NEWSLETTER

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Immuno Concepts® prides itself on the quality of ourproduct. Achieving this quality means that we strive to be a company with continuous improvement of our products as well as our services to you! Some of those services include educational support, technical support, pattern interpretation assistance, and research of literature to support our product. If we can be of any service to you please don't hesitate to contact us at technicalsupport@immunoconcepts.com or call us at 1-800-251-5115.

Technical Bulletin 2024 Q4
– PCNA, NuMA, Nuclear Dots

This technical bulletin will review four ANA patterns: PCNA, NuMA, Multiple Nuclear Dots (MND) and Few Nuclear Dots (FND). All four patterns are classified as expert-level patterns by the International Consensus on ANA Patterns (ICAP) (1). When encountered in a clinical setting, it's sufficient to call them under their umbrella categories: pleomorphic (PCNA), mitotic (NuMA), and discrete nuclear dots (MND and FND) (1).

AC-13: PCNA

The AC-13 pattern has a distinctive appearance caused by the staggered expression of the proliferating cellular nuclear antigen (PCNA) in interphase cells. For this cell cycle-dependent pattern, the size and intensity of nuclear speckling varies, depending on where each cell is at in the cell cycle (Figure 1). Given there are other patterns that also demonstrate cell cycle-dependent expression, it can be challenging to differentiate PCNA from these other patterns. For less confident readers, the more generic term "pleomorphic" can act as a catch-all to describe any such cell cycle-dependent pattern. Diseases associated with this pattern include systemic lupus erythematosus (SLE), autoimmune myositis (AIM), and systemic sclerosis (SSc) (2).

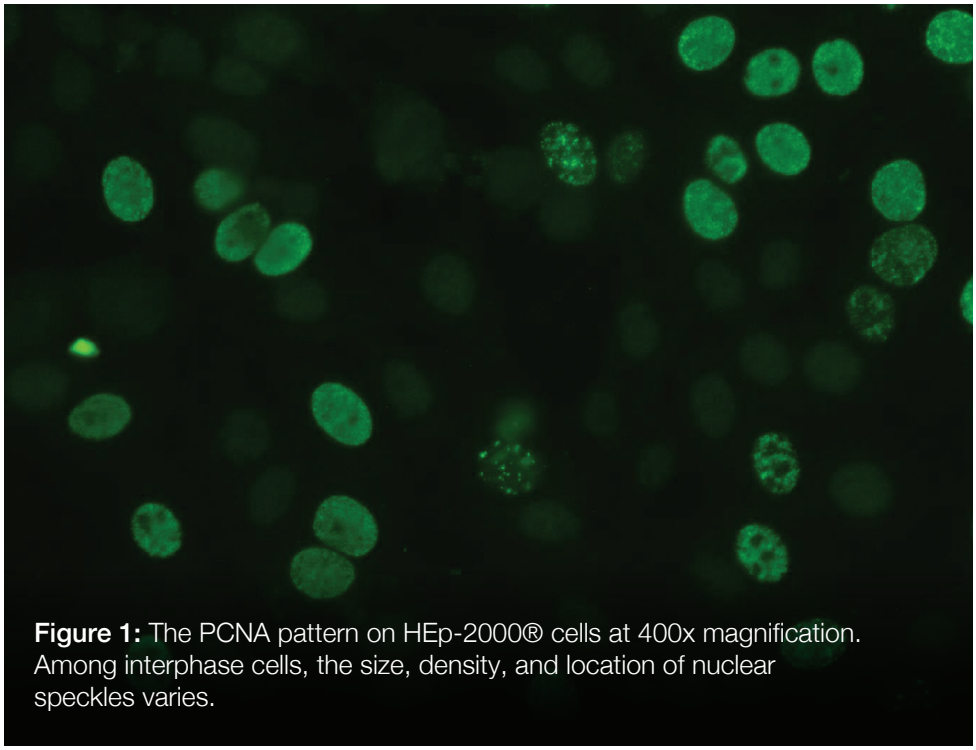

Figure 1: The PCNA pattern on HEp-2000® cells at 400x magnification. Among interphase cells, the size, density, and location of nuclear speckles varies.

Due to variations in staining intensity, at first glance the PCNA pattern may appear to resemble SSA-Ro staining on HEp-2000® cells. However there are clear differences between these two patterns of staining. SSA-Ro staining on HEp-2000® cells shows a ratio of ten to thirty percent of SSA-Ro hyper-expressing

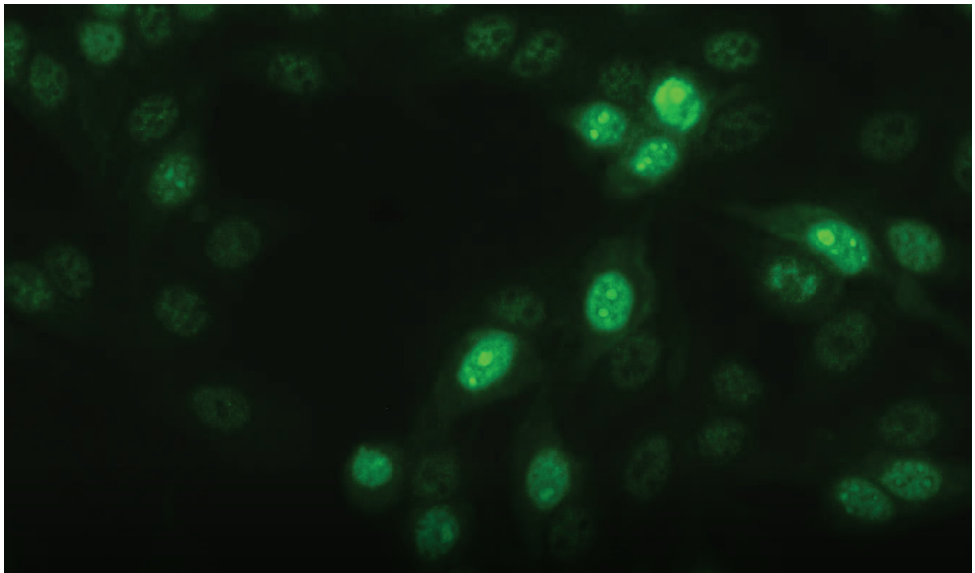

Figure 2: SSA-Ro staining on HEp-2000® cells at 400X magnification. Cells that hyper-express the SSA-Ro protein show more intense nuclear speckling and have stained nucleoli. Note the similar size and layout of nuclear speckling for all interphase cells.

cells; SSA-Ro hyper-expressing cells are stained more intensely and have the added feature of nucleolar staining compared to cells that are not hyper-expressing (Figure 2). In contrast, PCNA is not hyper-expressed on either HEp-2000® or HEp-2 substrates, rather its cycle of staining aligns with the concentration of the PCNA protein in the cell: increasing as the cell approaches division, but quickly disappearing as division begins. The size and density of nuclear speckles varies, and while there may be nucleolar speckling, it is not exclusive to the most intensely stained cells.

AC-26: NuMA

The AC-26 pattern is named for the Nuclear Mitotic Apparatus (NuMA) which aids in the cellular division process (3). While NuMA is a mitotic pattern, staining occurs in both mitotic and interphase cells. Mitotic cells show staining of the nuclear mitotic apparatus, including staining along the path of spindle fibers, which extend from the center to the poles of dividing cells. Interphase cells exhibit nuclear speckling (4) (Figure 3).

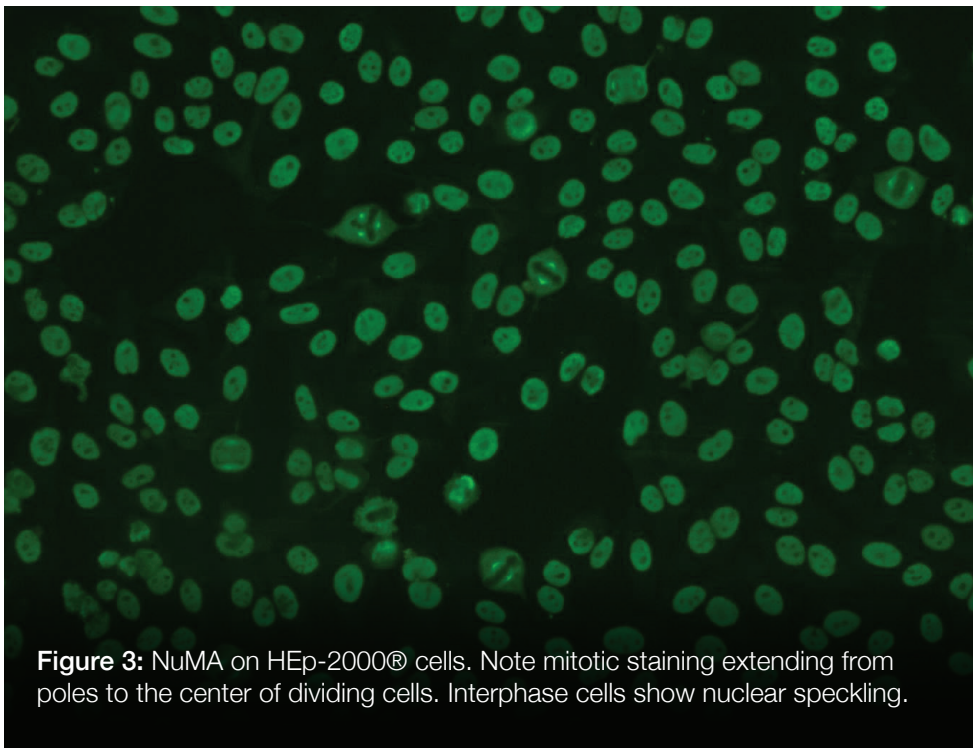

Figure 3: NuMA on HEp-2000® cells. Note mitotic staining extending from poles to the center of dividing cells. Interphase cells show nuclear speckling.

The NuMA pattern is associated with autoantibodies against several proteins involved in the nuclear mitotic apparatus, including NuMA1, NuMA2, and centrophilin (4,5). Patients who are seropositive for these antibodies may have symptoms of autoimmune diseases including SLE, limited systemic sclerosis (SSc), Sjogren's syndrome (SjS), and undifferentiated connective tissue disease (UCTD). However, the evidence linking this pattern to SARD is not particularly strong, which limits its ability to predict disease (4). Instead, it remains an area of active research.

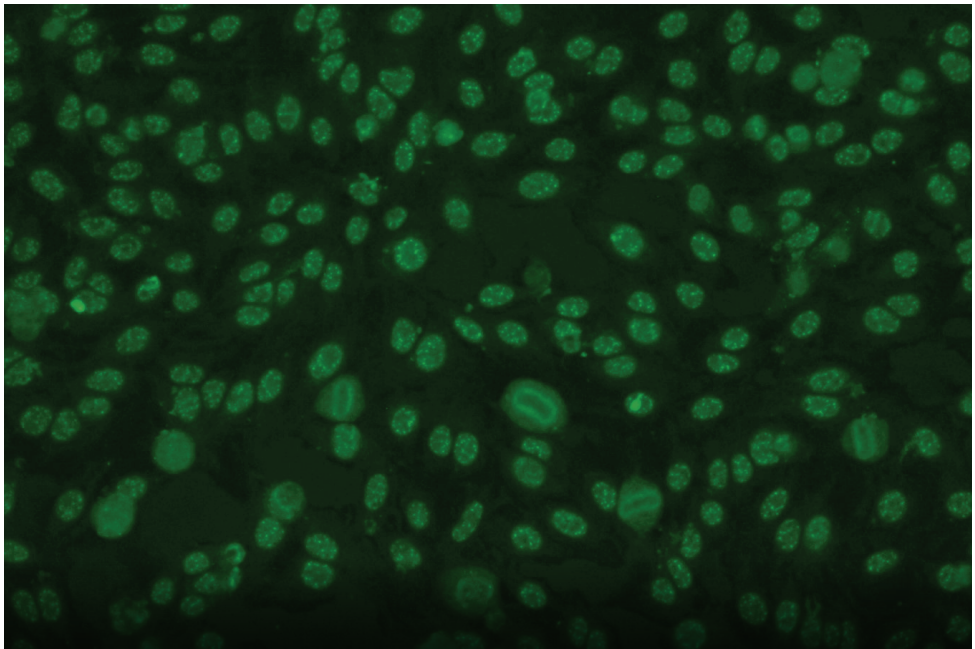

Figure 4: Multiple Nuclear Dots on HEp-2000® cells. Note the number of dots per cell is within the six to 20 range and the variation in dot size.

Multiple Nuclear Dots

The first of the discrete nuclear dots patterns is AC-6 Multiple Nuclear Dots (MND), previously known as promyelocytic leukemia (PML) bodies (6). This pattern is distinguishable based on the number of discrete nuclear speckles, ranging from six to 20 nuclear dots of varying size per cell. These nuclear dots localize in the nucleus of the interphase cell (7). Additionally, staining of the cytoplasm may be observed, but typically this does not occur (7). At first glance, one might mistake this pattern for the more common Anti-Centromere, however readers should note the absence of staining within the mitotic plane, which would otherwise have the classic zipper-like appearance if the centromere were fluorescing. According to ICAP, the pattern is seen in a wide-range of autoimmune diseases, including inflammatory conditions such as primary biliary cholangitis (PBC), autoimmune myopathy (AIM), and dermatomyositis (DM) (8). This pattern is associated with anti-Sp100 antibodies, anti-PML/Sp140 antibodies, and anti-MJ/NXP-2 antibodies (6).

Few Nuclear Dots

The second of the discrete nuclear dots patterns, the AC-7 Few Nuclear Dots (FND) pattern is distinguished based on a range of one to six nuclear dots per cell (9) (Figure 5). These dots, also known as Cajal bodies or coiled bodies, are typically found close to the nucleolus (7, 9). This pattern is associated with autoantibodies against p-80 coilin and the survival motor neuron complex (10, 11). Interestingly, cells that contain Cajal Bodies are usually in the late S/G2 phase of the cell cycle (7). There is not a clear disease association for this pattern (9).

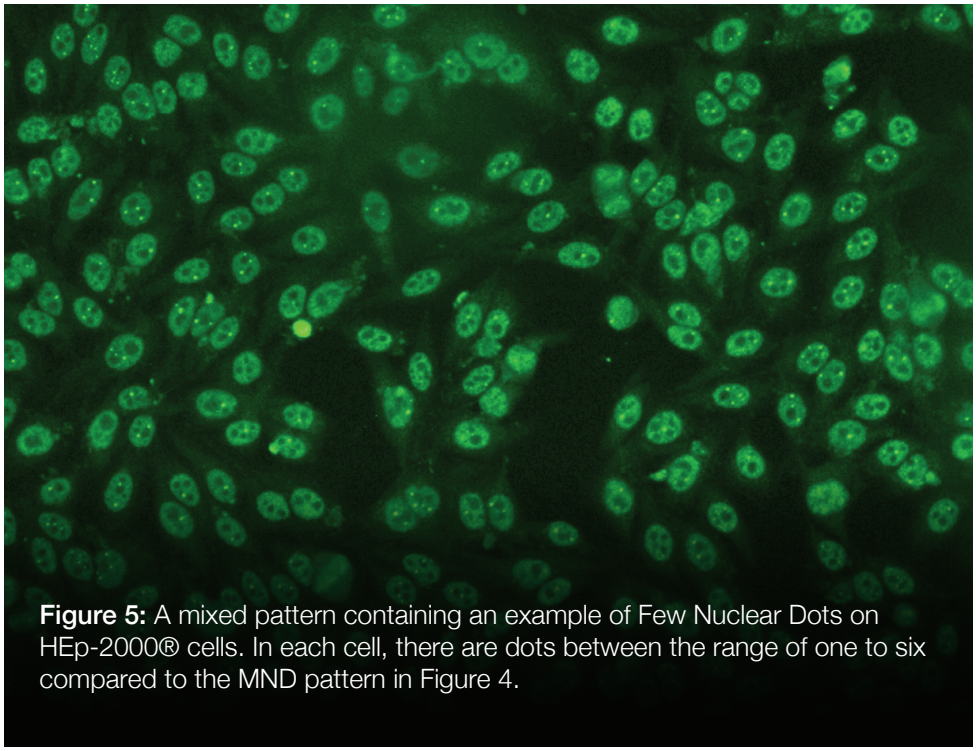

Figure 5: A mixed pattern containing an example of Few Nuclear Dots on HEp-2000® cells. In each cell, there are dots between the range of one to six compared to the MND pattern in Figure 4.

Conclusion

The Immuno Concepts Technical Support team hopes this bulletin has given you a better understanding of ANA patterns you are likely to encounter on immunofluorescence assays. In summary:

- The ANA patterns PCNA, NuMA, Multiple Nuclear Dots, and Few Nuclear Dots are expert level patterns.
- The generic terms “pleomorphic” (PCNA), “mitotic” (NuMA), and “discrete nuclear dots” (MND and FND) can also describe these patterns.
- PCNA staining is cycle-dependent and limited to interphase cells; the size, intensity, and density of nuclear speckling varies (2).
- NuMA is a mitotic pattern with staining in both mitotic and interphase cells. Interphase cells have nuclear speckling while mitotic cells are stained along the path of spindle fibers (4).
- MND has a range of six to 20 dots per cell and FND has a range of one to six dots per cell (6, 9).

References

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