



## ***ANA and Antibody Series***

### ***How to Do Scleroderma ANA and Antibody Testing Correctly (A Practical Guide for Physicians and Other Clinicians)***

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#### ***Background***

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 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 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 delay in making the 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 and less "hands on" methods such as ELISA or Multiplex. ANA testing by IFA can detect up to 100 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 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 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 sclerosis and the reference lab that will do the testing offers it, s/he may instead order a specific screening panel, for example, a sclerosis screening panel or a SLE (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 sclerosis patients in particular, consider the following: while all sclerosis-specific screening panels will include the two main antibodies that have been linked to Sclerosis for decades [anti-Scl70 (associated with one of the diffuse sclerosis variants) and anti-centromere (associated with one of the limited sclerosis variants)], these panels commonly leave out a number of other antibodies that have been linked to sclerosis based on recent research. In one recent study<sup>1</sup>, a typical autoimmune screening panel done by Multiplex resulted in an **almost 43% false negative rate for sclerosis 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 sclerosis-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 “sclerosis antibody”. So if you are a primary care clinician with little experience in diagnosing autoimmune disorders (much less a patient reading 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 “sclerosis antibody”, it is not at all surprising that accurate sclerosis 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”<sup>2</sup>. This is especially important when doing initial ANA and antibody screening for patients that may have some form of sclerosis, but also applies to other autoimmune diseases, including Lupus.

### **ANA Testing for Sclerosis – The Solution**

- The best way to do initial testing for sclerosis 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). 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 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 Multiplex, as long as s/he orders a reflex ANA by IFA 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 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 specific scleroderma antibody may have a different clinical presentation and prognosis. Since ANA by IFA can potentially detect up to 100 different antibodies, determining the specific antibody is the next critical step.
- Recent research has identified at least eight 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 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.
- 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 that include all of the above-mentioned antibodies: 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 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.<sup>4</sup> 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 patients presenting symptom profile may be appropriate rather than expensive comprehensive antibody screening. 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. For patients presenting with symptoms that are more consistent with a potential diagnosis of limited scleroderma (old term: CREST), a logical testing sequence might be: (1) ANA by IFA plus centromere, (2) Scl70, and (3) Th/To.

## **References**

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