

AMERICAN COLLEGE OF RHEUMATOLOGY

POSITION STATEMENT

SUBJECT: Methodology of Testing for Antinuclear Antibodies

PRESENTED BY: American College of Rheumatology

FOR DISTRIBUTION TO: Members of the American College of Rheumatology
Medical Societies
Managed Care Organizations/Third Party Carriers
Laboratories

SUMMARY:

- 1 • The immunofluorescence antinuclear antibody (ANA) assay is the gold standard for ANA testing
2 with greater sensitivity than solid phase assays.
3
- 4 • HEp-2 cells have approximately 100 to 150 possible autoantigens. These cells are used to detect
5 ANAs by the immunofluorescence (IF) method, in which both pattern and titer can be described,
6 and to display a variety of autoantigens not present in multiplex ANA tests.
7
- 8 • Many commercial laboratories and some hospital laboratories have switched their ANA screening
9 test to solid phase immunoassays, such as a multiplex platform. The latter technique can screen
10 and process large volumes of clinical specimens more quickly and at less cost than the traditional
11 immunofluorescence ANA test using fixed HEp-2 cells as substrate.
12
- 13 • These multiplex assays can detect only the specific autoantibodies directed against the limited
14 number (typically 8-10) autoantigens that are displayed.
15
- 16 • Laboratories should indicate the method used when reporting ANA results.
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BACKGROUND:

18 The methodology of the tests for the detection of antinuclear antibodies has changed over the years from
19 the LE cell prep, to immunofluorescence utilizing sections of various rodent organs (e.g. rat or mouse liver
20 or kidney, etc.) to cell lines, in particular HEp-2. HEp-2 cells contain approximately 100 to 150
21 autoantigens. These cells are used to detect ANA by the immunofluorescence method, in which both
22 pattern and titer can be described, and to display a variety of autoantigens not present in the multiplex ANA
23 tests. Researchers have seen the evolution of methodology of tests for the detection of particular ANAs
24 (anti-DNA, Sm, RNP, Ro/SS-A, La/SS-B, *etc.*) from immunodiffusion, complement-fixation,
25 hemagglutination, to various solid phase immunoassays.
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28 Over the years, numerous investigators and commercial organizations have attempted to develop solid
29 phase immunoassays for the detection of ANA and specific ANAs, which are easier and cheaper to perform
30 and standardize compared to immunofluorescence assays using fixed HEp-2 cells as a substrate. A review
31 of the literature by the committee indicates that up to 35% of patients with SLE and a positive ANA by
32 immunofluorescence (IF) were negative on solid phase assays^(1-4, 6-21, 23). Recent research would indicate
33 that the antinuclear antibody (ANA)-HEp-2 test results discriminate ANA-positive healthy individuals and
34 patients with autoimmune rheumatic diseases (ARDs). Many commercial laboratories and some hospital
35 laboratories have switched their antinuclear antibody (ANA) screening test to solid phase immunoassays,
36 such as a multiplex platform, for the reasons noted above, and since the latter technique can screen and
37 process large volumes of clinical specimens than the traditional immunofluorescence ANA test using fixed
38 HEp-2 cells as substrate.
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41 Various national and international organizations have also been involved in the standardization of these
42 tests for the harmonization of laboratory results. These include World Health Organization, Centers for
43 Disease Control, Dutch Red Cross and the International Union of Immunological Societies ⁽⁵⁾.
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45 Any laboratory test, to be most useful, must maximally distinguish patients with a particular disorder from
46 related disorders. It is understood that both commercial and hospital laboratories are interested and
47 committed to providing the best laboratory tests for the diagnosis of rheumatic diseases.
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49 The immunofluorescence ANA test is the gold standard for ANA testing. When performed with a history
50 and physical, it identifies almost all patients with systemic lupus erythematosus (sensitivity over 95%) ⁽²²⁾,
51 although the specificity of this assay is only 57% for SLE when compared to patients with related
52 rheumatic and autoimmune disorders ⁽²²⁾. In addition, the IF ANA is an important test for the screening and
53 diagnosis of systemic sclerosis (sensitivity 85%), polymyositis/dermatomyositis (sensitivity 61%), primary
54 Sjogren's syndrome (sensitivity 48%), juvenile idiopathic arthritis (sensitivity 57%), drug-induced lupus
55 (sensitivity 100%), mixed connective tissue disease (sensitivity 100%), and autoimmune hepatitis as well as
56 being important in monitoring and assessing prognosis in individuals with Raynaud's phenomenon.
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58 59 **RECOMMENDATIONS:**

- 60 • The immunofluorescence (IF) ANA test should remain the gold standard for ANA testing.
- 61 • Hospital and commercial laboratories using bead-based multiplex platforms or other solid phase
62 assays for detecting ANAs must provide data to ordering physicians on request that their assay has
63 the same or improved sensitivity and specificity compared to the IF ANA.
- 64 • In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-
65 La/SS-B, *etc.* should be standardized according to national (*e.g.*, CDC) and/or international (*e.g.*,
66 WHO, IUIS) standards.
- 67 • Laboratories should specify the methods utilized for detecting ANAs when reporting their results.
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