MAYO CLINIC LABORATORIES

Test Definition: ANA2

Antinuclear Ab, S

Overview

Useful For

Evaluating patients at-risk for antinuclear antibodies-associated systemic autoimmune rheumatic disease particularly systemic lupus erythematosus, Sjogren syndrome, and mixed connective tissue disease

Testing Algorithm

See Connective Tissue Disease Cascade (CTDC)

Special Instructions

- Connective Tissue Disease Cascade (CTDC)

Method Name

Enzyme-LinkedImmunosorbentAssay(ELISA)

NY State Available

Yes

Specimen

Specimen Type

Serum

Ordering Guidance

If suspicious of connective tissue disorder, see CTDC / Connective Tissue Disease Cascade, Serum.
If suspicious of autoimmune liver disease, see ALDG / Autoimmune Liver Disease Panel, Serum.

Specimen Required

Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Specimen Volume:0.5 mL

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-General Request (T239)
-Gastroenterology and Hepatology Client Test Request (T728)
-Renal Diagnostics Test Request (T830)

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK

Specimen Minimum Volume

0.4 mL

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	21 days	
	Frozen	21 days	

Clinical & Interpretive

Clinical Information

Measurement of antinuclear antibodies (ANA) in serum is the most commonly performed screening test for patients suspected of having a systemic autoimmune rheumatic disease (SARD), also referred to as connective tissue disease.(1) ANA occur in patients with various autoimmune diseases, both systemic and organ specific, but they are particularly common in SARDs, which include systemic lupus erythematosus (SLE), discoid lupus erythematosus, drug-induced lupus erythematosus, mixed connective tissue disease (MCTD), Sjogren's syndrome (SjS), systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia), and idiopathic inflammatory myopathies.

ANA can be detected by different technologies, including indirect immunofluorescence assay (IFA) and solid phase assays, such as enzyme immunoassays and multiplex bead immunoassays. In a study performed in the Mayo Clinic Antibody Immunology Laboratory, no significant differences were demonstrated between ANA IFA and ANA enzyme-linked immunosorbent assay (ELISA) for a cohort of patients with connective tissue disease consisting predominantly of patients with SLE, SjS, and MCTD. Weakly positive ANA ELISA results were not a strong indicator of SARD in this laboratory cohort. The likelihood of finding an autoantibody to a specific extractable nuclear antigen including double-stranded DNA on a second-order testing increased directly with the level of ANA: 88% of sera that had detectable autoantibodies on second-order testing had an ANA level greater than 3.0 U.(2)

Overall, an ANA ELISA result of greater than or equal to 3.0 U was demonstrated as the optimal cutoff for CTDC / Connective Tissue Disease Cascade, Serum. This algorithm is intended to evaluate patients with common connective tissue diseases such as SLE, SjS, and MCTD.

See Connective Tissue Disease Cascade (CTDC)

Reference Values

Negative: < or =1.0 U
Weakly positive: 1.1-2.9 U
Positive: 3.0-5.9 U
Strongly positive: > or =6.0 U
Reference values apply to all ages.

Interpretation

A large number of healthy individuals have weakly-positive (1.1 - 2.9 U) antinuclear antibody (ANA) enzyme-linked immunosorbent assay (ELISA) results, many of which are likely to be clinical false-positive results; therefore, second-order testing of all positive ANA yields a very low percentage of positive results to extractable nuclear antigens including double-stranded (ds) DNA.(2)

Positive ANA results greater than 3.0 U are associated with the presence of detectable autoantibodies to specific extractable nuclear antigens (SM, SS-A, SS-B, Sm/RNP or RNP 68 and RNP A, Jo-1, Scl-70) including dsDNA.

Cautions

Results for antinuclear antibodies (ANA) performed by enzyme-linked immunosorbent assay (ELISA) and traditional immunofluorescence assay (IFA) in the Mayo Clinic Antibody Immunology Laboratory show both methods to substantially equivalent for the evaluation of common connective tissue diseases (CTD; systemic lupus erythematosus, Sjogren syndrome , and mixed connective tissue disease).(2) Negative results do not rule out the diagnosis of other CTD, such as systemic sclerosis and inflammatory myopathies, and overlap syndromes associated with complex antigens optimally detected in with the ANA IFA and associated with nucleolar, certain cytoplasmic and/or speckled patterns. For autoimmune hepatitis and juvenile idiopathic arthritis, ANA IFA remains the optimal testing method as the target autoantigens are largely unknown and not included in the ELISA or other solid-phase immunoassays.(3-6)
ANA may also be detectable following viral illnesses, in chronic infections, or in patients treated with many different medications.

Clinical Reference

1. Agmon-Levin N, Damoiseaux J, Kallenberg C, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis. 2014 Jan;73(1):17-23. doi: 10.1136/annrheumdis-2013-203863
2. Deng X, Peters B, Ettore MW, et al: Utility of antinuclear antibody screening by various methods in a clinical laboratory patient cohort: J Appl Lab Med. 2016 Jul 1;1(1):36-46. doi: 10.1373/jalm.2016.020172
3. Sparchez M, Delean D, Samasca G, Miu N, Sparchez Z: Antinuclear antibody screening by ELISA and IF techniques: discrepant results in juvenile idiopathic arthritis but consistency in childhood systemic lupus erythematous. Clin Rheumatol. 2014 May;33(5):643-647. doi: 10.1007/s10067-014-2529-y
4. Bossuyt X, De Langhe E, Borghi MO, Meroni PL: Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases. Nat Rev Rheumatol. 2020 Dec;16(12):715-726. doi: 10.1038/s41584-020-00522-w
5. Bossuyt X, Claessens J, De Langhe E, et al: Antinuclear antibodies by indirect immunofluorescence and solid phase assays. Ann Rheum Dis. 2020 Jun;79(6):e65. doi: 10.1136/annrheumdis-2019-215443
6. Alsaed OS, Alamlih LI, Al-Radideh O, Chandra P, Alemadi S, Al-Allaf AW. Clinical utility of ANA-ELISA vs ANA-immunofluorescence in connective tissue diseases. Sci Rep. 2021 Apr 15;11(1):8229. doi: 10.1038/s41598-021-87366-w

Performance

Method Description

The method used to detect antinuclear antibody (ANA) is enzyme-linked immunosorbent assay (ELISA). A HEp-2 lysate supplemented with specific purified antigens (double-stranded deoxyribonucleic acid [dsDNA], histone, SS-A [Ro], SS-B [La] Smith, sm/RNP, Scl-70, Jo-1, and centromere B antigen) are coated onto microtiter plate wells. A dilution of patient serum is added to the well and incubated. After washing to remove unbound serum protein, an enzyme-conjugated antihuman-IgG antibody is added to detect human IgG bound to the microtiter plate well. After incubation and washing to remove unbound conjugate, a substrate to the enzyme is added to the well. After incubation, the enzyme substrate reaction is stopped. The complete assay is measured on a spectrophotometer plate reader. The optical density measured is proportional to the antibody present in the patient serum. Testing is performed on the Agility instrument by Dynex.(Package insert: ELISA kits. Bio-Rad Laboratories; 07/14)

PDF Report

No

Specimen Retention Time

14 days

Performing Laboratory Location

Rochester

Fees & Codes

Test Classification

This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information

86038