Overview

Useful For
Evaluation of patients with signs and symptoms compatible with connective tissue diseases

Profile Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA2</td>
<td>Antinuclear Ab, S</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CCP</td>
<td>Cyclic Citrullinated Peptide Ab, S</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>IM_01</td>
<td>Interpretation</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Reflex Tests

<table>
<thead>
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<th>Test ID</th>
<th>Reporting Name</th>
<th>Available Separately</th>
<th>Always Performed</th>
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</thead>
<tbody>
<tr>
<td>CMA</td>
<td>Centromere Ab, IgG, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>CASMT</td>
<td>ANA2 Cascade</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>RIB</td>
<td>Ribosome P Ab, IgG, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>ENAE</td>
<td>Ab to Extractable Nuclear Ag Eval, S</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>ADNAR</td>
<td>dsDNA Ab with Reflex, IgG, S</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Testing Algorithm

If antinuclear antibodies are $\geq 3.0$ U, then antibodies to double-stranded DNA (dsDNA), extractable nuclear antigen evaluation, ribosome P, and centromere are performed at an additional charge.

If result from dsDNA test is borderline, then dsDNA antibody by Crithidia IFA will be performed at an additional charge.

See Connective Tissue Disease Cascade (CTDC) in Special Instructions.

Special Instructions

- Connective Tissue Disease Cascade (CTDC)

Method Name

Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available

Yes
Test Definition: CTDC
Connective Tissue Disease Cascade,S

Specimen

Specimen Type
Serum

Specimen Required

Container/Tube:

Preferred: Serum gel
Acceptable: Red top

Specimen Volume: 1 mL

Forms
If not ordering electronically, complete, print, and send a General Request (T239) with the specimen.

Specimen Minimum Volume
0.7 mL

Reject Due To

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

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Refrigerated (preferred)</td>
<td>21 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>21 days</td>
<td></td>
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</table>

Clinical and Interpretive

Clinical Information

The following diseases are often referred to as connective tissue diseases: rheumatoid arthritis (RA), lupus erythematosus (LE), scleroderma (systemic sclerosis) CREST syndrome (calcinoïd, Raynaud phenomenon, esophageal hypomotility, sclerodactyly, and telangiectasia), Sjogren syndrome, mixed connective tissue disease (MCTD), and polymyositis. Connective tissue diseases (systemic rheumatic diseases) are characterized by immune-mediated inflammation that involves the joints, skin, and visceral organs. These diseases are also accompanied by antibodies to a host of nuclear and cytoplasmic autoantigens.

The diagnosis of a connective tissue disease is based on clinical signs and symptoms and characteristic radiographic, histopathologic, and serologic findings. Certain connective tissue diseases are characterized by
autoantibodies that are highly specific for individual diseases (see table). Connective tissue diseases often present clinically with signs and symptoms that are nonspecific, including constitutional signs (eg, fever, weight loss, fatigue, and arthralgias). Accordingly, consideration of the possibility of a connective tissue disease is common on initial clinical presentation and testing for antibodies to autoantigens associated with connective tissue diseases is often performed early in the evaluation of many patients.(1)

### Autoantibodies with High Specificity for Individual Connective Tissue Diseases

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic citrullinated peptide antibodies</td>
<td>RA</td>
</tr>
<tr>
<td>dsDNA antibodies</td>
<td>LE</td>
</tr>
<tr>
<td>Scl 70 antibodies (topoisomerase 1)</td>
<td>Scleroderma</td>
</tr>
<tr>
<td>Jo 1 antibodies (histidyl tRNA synthetase)</td>
<td>Polymyositis</td>
</tr>
<tr>
<td>SSA/Ro and SSB/La antibodies</td>
<td>Sjogren syndrome</td>
</tr>
<tr>
<td>RNP antibodies (in isolation)</td>
<td>MCTD</td>
</tr>
<tr>
<td>Sm antibodies</td>
<td>LE</td>
</tr>
<tr>
<td>Ribosome P antibodies</td>
<td>LE</td>
</tr>
<tr>
<td>Centromere antibodies</td>
<td>CREST syndrome</td>
</tr>
</tbody>
</table>

In this test, serum is tested initially for the presence of antinuclear antibodies (ANA) and for cyclic citrullinated peptide (CCP) antibodies. The presence of CCP antibodies indicates a strong likelihood of RA.(2) The presence of ANA supports the possibility of a connective tissue disease, and the level of ANA is used to identify sera for second-order testing for antibodies to double-stranded DNA (dsDNA) and the other autoantigens. The decision threshold for performing the second-order tests is based on empirical data derived from testing patients with varying levels of ANA and was chosen to minimize testing when positive results for dsDNA and other antibodies are very unlikely.(3)

The testing algorithm is useful in the initial evaluation of patients and performs best in clinical situations in which the prevalence of disease is low.(4)

### Reference Values

ANTINUCLEAR ANTIBODIES (ANA)

- \(< \text{or } =1.0 \text{ U (negative)}\)
- \(1.1-2.9 \text{ U (weakly positive)}\)
- \(3.0-5.9 \text{ U (positive)}\)
- \(> \text{or } =6.0 \text{ U (strongly positive)}\)

Reference values apply to all ages.

CYCLIC CITRULLINATED PEPTIDE ANTIBODIES, IgG

- \(<20.0 \text{ U (negative)}\)
- \(20.0-39.9 \text{ U (weak positive)}\)
40.0-59.9 U (positive)
> or =60.0 U (strong positive)

Reference values apply to all ages.

**Interpretation**
Interpretive comments are provided.

See individual unit codes for additional information.

**Cautions**

Results must be interpreted in the context of the complete clinical picture.

The presence of detectable levels of antinuclear antibodies (ANA) is not specific for connective tissue disease. False-positive results for ANA occur in approximately 15% of women over age 40.(1) Weakly positive results for cyclic citrullinated peptide antibodies may occur in patients with connective tissue diseases other than rheumatoid arthritis.

This test is not recommended for:

- Testing in clinical situations with a high prevalence of connective tissue diseases (eg, rheumatology specialty practice)
- Follow-up evaluation of patients with known connective tissue diseases

**Clinical Reference**


**Performance**

**Method Description**

Antinuclear Antibodies (ANA):

The method used to detect antinuclear antibody (ANA) is enzyme-linked immunosorbent assay (ELISA). A HEp-2 lysate supplemented with various purified antigens (double-stranded deoxyribonucleic acid (dsDNA), histone, SS-A (Ro), SS-B (La) Smith, RNP, Scl-70, Jo-1, plus centromere 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, Hercules CA 7/2014)

Cyclic Citrullinated Peptide (CCP):
Cyclic citrullinated peptide (CCP) antibodies in serum are detected by binding to the wells of a commercial microtiter plate coated with synthetic CCP (Quanta Lite CCP3 IgG ELISA, INOVA Diagnostics). During the first incubation, serum antibodies bind to adsorbed, solid phase CCP. The wells are then washed to remove unbound serum constituents, and horse radish peroxidase-labeled goat anti-human IgG antibody is added. After further incubation and washing to remove unbound conjugate, substrate (3,3',5,5' tetramethylbenzidine) is added and allowed to incubate. The reaction between enzyme and substrate is stopped and color in the wells is measured in a microtiter plate reader. The concentration of CCP antibodies is determined by comparison to a 5 point standard curve (15.6-250 U). Testing is performed on the Agility instrument by Dynex. (Package insert: Quanta Lite CCP3 IgG ELISA. INOVA Diagnostics. San Diego, CA 11/2010)

Double-Stranded DNA (dsDNA):
dsDNA antibodies are measured by a commercial ELISA. Microwells are precoated with calf thymus double-stranded DNA (dsDNA) antigen. The calibrators, controls, and diluted patient samples are added to the wells and autoantibodies recognizing the dsDNA antigen bind during the first incubation. After washing the wells to remove all unbound proteins, purified peroxidase-labeled goat antihuman IgG conjugate is added. The conjugate binds to the captured human autoantibody and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualized with 3,3’,5,5’ tetramethylbenzidine (TMB) substrate, which gives a blue reaction product, the intensity of which is proportional to a concentration of autoantibody in the sample. Sulfuric acid is added to each well to stop the reaction. This produces a yellow end-point color, which is read at 450 nm. (Package insert: QUANTA Lite dsDNA SC ELISA, INOVA Diagnostics Inc, San Diego, CA 07/2012)

Confirmatory testing for borderline dsDNA by ELISA testing is performed by immunofluorescence assay (IFA). Autoantibodies in a test sample directed against dsDNA bind to antigens in the substrate placed on the slide, which, in this case, is Crithidia luciliae. Washing removes excess serum from the substrate. Fluorescein-conjugated (FITC) antisera added to the substrate attaches to the bound autoantibody. After a second washing step to remove excess conjugate, the substrate is coverslipped and viewed for fluorescent patterns with a fluorescent microscope. Observation of specific fluorescent patterns on the substrate indicates the presence of autoantibodies in the test sample. (Package insert: Bio-Rad Kallestad Crithidia luciliae Substrate. Bio-Rad Laboratories, Hercules, CA 07/2009)

SS-A/Ro, SS-B/La, RNP, Sm, ScI 70, Jo 1, ribosome P, and centromere antibodies are measured by a commercial multiplex flow immunoassay system. Recombinant or purified antigens are coupled covalently to polystyrene microspheres that are impregnated with fluorescent dyes to create unique fluorescent signatures, 1 microsphere type for each antigen. Diluted sera, calibrators, and controls are added to a mixture containing the antigen-coupled microspheres. Antibodies to each antigen bind to their homologous antigen-coupled microspheres. The microspheres are washed to remove extraneous serum proteins. Phycoerythrin (PE)-conjugated antihuman IgG antibody is then added to detect IgG antibodies bound to the microspheres. The microspheres are washed to remove unbound conjugate, and bound conjugate is detected by laser photometry. A primary laser determines the fluorescent signature of each microsphere, and a secondary laser reveals the level of PE fluorescence associated with the microsphere surface. Results are calculated for each antigen-coated microsphere type by comparing the median fluorescence response to a series of multipoint calibration curves. (Package insert: BioPlex 2200 ANA Screen. Bio-Rad Laboratories, Hercules, CA 03/2012)
Test Definition: CTDC
Connective Tissue Disease Cascade,S

Monday through Saturday; 4 p.m.

Analytic Time
3 days

Maximum Laboratory Time
4 days

Specimen Retention Time
14 days

Performing Laboratory Location
Rochester

Fees and 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 or approved by the U.S. 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
86200
83516-Centromere (if appropriate)
83516-Ribosome (if appropriate)
86225-ds-DNA Ab with Reflex (if appropriate)
86255-ds-DNA Ab by Crithidia IFA (if appropriate)
86235 x 6-RNP, Sm, SS-B, SS-A, Jo 1, and Scl 70 (if appropriate)

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<tbody>
<tr>
<td>CTDC</td>
<td>Connective Tissue Disease Cascade,S</td>
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Document generated April 7, 2020 at 5:28pm CDT
## Test Definition: CTDC
Connective Tissue Disease Cascade, S

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<tr>
<th>Result ID</th>
<th>Test Result Name</th>
<th>Result LOINC Value</th>
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<tbody>
<tr>
<td>ANA2</td>
<td>Antinuclear Ab, S</td>
<td>5047-6</td>
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<tr>
<td>CCP</td>
<td>Cyclic Citrullinated Peptide Ab, S</td>
<td>33935-8</td>
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<tr>
<td>IM_01</td>
<td>Interpretation</td>
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