The Scleroderma Education Project™

ANA and Antibody Series

How to Do Scleroderma ANA and Antibody Testing Correctly

(A Practical Guide for Clinicians)

Background

When a clinician has a reason to suspect that a patient may have an autoimmune disease, for example, systemic lupus erythematosus (SLE/lupus), rheumatoid arthritis, systemic sclerosis (scleroderma), or Sjogrens syndrome, s/he will usually order an antinuclear antibody (ANA) test as the first step. If the result of that test is positive, this can be a strong marker for the presence of an autoimmune disease. If the result is negative, an autoimmune disease is much less likely but still a possibility. Following a positive ANA result that has been correctly done, the next diagnostic step is to look at the patient's symptoms in combination with lab test results that look for the presence of individual autoantibodies, especially for autoimmune diseases that tend to be strongly associated with specific autoantibodies.

If the ANA and antibody testing is done correctly, this can be a powerful tool for helping a clinician quickly and correctly diagnosis an autoimmune disease at an earlier stage, when it is often easier to treat. Unfortunately, for a variety of reasons, including recent changes in standard laboratory testing procedures that have been implemented to save money (often at the expense of diagnostic accuracy), it is increasingly likely that clinicians (who are not typically aware of these testing problems) will receive incorrect lab results, sometimes resulting in years of delay in making the correct clinical diagnosis.

This article is a short, bullet point summary of the more detailed article titled "Scleroderma ANA and Antibody Testing Basics" – one of the articles in the ANA and Antibody series that is part of this website. It is intended to educate clinicians as well as patients. Key references that document the issues raised in this article are included at the end.

ANA Testing for Scleroderma – The Problem

- Historically, all ANA testing was done by a method called indirect immunofluorescence (IFA or IIF). Now, however, most ANA testing is done (by default) using newer, less expensive and less "hands on" methods such as ELISA or Multiplex. ANA testing by IFA can detect up to 150 different antigens. In contrast, typical ANA testing by ELISA detects 8 to 10 antigens, and testing by Multiplex detects 11 to 13 antigens, potentially missing key antigens critical for correct diagnosis.

- ANA testing by ELISA or Multiplex is very accurate IF the patient has one of the antibodies included in the panel*. However, if the patient has an antibody that is *not* included in the testing panel, the ANA result itself will be falsely reported as negative, suggesting that the patient does not have an autoimmune disease.
- When a clinician suspects that a patient has an autoimmune disease, but the early symptoms are ambiguous enough so that s/he is unsure as to which autoimmune disease the patient may have (this is frequently the case since there are several symptoms/signs that overlap with certain autoimmune diseases), s/he will typically order a general ANA screening panel. This is sometimes referred to as an Extractable Nuclear Antigen (ENA) panel, an ANA profile, an Autoimmune Disorders Panel, or an Autoimmune Cascade. In other cases, if s/he suspects a specific disease, e.g., systemic scleroderma, and the reference lab that will do the testing offers it, s/he may instead order a specific screening panel, for example, a scleroderma screening panel or a lupus screening panel. Unfortunately, there is absolutely no standardization of what is included in any of these disease-specific panels.
- To illustrate the problem for scleroderma patients in particular, consider the following: While all scleroderma-specific screening panels will include the two main antibodies that have been linked to scleroderma for decades [anti-Scl70 (associated with one of the diffuse scleroderma variants) and anti-centromere (associated with one of the limited scleroderma variants)], these panels commonly leave out a number of other antibodies that have been linked to scleroderma based on recent research. In one recent study[1], a typical autoimmune screening panel done by Multiplex resulted in an almost 43% false negative rate for scleroderma patients. Notably, one of these typically omitted antibodies – anti-RNA polymerase III - has an incidence rate of about 20%, comparable to the general rates for anti-Scl70 and anti-centromere antibodies.
- The problem is even worse for general autoimmune disease screening panels. It is very common for these panels to *only* include one scleroderma-related antibody – anti-Scl70 - and leave out the next most frequent, anti-centromere antibody. Moreover, some of these panels refer to the anti-Scl70 antibody as the "scleroderma antibody." So if you are a primary care clinician with little experience in diagnosing autoimmune disorders (no less a patient reading his/her lab results), when you get a (potentially false) negative ANA result from a general autoimmune screening panel that includes a negative result for the "scleroderma antibody," it is not at all surprising that an accurate scleroderma diagnosis can be delayed for years.
- Because of these issues, in a 2011 Position Statement, the American College of Rheumatology strongly recommends that ANA testing by indirect immunofluorescence (IFA) "should remain the gold standard for ANA testing."[2] This is especially important when doing initial ANA and antibody screening for patients who may have some form of scleroderma, but also applies to other autoimmune diseases, including lupus.

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* There is a known problem with false-positive Scl-70 antibody results when done by ELISA or Multiplex assay. These will usually be low positive results. See the separate article in this series titled "False-positive Scl-70 (Topoisomerase) Antibody Testing: A Major Problem in Systemic Sclerosis Diagnosis" for more information about this important issue.

ANA Testing for Scleroderma – The Solution

- The best way to do initial testing for scleroderma is to order an ANA done by IFA using human HEp-2 substrate. To the best of our knowledge, all major labs in the US now use HEp-2 substrate for ANA/IFA testing. However, this may not be true in other parts of the world. If you are unsure about this, check with the lab before ordering the test. If the lab is using a different substrate, e.g., rodent substrate, centromere antibodies are not reliably detected, so it is best to order a separate anticentromere antibody test in this situation.
- It is perfectly reasonable for a clinician to order instead an ANA test and reflex scleroderma screening panel that is done using ELISA or Multiplex, as long as s/he orders a confirming reflex ANA by IFA if the panel yields a negative result. If the ANA result turns out positive, then it is most likely correct (however, see note above about false-positive Scl-70 results), and since very few scleroderma patients have more than one scleroderma-related antibody, the test results will give the best information that the clinician needs to help make a correct diagnosis. Note that labs never do a reflex ANA by IFA test automatically upon getting a negative result – this is something that the clinician needs to be aware of and to order, either as part of the original test order or in a subsequent testing round. (Ironically, with many reference labs, if the ANA result done by ELISA or Multiplex is positive, then the lab will often automatically re-run the ANA testing using IFA since this procedure yields additional information that may be useful to the clinician.)

Scleroderma Antibody Testing – The Final Piece of the Puzzle

- Assuming that the clinician has ordered an ANA test that has been done by IFA using human HEp-2 cell substrate and has received a positive result, the next step is to try to determine which specific scleroderma-related antibody the patient might have, since each scleroderma antibody may have a different clinical presentation and prognosis. Since ANA by IFA can potentially detect up to 150 different antibodies, determining the specific antibody is the next critical step.
- Recent research has identified at least ten different antibodies that are associated with specific variants of scleroderma. Some of these fall into a category called "overlap syndromes," where the patient can have symptoms of more than one autoimmune disease. (See the Antibody section of the *Scleroderma FAQ* on this website for a list of these ten antibodies and disease associations.) Unfortunately, only a very small number of reference labs even test for the majority of these scleroderma antibodies. If the clinician is not aware of this and just orders a scleroderma antibody screening panel, the number of antibodies included in this panel can be only the two originally identified antibodies, anti-Scl70 and anti-centromere, entirely missing other important antibodies.
- At a bare minimum, if the clinician suspects scleroderma, the following three antibodies should be included in the panel or ordered separately if not included: Scl70, centromere, and RNA Polymerase III. These three antibodies are present in between 60% and 80% of patients with diagnosed systemic sclerosis.

- If these antibodies are negative, then the next group of antibodies that should be tested for include: Th/To, PM-Scl, U3-RNP, U1-RNP (Mixed Connective Tissue Disease), and Ku. Unfortunately, to the best of our knowledge, no commercial testing is yet available for U11/U12-RNP or RuvBL1/2 antibodies.
- At least two reference labs in the US offer comprehensive scleroderma screening panels that include almost all of the above-mentioned antibodies: RDL and ARUP. Note that the Ku antibody is not included in the standard scleroderma screening panel but it can be added by request if the initial seven antibodies are all negative. Unfortunately, we currently have no information on the availability of comprehensive scleroderma screening panels in other countries.
- It is important to note that antibodies are currently used diagnostically to identify specific disease subsets in order to better monitor and manage potential disease complications or progression. There is no currently identified clinical need to do antibody level trending as antibody levels are generally stable over time and are not thought to be directly correlated with symptom severity in systemic scleroderma.
- If ANA testing by IFA yields a centromere staining pattern, many labs may not do additional testing for Scl70, RNA Polymerase III, or other scleroderma related antibodies. While in most cases the IFA centromere pattern interpretation is correct, reading ANA staining patterns is subjective and has been shown to be insufficient clinically and in some cases incorrect.[4] Because of this concern, it is recommended that centromere staining patterns be confirmed with separate anti-centromere antibody testing.
- If there are cost constraints, stepwise antibody testing tailored to the patient's presenting symptom profile may be appropriate rather than expensive comprehensive antibody screening. Here is a chart that suggests sequential antibody testing. Note that the first step is always an ANA/IFA. If that is negative, additional antibody testing should not be done.

Symptom Profile	Antibody Testing Level 1	Antibody Testing Level 2
Potential diffuse SSc: severe pain and fatigue, Raynaud's, skin thickening, GI symptoms	Scl-70, RNA Polymerase III	U3-RNP, Centromere, Th/To
Potential limited SSc: Raynaud's, GI symptoms, swollen fingers, telangiectasia	Centromere	Th/To, U1-RNP
Overlap syndrome: myositis, SLE symptoms	Centromere, U1-RNP	PM/Scl, Ku

References

1. Shanmugam VK, Swistowski DR, Saddic N, Wang H, Steen VD. Comparison of indirect immunofluorescence and multiplex antinuclear antibody screening in systemic sclerosis. *Clinical Rheumatology* Oct 2011 30(10): 1363-1368.
2. American College of Rheumatology Position Statement. Methodology of testing for antinuclear antibodies. www.rheumatology.org/practice/ana_position_stmt.pdf. Approved by Board of Directors: Aug 2011.
3. Beutner EH, Krasny SA, Chorzelski TP, Rodnan G, Jablonska S, Kumar V. Evaluation of methods for detection of anticentromere antibodies and other antinuclear antibodies. *J Am Acad Dermatol* 1985; 12: 289-95.
4. Pham, BN, Albarede S, Guyard A, Burg E, Maisonneuve P. "Impact of external quality assessment on antinuclear antibody detection performance." *Lupus* 14.2 (2005): 113-119.

Acknowledgement

The author wishes to thank Allan L. Metzger, MD (RDL Reference Laboratory) for his invaluable assistance in helping to make the information in this article as accurate and up-to-date as possible.