



CPAL

Central Pennsylvania Alliance
Laboratory

Technical Bulletin

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Method Change - Anti Nuclear Antibodies(ANA) Screen

Explanation of Change:

Starting in April, 2002, the method for ANA screen test will be changed to the Rhigene's Titer-Fluor Anti-Nuclear Antibody Test System. The Rhigene ImageTiter System will allow us to determine the pattern and the titer simultaneously. Therefore, results of both the pattern and the titer of a sample can be reported on the same day.

Background:

Antinuclear antibodies (ANA) are a group of autoantibodies characterized by specificity for numerous antigenic determinants of cell nuclei. They are quite useful as disease markers, primarily for diagnostic screening and also to monitor the course of connective tissue disease. The most commonly used analytical method to test for ANA is indirect immunofluorescent (IFA) performed on substrate slides made from mounted tissue culture cells (HEp-2). IFA can be used to screen for a wide variety of known and unknown autoantibodies. Through pattern recognition, IFA offers insights into the probable identity of the antigen and associated autoimmune disease.

Medical Use:

ANAs are commonly found in the following diseases: systemic lupus erythematosus (SLE), discoid lupus erythematosus (DLE), drug induced lupus erythematosus (LE), mixed connective tissue disease (MCTD), Sjogren syndrome, scleroderma (systemic sclerosis) / CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) syndrome, rheumatoid arthritis, and polymyositis/ dermatomyositis. Test results for autoantibodies alone are insufficient to establish the diagnosis of a systematic rheumatic disease; they must always be interpreted in the clinical context.

Method:

The Rhigenes Titer-Fluor Anti-Nuclear Antibody Test System is an indirect fluorescent antibody staining method using human tumor cell line substrate (HEp-2) to capture ANA in a serum sample and an image titer system to visualize the slides. The Rhigene's ImageTiter system, consisting of a specialized camera and shutter device, acquires multiple images of a single dilution of patient sample with various exposure times and processes these images to simulate conventional titration. Therefore, we can combine the ANA screen and ANA titer in one procedure.

Supportive

Data:

Rhigene ImageTiter system was evaluated using CDC's reference sample (ANA human reference serum #01-#11). The patterns and titers of these samples obtained by technologists at CPAL agreed with Rhigene's results within the acceptable variations of titers. Rhigene's ImageTiter system was also compared with ImmunoConcepts IFA using patient specimens. In an initial study, about 81

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negative (IC/IFA) and 49 positive (IC/IFA) specimens were used. The patterns of the positive specimens included homogeneous, speckled, nucleolar, centromere, and SSA-Ro. The titer ranges from 1:40 to \geq 1:2560 which resembles CPAL's daily patient run. The sensitivity, specificity, predictive value of a positive test (PVP) and predictive value of a negative test (PVN) of ImageTiter are: Sensitivity = 77.6 %, Specificity = 96.3 %, PVN = 87.6 %, PVP = 92.7 %

In an additional study, 94 positive ANA samples were tested using both Rhigene ImageTiter system and IC/IFA. Both methodologies agreed on all the patterns except SSA-Ro. Immunoconcepts IFA uses a genetically transfected Hep-2 cell line which allows for the specific identification of autoantibodies to the SSA-Ro antigen, whereas Rhigene does not. Results of this study are listed in the following table:

	Rhigene positive (within 2x dilution)	Rhigene Negative (including 1:40)
IFA Positive (within 2x dilution)	28	0
IFA Negative (including 1:40)	0	66

Cautions : SSA-Ro will no longer be reported. Instead, they will be reported as “speckled pattern.” An ENA test using the SSA-Ro antigen-specific immunodiffusion method is available for the final determination of SSA-Ro. When multiple patterns are present in a sample, there will be a delay of 1-2 days because of the need for manual dilution before the titer can be determined for each pattern.

References: Kavanaugh, A., R. Tomar, J. Reveille et. Al. 2000. Guidelines for clinical use of the antinuclear antibody tests for specific autoantibodies to nuclear antigens. Arch. Pathol. Lab. Med. 124:71-81.

Bartholemew, B.A. 1974. "Antinuclear antibody tests as a clinically selected screening procedure." Am. J. Clin. Pathol. 81: 495-499.

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Package insert, Rhigene Titer-Fluor Anti-Nuclear Antibody Test System.

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