Antinuclear Antibody Testing

Version: 1.0

Date: June 2, 2025

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SYNOPSIS AND RELEVANCE

Laboratory testing for antinuclear antibodies (ANAs) can be complicated by different methodologies as well as the availability of numerous available individual antigen-specific tests. Adherence to the strategies in this module will:

1. Ensure that an ANA test is ordered before any subsequent antigen-specific testing, and only in the setting of clinical suspicion for a rheumatologic disease.
2. Understand the general differences in basic ANA testing methodology

OBJECTIVES

1. Discuss the use of ANA testing and antigen-specific autoantibody panels in the diagnosis of rheumatologic disease.
2. Review the two main testing methodologies for ANA detection, along with their advantages and disadvantages
3. Understand the role of individual antigen testing in the setting of a positive ANA screen

BACKGROUND

Antinuclear antibody (ANA) testing is familiar to the clinical lab and is an important initial test when the patient has a suspected systemic autoimmune rheumatic disease, such as systemic lupus erythematosus (SLE). A positive antinuclear antibody (ANA) can be due to autoantibodies against many different detected antigens, such as double-stranded DNA (dsDNA), DNA histone complexes, u1RNP, Sm, La, and fibrillin, to name a few. Although the negative predictive value of positive ANA tests is quite high, the positive predictive value is relatively low, as up to 30% of the healthy population can demonstrate a positive ANA.[1-3] Additionally, the availability of individual antibody testing, such as anti-dsDNA, anti-Sm, and anti-La, complicates the testing menu for the serologic diagnosis of rheumatologic diseases. In 2013, the American College of Rheumatology partnered with the Choosing Wisely campaign to help guide the use of ANA testing. They recommended not ordering individual autoantibody panels or more specific ANA subserologies unless: 1) an ANA screening test is positive, AND 2) there is clinical suspicion and evidence of an immune-mediated or rheumatologic disease.[4]

In addition to the guidelines for judicious test utilization, different methodologies are available for ANA testing, and can generate different results, especially when testing is done on different analytical platforms. There are two major methodologies for ANA screening. The traditional method, which is currently generally considered the reference method, is the use of indirect immunofluorescence (IFA).[3] With this methodology, patient serum is incubated with HEp-2 cells, a human cell line with a large nucleus that makes it easy to visualize various staining patterns. After incubation, the cells are incubated with a fluorescent-conjugated antibody against human IgG. Specific positive staining patterns, whether homogeneous, speckled, centromeric, nucleolar, or a combination of these, can be reported. This can be helpful to guide the next steps, although the association between staining pattern and disease specific autoantibodies is relatively weak. Of note, standardization of such patterns is difficult, although the International Consensus on Antinuclear Antibody Patterns (ICAP) has attempted to achieve consensus, and labs should be familiar with these guidelines if reporting ANA results using IFA.[5-8]

The second, more recent methodology for detecting ANAs is the use of solid phase immunoassays. With this methodology, cell extracts, or more commonly a panel of recombinant antigens, are prepared and immobilized on a solid surface to which patient serum is added. Based on the serum binding to one or more of these specific antigens, the exact autoantibody in the patient's serum is identified.

Overall, the advantage of the IFA methodology is that it allows for a large number of autoantibodies to be detected (given the presence of over 150 antigens) and is therefore more sensitive. The disadvantages are, however, that it is labor intensive, requires trained personnel to interpret staining patterns, lacks reference sera for staining patterns, needs to be titered to enable autoantibody semiquantitative reporting , and does not identify specific autoantibodies

(subserologies).[3] Solid phase immunoassays, on the other hand, have high throughput, detect specific autoantibodies, and are semiquantitative, but lack sensitivity, since the antigens tested are limited to those immobilized on the solid surface. Of note, although both methods are useful in many scenarios, the American College of Rheumatology considers HEp-2 based IFA testing to be the gold standard for the detection of ANAs.

With IFA-based ANA testing, the titer reported is generally the dilution prior to endpoint, which is the point at which less than half of cells show fluorescence. Many large center studies of healthy volunteers have demonstrated positive ANAs at relatively low-level titers among healthy volunteers[1,9]; in one study, for example, approximately 32% of the population aged 20-60 had a positive ANA at a 1:40 titer; however, only 5% of the population had a positive ANA at a 1:160 titer.[10] A general suggestion has been to use an initial screening dilution that results in only 5% positivity in normal controls as the cutoff for a positive reaction. However, even at 5%, there is a high tendency towards false positives, so pre-test probability should be high before ANA testing is performed (as recommended by the Choosing Wisely campaign); otherwise, a lengthy, non-diagnostic/specific, and potentially expensive workup may ensue.

For laboratories utilizing IFA as their initial screen, if a patient has clinical suspicion for an autoimmune disease and the testing is positive, additional testing for specific autoantibodies using solid phase immunoassay may be helpful to further elucidate the diagnosis. The exact identification of these subserologies can help hone in on specific disease states; for example, whereas antibodies against dsDNA are generally associated with SLE, antibodies against Ro and La are associated more strongly with Sjogren's syndrome. This "reflex" testing can be all-inclusive or can specifically target certain autoantibodies based on the original IFA staining pattern. One must be circumspect in interpreting negative IFA results, since some antibodies, including anti-La/SS-B and anti-Jo-1, which exhibits cytoplasmic staining, may be undetectable or unreported in HEp-2 cells, limiting its sensitivity.[3]

Once a patient has been diagnosed with an ANA-associated autoimmune disease, further ANA titers after the diagnosis is established are generally not helpful.

INSIGHTS

1. ANA testing should not be performed unless there is clinical suspicion for a rheumatologic disease.
2. Antigen-specific testing generally should not be performed unless the patient has a positive ANA screen.
3. ANA screening can be performed either by IFA or solid phase immunoassay; both have their advantages and disadvantages from a clinical and laboratory perspective.
4. Certain reflex testing may be useful subsequent testing after an initial positive ANA screen to establish the exact autoantibody present.

INTERVENTIONS

1. Depending on the ANA testing available, work with patient-facing colleagues to determine an acceptable algorithm for follow-up testing after an initial positive ANA. For instance, the "reflex" testing can be all-inclusive or can specifically target certain antigens based on the original IFA staining pattern.
2. Implement order-entry intervention in electronic health system; options could include:
    a. Implement the reflex algorithm as a single (or minimal) number of orderables to simplify order-entry.
    b. Create a pop-up or other alert message (soft-stop) that recommends canceling individual autoantibody antigen testing for patients who do not have a positive ANA screen or refers the provider to the reflexive panel.
    c. Hide the ability to order large autoantibody panels.
    d. Create an order panel that maps clinical indication to specific orderables, eg, "Initial autoimmune evaluation" checkbox is ANA only or "Evaluate for Sjogren's autoantibodies" maps to Ro and La.
3. If bringing in ANA testing to your laboratory, consider the benefits and downsides of HEp-2 IFA vs solid phase immunoassay-based testing.

INTERVENTION ANALYSIS

Collect data on ANA and subsequent specific antigen-based autoantibody testing from the laboratory or hospital information system. It is easiest to use the same time period for before and after the intervention to do the assessment, for example, 3 months for pre-intervention and 3 months post-intervention. A correction factor must be applied if different time periods are used.

APPENDIX A: CALCULATING THE INTERVENTION IMPACT

Collect data in the table below, preferably using the same measurement period of time before and after implementing interventions (eg, 3 months). Correct the volume accordingly if different time periods are used for pre-intervention and post-intervention studies.

Pre-Intervention Column

Prior to taking any interventions steps, determine the total of :

- ANA screens ordered (A1).
- Individual antigen-based autoantibody tests, such as anti-dsDNA and the extracted nuclear antigens (anti-Sm, anti-La, anti-SS-A, anti-SS-B, anti-RNP, anti-Scl 70, and anti-Jo-1) ordered (B1)
- Separate individual antigen-based antibody tests ordered at the same time as the initial ANA screen (C1).
- NOTE: C1 should be less than B1, since B1 should represent all the individual tests and C1 should only represent those tests ordered at the same time as the initial ANA.

Post-Intervention Column
After the interventions steps are taken, determine the total of:
- ANA screens ordered (A2).
- Individual antigen-based autoantibody tests, such as anti-dsDNA and the extracted nuclear antigens (anti-Sm, anti-La, anti-SS-A, anti-SS-B, anti-RNP, anti-aScl 70, and anti-Jo-1) ordered (B2)
- Separate individual antigen-based antibody tests ordered at the same time as the initial ANA screen (C2).

Volume Change Column: Calculate the volume of tests after interventions have been established for each row. (A3, B3, C3).

Percent Volume Change Impact (%) Column: Calculate the percent change in the pre-intervention and post-intervention test volumes to find the impact of the change(s) instituted by your laboratory. (A4, B4, C4). With effective intervention, C4 should represent a decrease (in percentage) of testing ordered at the same time as an ANA. B4 might ideally also be less than 100% since total testing may be decreased as well, as many ANAs ordered will likely be negative. If A4 is close to zero, no adjustment factor needs to be made; however, if A4 is significantly higher or lower than zero, then the calculations will need to be adjusted for the difference in volume of total ANAs ordered.

Laboratory Test Volume Outcomes and Opportunities				
Description	**Pre-Intervention**	**Post-Intervention**	**Proportion of**	**Percent Volume Change Impact (%)**
Number of total ANA screens ordered	A1	A2	A1 - A2 = A3	A3/A1 x 100% = A4%
Number of total individual antigen-based autoantibody tests	B1	B2	B1 - B2 = B3	B3/B1 x 100% = B4%
Number of total individual antigen-based autoantibody tests ordered at the same time as the initial ANA screen.	C1	C2	C1 - C2 = C3	C3/C1 x 100% = C4%

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE
Understand the use of ANA testing, including its negative and positive predictive value, and the usage of specific antigen autoantibody panels in the diagnosis of rheumatologic disease.

QUESTION 1
A 35-year-old woman sees her primary care doctor and states she has been having some recent joint paint and fatigue. The primary care doctor orders an ANA IFA which returns at 1:20. The best interpretation for the result is:

A. Given the presence of some anti-nuclear antibody reactivity, the patient is likely to have a rheumatologic disease such as lupus.
B. The doctor should order additional specific autoantibody testing, such as antibodies to dsDNA, to further rule out a diagnosis of lupus.
C. It is most likely that the patient does not have a diagnosis of lupus.
D. The doctor should repeat the ANA test.

The correct answer is C. At 1:20, the ANA is essentially negative. ANA testing has a high negative predictive value, although suffers from a low positive predictive value. In this case, it is most likely that the patient does not have lupus, although it is not completely ruled out.
A is incorrect. ANA testing has a high negative predictive value.
B is incorrect. It is recommended that ANA be an initial screen for rheumatologic disease, and that additional further testing is not generally ordered unless there is clinical suspicion as well as a positive ANA test.
D is incorrect. There is no evidence to suggest that an ANA test should be repeated in this clinical scenario.

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QUESTION 2 OBJECTIVE
Understand the two main testing methodologies for ANA detection, along with their advantages and disadvantages.

QUESTION 2
A local laboratory serving a large rheumatologic clinic is considering bringing ANA testing in-house. Which of the following is true:
A. The laboratory should bring in a solid phase immunoassay because of its high throughput and increased sensitivity over IFA methodology.
B. The laboratory should bring in IFA methodology so it can identify the specific antigens when positive.
C. The laboratory should bring in both IFA and solid phase methodology.
D. The laboratory should work with patient-facing colleagues to determine an appropriate reflex algorithm, if needed, in cases of positive ANA IFA screens.

The correct answer is D. Positive ANA IFA results can be followed up with individual antigen identification, according to predetermined algorithms, in many clinical situations.
A is incorrect. Solid phase immunoassay is high throughput but potentially has less sensitivity than IFA.
B is incorrect. Although IFA technology can be used to identify morphologic patterns of ANA reactivity, the specific antigen cannot be identified.
C is incorrect. Although some labs may offer both IFA and solid phase immunoassay ANA testing, as there are pros and cons to each methodology, this may not be necessary, and laboratorians should work with their patient-facing colleagues to determine what the best testing strategy is for their patient population.

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QUESTION 3.
Understand the role of individual antigen testing in the setting of a positive ANA screen

QUESTION 3
A 35-year-old woman with fever, weight loss, and facial rash, is suspected by her doctor to have a rheumatologic disease. She has a positive ANA titer of 1:1024. An additional panel is ordered, which demonstrates positivity for anti-dsDNA and anti-Sm. The most probable diagnosis is:
A. Lupus
B. Sjogren's syndrome
C. System sclerosis
D. Rheumatoid arthritis
The correct answer is A. Lupus is usually associated with a positive ANA screen, and anti-dsDNA and anti-Sm are highly specific for lupus.
B is incorrect. Sjogren's syndrome is usually associated with anti-SSA or anti-SSB immunoreactivity.
C is incorrect. System sclerosis is often associated with anti-Scl70 immunoreactivity, anticentromere antibody (ACA), and/or anti-RNA polymerase III antibody
D is incorrect. Although rheumatoid arthritis can be associated with a positive ANA, it is more commonly associated with positive rheumatoid factor and anti-cyclic citrullinated peptide.

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