MAYO CLINIC LABORATORIES

Test Definition: CTDC

Connective Tissue Diseases Cascade, Serum

Overview

Useful For

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

Initial evaluation of patients in clinical situations in which the prevalence of disease is low (6)

This test is **not recommended for**:
-Testing in clinical situations in which there is a high prevalence of connective tissue diseases (eg, rheumatology specialty practice)
-Follow-up evaluation of patients with known connective tissue diseases

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
ANA2	Antinuclear Ab, S	Yes	Yes
CCP	Cyclic Citrullinated Peptide Ab, S	Yes	Yes
IM_01	Interpretation	No	Yes

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
CMA	Centromere Ab, IgG, S	Yes	No
CASMT	ANA2 Cascade	No	No
RIB	Ribosome P Ab, IgG, S	Yes	No
ENAE	Ab to Extractable Nuclear Ag Eval,S	Yes	No
ADNA1	dsDNA Ab, IgG, S	Yes	No

Testing Algorithm

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

For more information see Connective Tissue Disease Cascade.

Special Instructions

- Connective Tissue Disease Cascade

Method Name

Enzyme-Linked Immunosorbent Assay (ELISA)

NY State Available
Yes

Specimen

Specimen Type
Serum

Specimen Required
Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Collection Container/Tube:
Preferred: Serum gel
Acceptable: Red top
Submission Container/Tube: Plastic vial
Specimen Volume: 1 mL
Collection Information: Centrifuge and aliquot serum into plastic vial.

Specimen Minimum Volume
0.7 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information
Connective tissue diseases (CTD) constitute diverse disorders affecting the joints, skin, eyes, heart, lungs, and gastrointestinal tract.(1) The diseases may be caused mainly by inheritable disorders of the connective tissue or autoimmune mechanisms.(1,2) The most common autoimmune-mediated CTD include rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) including CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), Sjogren syndrome (Sjs), mixed connective tissue disease (MCTD), and idiopathic inflammatory myopathies (IIM).(1-3) Collectively, these diseases are also referred

to as antinuclear antibody (ANA)-associated CTD or ANA-associated systemic autoimmune rheumatic diseases (ASARD). These diseases are generally accompanied by antibodies to nuclear and cytoplasmic autoantigens when tested by indirect immunofluorescence assay (IFA) using HEp-2 substrate (HEp-2 IFA).(2,3) In addition to the ANA-CTD, RA (another CTD with overlapping clinical features) is an important consideration in the early detection of patients at-risk for CTD.(2,4,5)

The diagnosis of any CTD is based on the patient's personal and family medical histories, clinical presentation, radiographic and histopathologic features, presence of specific autoantibodies, and other laboratory findings.(6-8) Certain CTD subsets are characterized by autoantibodies that are highly specific for individual diseases as outlined in the Table below. The detection of ANA in certain clinical subsets of CTD is generally dependent on the type of immunoassay employed and the reference intervals.(5,9) Clinically, patients with CTD often present with signs and symptoms that are nonspecific such as fever, weight loss, fatigue, and arthralgias.

Table. **Autoantibodies and Clinical Associations with Specific Connective Tissue Diseases**

Autoantibody	**Connective tissue disease specificity**
Cyclic citrullinated peptide antibodies	Rheumatoid arthritis (RA)
Double-Stranded DNA (dsDNA)	Systemic lupus erythematosus (SLE)
Smith (Sm)	SLE
Ribosome P	SLE
SS-B/La	Sjogren syndrome (SjS)
SS-A/Ro (Ro52 or Ro60)	SjS, SLE, systemic sclerosis (SSc), antisynthetase syndrome
RNP 68 and A (RNP)	Mixed connective tissue disease
Topoisomerase 1 (topo-1, Scl-70)	SSc (commonly the diffuse cutaneous SSc, dsSSc)
Histidyl tRNA synthetase (JO1)	Idiopathic inflammatory myositis (IIM), commonly associated with anti-synthetase syndrome
Centromere B	SSc (commonly limited cutaneous lcSSc)

In the Connective Tissue Cascade, serum is tested initially for the presence of ANA and for cyclic citrullinated peptide (CCP) antibodies using solid-phase immunoassays (SPAs). The presence of significantly elevated CCP antibodies is suggestive of RA or risk for developing disease in at-risk individuals.(5,6) However, additional testing for rheumatoid factor (RF) and inflammatory markers, which are not included in the cascade, are important for optimal diagnosis as per the 2010 American College of Rheumatology/European League Against Rheumatism RA classification criteria.(6) The presence of ANA detected with SPAs such as enzyme-linked immunosorbent assay maybe useful in identifying patients with specific CTD subsets (ANA-associated CTD), notably Sjogren's syndrome, mixed connective tissue disease MCTD), systemic lupus erythematosus.(2,3) The CTD cascade employs a cut-off based on degree of ANA positivity to identify sera for second-order testing for first-line antibodies (dsDNA. SSA, SSB, Scl 70. Centromere B, U1RNP, Smith) and anti-CCP antibodies allowing for identification of the common autoantibodies in the classification criteria for RA, SLE and SSc.(6-8) The decision threshold for performing second-order tests is based on empirical data derived from testing patients with varying levels of ANA and is chosen to minimize testing in situations in which positive results for dsDNA and other antibodies is less likely.(9) However, a negative ANA enzyme immunoassay result does not rule out a diagnosis of CTD as has been reported in a number of studies.(reviewed in 3,10) Therefore, in patients with a strong clinical suspicion of CTD, testing for ANA using HEp-2 IFA may be warranted.(3,10)

Reference Values

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

CYCLIC CITRULLINATED PEPTIDE ANTIBODIES, IgG
<20.0 U (Negative)
20.0-39.9 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.

Differential testing for Ro52 and Ro60 antibodies in SS-A/Ro positive patients may be useful in the diagnosis of specific CTD clinical subset, disease stratification, and prognosis. Consider testing for Ro52 and Ro60 antibodies (ROPAN / Ro52 and Ro60 Antibodies, IgG, Serum) if the patient is positive for SS-A/Ro.

Cautions

The results of tests for autoantibodies must be interpreted in the appropriate clinical context.

The presence of detectable levels of antinuclear antibodies (ANA) in solid-phase immunoassays, such as enzyme-linked immunosorbent assay (ELISA), is not specific for connective tissue disease.

Positive results may occur prior to onset of clinical diseases as well as in the absence of connective tissue disease.

Negative ANA by ELISA results may not be reliable for the evaluation of patients at-risk for systemic sclerosis and inflammatory myopathies.

Weak-to-moderate positive results for cyclic citrullinated peptide antibodies may occur in patients with connective tissue diseases other than rheumatoid arthritis.

Clinical Reference

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4. Stinton LM, Fritzler MJ. A clinical approach to autoantibody testing in systemic autoimmune rheumatic disorders. Autoimmun Rev. 2007;7(1):77-84
5. Anaparti V, Smolik I, Meng X, et al. Expansion of Alternative Autoantibodies Does Not Follow the Evolution of Anti-Citrullinated Protein Antibodies in Preclinical Rheumatoid Arthritis: An Analysis in At-Risk First Degree Relatives. Arthritis Rheumatol. 2021;73(5):740-749. doi:10.1002/art.41675

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7. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: An American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum. 2013;65(11):2737-2747. doi:10.1002/art.38098
8. Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol. 2019; 71(9):1400-1412. 3
9. 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;1(1):36-46
10. Orme ME, Andalucia C, Sjölander S, Bossuyt X. A comparison of a fluorescence enzyme immunoassay versus indirect immunofluorescence for initial screening of connective tissue diseases: Systematic literature review and meta-analysis of diagnostic test accuracy studies. Best Pract Res Clin Rheumatol. 2018; 32(4):521-534

Performance

Method Description

Antinuclear Antibodies:
The method used to detect antinuclear antibodies (ANA) is enzyme-linked immunosorbent assay (ELISA). A HEp-2 lysate supplemented with specific purified antigens (double-stranded [ds] DNA, 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; 07/2014)

Cyclic Citrullinated Peptide:
Cyclic citrullinated peptide (CCP) antibodies in serum are detected by binding to the wells of a commercial microtiter plate coated with synthetic CCP. 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; v9 02/2020)

dsDNA:
The test kit contains 12 micotiter strips each with 8 break-off reagent wells coated with double-stranded DNA (dsDNA). In the first reaction step, diluted patient samples, calibrators and controls are incubated in the wells. Anti-dsDNA antibodies will bind to the antigens coated in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, rabbit anti-human IgG HRP enzyme conjugate is added to each well. The enzyme conjugate will bind to any wells that have human IgG binding to the dsDNA antigen. The wells

are washed to remove any unbound HRP enzyme conjugate. 3,3,5,5, tetramethylbenzidine (TMB) enzyme substrate is added. If the HRP enzyme is present in the well (positive reaction), the HRP enzyme will react with the TMB substrate and produce a blue color. After an additional incubation time to allow the color development, a stop solution is added which turns the blue color yellow and inhibits further color development to allow for stable spectrophotometric reading. The test strips are placed in a microplate reader and the optical density of the color is measured. The amount of antigen specific bound antibody is proportional to the color intensity.(Package insert: Anti-dsDNA-NcX ELISA (IgG). EUROIMMUN; 7/8/2020)

SS-A/Ro, SS-B/La, RNP, Sm, Scl 70, Jo 1, ribosome P, and centromere B 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, one 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; 02/2019)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
3 to 4 days

Specimen Retention Time
14 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Superior Drive

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 account representative. 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
86200
83516-Centromere (if appropriate)
83516-Ribosome (if appropriate)
86225-ds-DNA AB IgG, Serum (if appropriate)
86235 x 6-RNP, Sm, SS-B, SS-A, Jo 1, and Scl 70 (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
CTDC	Connective Tissue Disease Cascade,S	95267-1

Result ID	Test Result Name	Result LOINC® Value
ANA2	Antinuclear Ab, S	94875-2
CCP	Cyclic Citrullinated Peptide Ab, S	33935-8
IM_01	Interpretation	69048-7