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
Other interested parties

POSITIONS

1. The ACR supports the immunofluorescence antinuclear antibody (ANA) test using Human Epithelial type 2 (HEp-2) substrate, as the gold standard for ANA testing.

2. Hospital and commercial laboratories using alternative bead-based multiplex platforms or other solid phase assays for detecting ANAs must provide data to ordering healthcare providers on request that the alternative assay has the same or improved sensitivity compared to IF ANA.

3. In-house assays for detecting ANA as well as anti-DNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-La/SS-B, etc., should be standardized according to national (e.g, CDC) and/or international (e.g., WHO, IUIS) standards.

4. Laboratories should specify the methods utilized for detecting ANAs when reporting their results.

BACKGROUND

The methodology of the tests for the detection of (ANA) has changed over the years from the LE cell prep, to immunofluorescence utilizing sections of various rodent organs (e.g. rat or mouse liver or kidney, etc.) to cell lines, in particular HEp-2. HEp-2 cells contain approximately 100 to 150 autoantigens. These cells are used to detect ANA by the immunofluorescence method, in which both pattern and titer can be described, and to display a variety of autoantigens not present in the multiplex ANA tests. These multiplex assays can detect only the specific auto-antibodies directed against the limited number (typically 8-10) of auto-antigens that are displayed. Researchers have seen the evolution of methodology of tests for the detection of particular ANAs (anti-DNA, Sm, RNP, Ro/SS-A, La/SS-B, *etc*.) as well from immunodiffusion, complement-fixation, hemagglutination, to various solid phase immunoassays.

Over the years, numerous investigators and commercial organizations have attempted to develop solid phase immunoassays for the detection of ANA and specific ANAs, which are easier and cheaper to perform and standardize compared to immunofluorescence assays using fixed HEp-2 cells as a substrate. A review of the literature indicates that up to 35% of patients with SLE and a positive ANA by immunofluorescence (IF) were negative on solid phase assays [1-4, 6-21, 23]. Recent research would indicate that the (ANA)-HEp-2 test results discriminate ANA-positive healthy individuals and patients with autoimmune rheumatic diseases (ARDs). Many commercial laboratories and some hospital laboratories have switched their ANA screening test to solid phase immunoassays, such as a multiplex platform, for the reasons noted above, and since the latter technique can screen and process larger volumes of clinical specimens than the traditional immunofluorescence ANA test using fixed HEp-2 cells as substrate.

Various national and international organizations have also been involved in the standardization of these tests for the harmonization of laboratory results. These include World Health Organization, Centers for Disease Control, Dutch Red Cross and the International Union of Immunological Societies [5].

Any laboratory test, to be most useful, must maximally distinguish patients with a particular disorder from related disorders. It is understood that both commercial and hospital laboratories are interested and committed to providing the best laboratory tests for the diagnosis of rheumatic diseases. Laboratories should indicate the method used when reporting ANA results.

The immunofluorescence ANA test is the gold standard for ANA testing. When performed with a history and physical, it identifies almost all patients with systemic lupus erythematosus (sensitivity over 95%) [(22)], although the specificity of this assay is only 57% for SLE when compared to patients with related rheumatic and autoimmune disorders [(22)]. In addition, the IF ANA is an important test in the evaluation of systemic sclerosis (sensitivity 85%), polymyositis/dermatomyositis (sensitivity 61%), primary Sjogren's syndrome (sensitivity 48%), juvenile idiopathic arthritis (sensitivity 57%), drug-induced lupus (sensitivity 100%), mixed connective tissue disease (sensitivity 100%), and autoimmune hepatitis as well as being important in monitoring and assessing prognosis in individuals with Raynaud's phenomenon. Finally, ANA testing is an important prognostic marker for uveitis in patients with juvenile inflammatory arthritis and is an essential element in determining the frequency of required ophthalmologic exams as per published guidelines.

Healthcare providers should avoid ordering panels of ANA subserologies (double stranded DNA, Smith, RNP, SS-A, SS-B Scl-70, centromere) when not appropriately indicated. Instead, ordering healthcare professionals should select specific ANA subserologies based on a patient's signs and symptoms and when there is a high pretest suspicion for a specific condition.

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Approved by the Committee on Rheumatologic Care: 01/2009 06/2011
Approved by the Board of Directors: 02/2009 08/2011 08/2015