



Technical Article Series

How to Do Scleroderma ANA and Antibody Testing Correctly

When a physician has a reason to suspect that a patient may have an autoimmune disease, for example, SLE (Lupus), Rheumatoid Arthritis, Systemic Scleroderma, or Sjogrens Syndrome, s/he will usually order an antinuclear antibody (ANA) test as a 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 less likely but still a possibility. Following a positive ANA result, the next diagnostic step is to look at the patient's symptoms combined with lab test results that look for the presence of individual autoantibodies, especially those that tend to be strongly associated with a particular autoimmune disease.

If the ANA and antibody testing is done correctly, this can be a powerful tool for helping a physician quickly and correctly diagnosis an autoimmune disease earlier 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 physicians (who are not typically aware of these testing problems) will receive incorrect lab results, sometimes resulting in years of delays in correct clinical diagnosis.

This article is a bullet point summary of the detailed Technical Article on Scleroderma ANA and Antibody Testing that can be found in the Additional Articles section of the Scleroderma Education Project website (SclerodermaInfo.org). 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 (by default) done using newer, less expensive methods such as ELISA or Multiplex. ANA testing by IFA can detect up to 150 different antigens. Typically, ANA testing by ELISA detects 8 to 10 antigens and testing by Multiplex detects 11 to 13 antigens.
- 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 physician 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 is a lot of symptom overlap with certain autoimmune diseases), s/he will typically order a general screening panel. This is sometimes referred to as an Extractable Nuclear Antigen (ENA) panel or 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 SLE (Lupus) screening panel. Unfortunately, there is absolutely no standardization of what is included in any of these panels, which can create a lot of problems for clinicians.

- 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 diffuse scleroderma) and anti-centromere (associated with limited scleroderma), these panels commonly leave out a number of other antibodies that have been linked to scleroderma based on recent research. In one recent study¹, a typical autoimmune screening panel done by Multiplex resulted in an ***almost 43% false negative rate for scleroderma patients*** since these other antibodies were not included in the panel. Notably, one of these 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 very common anti-centromere antibody. And to make things even more confusing, some of these panels call the anti-Scl70 antibody the “scleroderma antibody”. So if you are a primary care clinician with little experience in diagnosing autoimmune disorders (much less a patient reading a report of their 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 accurate scleroderma diagnosis can be delayed for years.
- Because of these issues, in a 2011 Position Statement, the American College of Rheumatology 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 that may have some form of scleroderma, but also applies to other autoimmune diseases, including Lupus.

ANA Testing for Scleroderma – The Solution

- The best way to do initial testing for scleroderma is to order an ANA done by IFA, but it is critical that the lab does the ANA /IFA testing using human HEp-2 substrate. Some labs include this information in their test catalogs, but others do not. The reason for this is that ANA testing, even when done by IFA, is likely to miss the very common anti-centromere antibodies if the test is done using rodent cells (substrate) instead of human cells (HEp-2 substrate)³. For this reason, it is very important to verify that the ANA IFA test is done using HEp-2 or alternatively order a separate anti-centromere B test along with the ANA test.
- It is perfectly reasonable for a clinician to instead order an ANA test and reflex scleroderma screening panel that is done using ELISA or Mutiplex, **as long as**

s/he orders a reflex ANA by IFA done using HEp-2 substrate if the panel yields a negative result. If the ANA result turns out positive, then it is almost always correct and since very few scleroderma patients have more than one scleroderma related antibody, the test results will give the correct 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 human cell substrate and has received a positive result, the next step is to try to determine which specific scleroderma-related antibody the patient has, since each scleroderma antibody-specific subtype has a different clinical picture. If the ANA test was done using ELISA or Multiplex screening, then they would already have this information, but since ANA by IFA can potentially detect up to 150 different antibodies, determining the specific antibody is a critical next step.
- Recent research has identified at least nine 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 Table 2 in the Scleroderma FAQ section of the Scleroderma Education Project website at SclerodermaInfo.org for a list of these antibodies.). Unfortunately, only a very small number of reference labs test for even 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 as few as the two originally identified antibodies: anti-Scl70 and anti-centromere.
- At a bare minimum, if the clinician suspects scleroderma, the following three antibodies must be included in the panel, or ordered separately if not included: Scl70, centromere B, and RNA Polymerase III.
- If these antibodies are negative, then the next group of antibodies that should be tested for include: Th/To, PM-Scl, U3-RNP, and U1-RNP.
- At least two reference labs in the US offer comprehensive scleroderma screening panels: RDL and ARUP. Others may as well. 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 only used diagnostically to identify specific disease subsets in order to better monitor and manage likely symptom progression patterns associated with a particular disease subset.

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, it is often the case that many labs interpret this finding as an indirect indicator of the presence of centromere antibodies and consequently may not do additional testing for Scl70, RNA Polymerase III, or other scleroderma related antibodies. While in most cases the centromere pattern interpretation is correct, ANA staining pattern interpretation is subjective and has been shown to be subject to misinterpretation in some studies.⁴ For this reason, 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 patients presenting symptom profile may be appropriate rather than comprehensive antibody screening, which can be significantly more expensive. For example, with patients presenting with symptoms consistent with a potential diagnosis of diffuse scleroderma, a logical testing sequence might be: (1) ANA by IFA combined with Scl70, (2) RNA Polymerase III, and (3) centromere B. For patients presenting with symptoms that are more consistent with a potential diagnosis of limited scleroderma, a logical testing sequence might be: (1) ANA by IFA plus centromere B, (2) Scl70, and (3) Th/To.

References

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Publication Date: October 7, 2014

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