrillarin | SSc |
| | Punctate (AC-10) | Nucleolar speckled | RNA polymerase I, hUBF/NOR-90 | SSc, SjS |
| | **Nuclear envelope** (AC-11,12) | | | |
| | Smooth nuclear envelope (AC-11) | Nuclear rim, nuclear membrane, membranous | Lamins A,B,C, or lamin-associated proteins | SLE, SjS, seronegative arthritis |
| | Punctate nuclear envelope (AC-12) | Nuclear membrane pores | Nuclear pore complex proteins (i.e., gp22) | PBC |
| | **Pleomorphic** (AC-13,14) | | | |
| | PCNA-like (AC-13) | None | PCNA | SLE, other conditions |
| | CENP-F-like (AC-14) | MSA-3, NSp-II | CENP-F | Cancer, other conditions |

Figure 3: **Synonyms for Cytoplasmic Patterns and Association with Specific Antigens and Diseases**

| | | Synonyms | Antigen associations | Disease association |
|---|---|---|---|---|
| Cytoplasmic patterns | ***Fibrillar*** (AC-15,16,17) | | | |
| | Linear/actin (AC-15) | Actin-like | Actin, non-muscle myosin | MCTD, chronic active hepatitis, liver cirrhosis, myasthenia gravis, Crohn's disease, PBC, long-term hemodialysis, rare in SARD other than MCTD |
| | Filamentous/ microtubules (AC-16) | | Vimentin, cytokeratins | Infectious or inflammatory conditions, long-term hemodialysis, alcoholic liver disease, SARD, psoriasis, healthy controls |
| | Segmental (AC-17) | | Alpha-actinin, vinculin, tropomyosin | Myasthenia gravis, Crohn's disease, ulcerative colitis |
| | ***Speckled*** (AC-18–20) | | | |
| | Discrete dots (AC-18) | GW body, processing body, lysosome* | GW182, Su/Ago2, Ge-1 | PBC, SARD, neurological and autoimmune conditions |
| | Dense fine speckled (AC-19) | Homogeneous | PL-7, PL-12, ribosomal P proteins | "anti-synthetase syndrome," PM/DM, SLE, juvenile SLE, neuropsychiatric SLE |
| | Fine speckled (AC-20) | Speckled | Jo-1/histidyl-tRNA synthetase | Anti-synthetase syndrome, PM/DM, limited SSc, idiopathic pleural effusion |
| | ***Reticular/AMA*** (AC-21) | Mitochondrion-like | PDC-E2/M2, BCOADC-E2, OGDC-E2, E1α subunit of PDC, E3BP/protein X | Common in PBC, SSc, rare in other SARD |
| | ***Polar/Golgi-like*** (AC-22) | | Giantin/macrogolgin, golgin-95/GM130, golgin-160, golgin-97, golgin-245 | Rare in SjS, SLE, RA, MCTD, GPA, idiopathic cerebellar ataxia, paraneoplastic cerebellar degeneration, viral infections |
| | ***Rods and rings*** (AC-23) | | IMPDH2, others | HCV patients post-IFN/ribavirin therapy, rare in SLE, Hashimoto's and healthy controls |

*These disease associations are primarily based on the antigens recognized by antibodies that reveal this particular ANA pattern. Amber background are recommended as competent-level reporting, whereas all others (Olive green) are considered for expert-level reporting.*
**no molecular evidence to support this pattern is associated with lysosomal targets.*

Figure 4: **Synonyms for Mitotic Patterns and Association with Specific Antigens and Diseases**

**TABLE 4 | Synonyms for mitotic patterns and association with specific antigens and diseases.**

| | | Synonyms | Antigen associations | Disease association |
|---|---|---|---|---|
| Mitotic patterns | Centrosome (AC-24) | Centrioles | Pericentrin, ninein, Cep250, Cep110, enolase | Rare in SSc, Raynaud's phenomenon, infections (viral and mycoplasma) |
| | Spindle fibers (AC-25) | MSA-2 | HsEg5 | Rare in SjS, SLE, other SARD |
| | NuMA-like (AC-26) | MSA-1 | Centrophilin | SjS, SLE, other |
| | Intercellular bridge (AC-27) | Stem body, midbody | Aurora kinase B, CENP-E, MSA-2, KIF-14, MKLP-1 | Rare in SSc, Raynaud's phenomenon, malignancy |
| | Mitotic chromosome coat (AC-28) | Chromosome coat protein, dividing cell antigen, mitotic chromosome autoantigen (MCA) | Modified histone H3, MCA-1 | Rare in discoid lupus erythematosus, chronic lymphocytic leukemia, SjS, and polymyalgia rheumatica |