

ANA Negative Patterns

Proper Identification and Tips

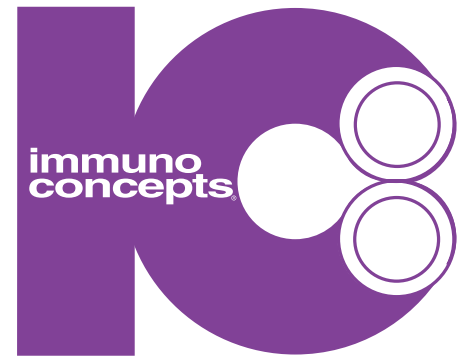
When the body's internal defense system makes a mistake and targets itself, autoimmunity arises. Autoimmune diseases affect as much as 4.5 percent of the world's population and are most likely to afflict women more than men (1). Many affected will exhibit symptoms like fever, joint pain, weight loss, skin rash, and/or tiredness. To evaluate if a patient is suffering from an autoimmune disease, clinicians can consult an Antinuclear antibody (ANA) test. ANA tests, which detect antibodies associated with autoimmune diseases, can play an instrumental role in moving forward with a patient's treatment plan.

Creating a treatment plan involves many aspects including a patient's clinical presentation, other underlying conditions, and drugs the patient is taking, accompanied with a positive or negative ANA result. When testing for ANA, up to 85% of patients are negative (2). This percentage is highly dependent upon the patient population. When clinicians are presented with negative patterns, this still does not rule out autoimmune disease. Clinicians will consider the negative result, and the patient's symptoms, to determine the need for follow-up testing to further evaluate for disease (3).

When reporting negative patterns, many are easily interpreted, but there can be borderline samples. The examples in Figures 1 and 2 (next page) represent borderline samples and intend to illustrate a more difficult type of negative sample. When encountering borderline samples, there are some steps to follow to assist in interpretation.

Adjustments to screen brightness settings are also a consideration when viewing and interpreting images using a computer. Settings may vary from computer to computer and screen optimization is necessary for appropriate interpretation. The screen should match the appearance of the cell substrate through the oculars. The Image Navigator® monitor is fully formatted with settings to properly display images and should never be adjusted.

In recent years, the International Consensus on ANA Patterns (ICAP) website has attempted to promote agreement and further standardize ANA patterns. The ICAP defines negative ANA patterns as AC-0 (4) and provides excellent examples of patterns that show negative results despite diffuse non-specific staining. These photos, and additional information, can be found at www.ANAPatterns.org.



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Step-by-Step Process

- A.** Begin with looking at the sample at 200x total magnification (20x objective with 10x oculars). Do not stare so long that a negative pattern is misinterpreted as a positive result.
- B.** First, review interphase cells. If interphase cells are negative, confirm that the metaphase mitotic cells do not express positive results.
 - i.** In Figures 1 and 2 (next page), notice there is no clear discernible pattern in the interphase cells (a). The metaphase mitotic cells (b) are demonstrating some weak staining, but without a clearly discernible pattern in the interphase cell nucleus, and no defined staining of subcellular structures, the sample is ANA negative. For borderline negative samples at 200x, when viewed at 400x, one can often convince oneself a pattern is present. At this point, you are over-reading the ANA.
- C.** When reading and viewing a sample, regardless of the staining intensity, if there is no clearly discernable pattern in any given subcellular structure, interpret as negative.

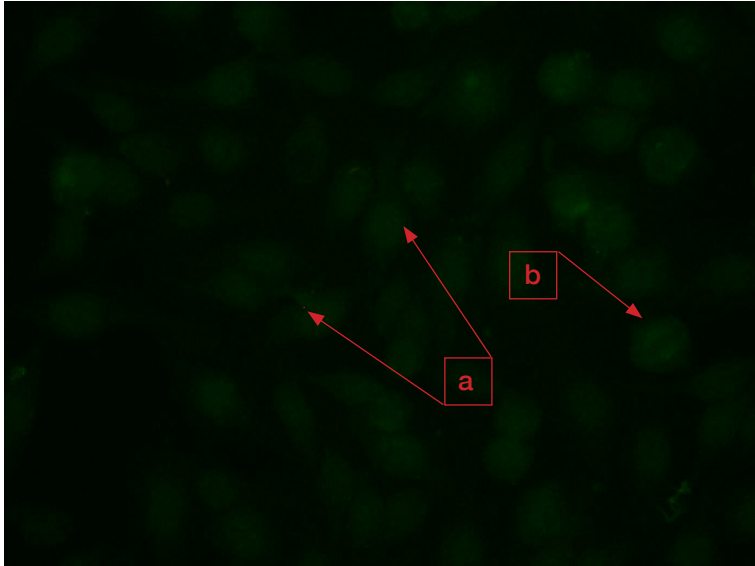


Figure 1 - ANA HEp-2000® fluorescence antibody negative. (a) interphase cell exhibit negative staining, with no clear, discernable pattern. (b) mitotic cell exhibits some weak staining, but since interphase cells have no clear discernable pattern, result is ANA negative.

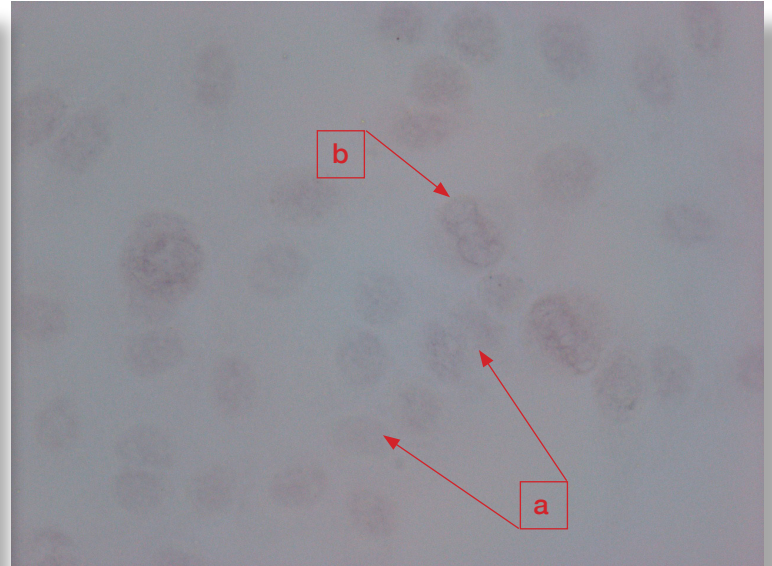


Figure 2 - ANA HEp-2000® Colorzyme negative. (a) interphase cell exhibit negative staining, with no clear, discernable pattern. (b) mitotic cell exhibits some weak staining, but since interphase cells have no clear discernable pattern, result is ANA negative.

Summary of tips when interpreting a negative ANA pattern:

- To interpret positive/negative results, start at 200x total magnification.
- Staring too long could create a false positive in the mind of the observer.
- Picking out one or two nuclei and concentrating on them can create a false positive. With rare exceptions the pattern should be seen in the majority of interphase nuclei.
- No discernible pattern in interphase cells indicates a negative ANA.
- Mitotic cells may be hard to discern in a negative ANA.
- Do not confuse intensity of background staining with positive ANA results.
- When using automation, standardize and designate computer screen use and brightness settings. Consult the manufacturer of the automated system before changing any factory settings.

References:

1. Bender M, Christiansen J, Quick M. Autoimmune disease, by the numbers. *Scientific American* 2021;325, 3, 31-33.
2. University of Rochester Medical Center. Antinuclear Antibody. https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=antinuclear_antibodies (Accessed November 2022).
3. Hoy, E et al. HEp-2000® Fluorescent ANA-Ro Test System. <https://immunoconcepts.com/wp-content/uploads/2020/11/HEp-2000%C2%AE-FA-ANA-Ro-En.pdf> (Accessed December 2022).
4. ICAP International Consensus on ANA Patterns. <https://anapatterns.org/> (Accessed November 2022).

technicalsupport@immunoconcepts.com



www.ImmunoConcepts.com
technicalsupport@ImmunoConcepts.com

