Test Definition: ANA2
Antinuclear Antibodies (ANA), Serum

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-Linked Immunosorbent Assay (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)

Specimen Minimum Volume
0.4 mL
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Reject Due To

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross lipemia</td>
<td>Reject</td>
</tr>
<tr>
<td>Gross icterus</td>
<td>OK</td>
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</tbody>
</table>

Specimen Stability Information

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<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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<tbody>
<tr>
<td>Serum</td>
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<td>21 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
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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
**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 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**


**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

Day(s) Performed
Monday through Saturday

Report Available
1 day

Specimen Retention Time
14 days

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

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

LOINC® Information

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<th>Test Order Name</th>
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<td>Antinuclear Ab, S</td>
<td>94875-2</td>
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### Test Definition: ANA2
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