MAYO CLINIC LABORATORIES

Test Definition: ANCA

Cytoplasmic Neutrophil Antibodies, Serum

Overview

Useful For

Evaluating patients with clinical features of ANCA-associated vasculitis, specifically granulomatosus with polyangiitis, microscopic polyangiitis, and eosinophilic granulomatosus with polyangiitis

Testing Algorithm

For more information see:
-First-Line Screening for Autoimmune Liver Disease Algorithm.
-Acquired Neuropathy Diagnostic Algorithm

Special Instructions

- First-Line Screening for Autoimmune Liver Disease Algorithm
- Acquired Neuropathy Diagnostic Algorithm

Method Name

Indirect Immunofluorescence (IIF)

NY State Available

Yes

Specimen

Specimen Type

Serum

Additional Testing Requirements

When used for diagnosis, it is recommended that specific tests for proteinase 3 antibodies and myeloperoxidase antibodies be performed first, with additional testing for anti-neutrophil cytoplasmic antibodies only needed in certain circumstances.(3) A testing algorithm based on these recommendations is available. For more information see VASC / Antineutrophil Cytoplasmic Antibodies Vasculitis Panel, 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: 0.8 mL
Collection Instructions: Centrifuge and aliquot serum into plastic vial.

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-General Request (T239)

-Renal Diagnostics Test Request (T830)

-Kidney Transplant Test Request

Specimen Minimum Volume

0.4 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	OK
Heat-treated specimen	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides are characterized by a pauci-immune inflammation within the walls of small blood vessels (1). There are 3 specific diseases which are identified as ANCA-associated vasculitides: microscopic polyangiitis (MPA), granulomatosus with polyangiitis (GPA), and eosinophilic granulomatosus with polyangiitis (EGPA). The serological hallmark of these disorders is the presence of ANCA, which are antibodies that bind to cytoplasmic antigens found in the granules of neutrophils (2). Patients with GPA frequently have antibodies specific for proteinase 3 (PR3), while individuals with MPA or EGPA are more likely to have antibodies that bind to myeloperoxidase (MPO). The presence of PR3-ANCA and MPO-ANCA can be detected using antigen-specific immunoassays or indirect immunofluorescence (IIF). IIF is performed most commonly using ethanol-fixed neutrophils. Using this substrate, anti-PR3 antibodies produce a granular cytoplasmic staining pattern, which is referred to as cANCA. In comparison, due to an artefact that is a result of the fixation process, anti-MPO antibodies display a perinuclear pattern, or pANCA.

Patients with suspected ANCA-associated vasculitis should be evaluated for the presence of PR3-ANCA, MPO-ANCA and ANCA by IIF. A consensus guideline published in 2017 recommends that patients with possible GPA or MPA be tested for PR3-ANCA and MPO-ANCA using antigen-specific immunoassays (3). ANCA by IIF should then be used in cases where there is a high degree of suspicion for GPA or MPA but the PR3-ANCA and MPO-ANCA testing is negative. This guideline also suggests that ANCA may be used in situations where a low positive PR3-ANCA or MPO-ANCA is detected, to improve specificity of the testing. The classification criteria for MPA, GPA and EGPA published by the American College of Rheumatology and the European Alliance of Associations for Rheumatology include PR3-ANCA and MPO-ANCA detected

by either antigen-specific immunoassay or IIF (4-6). These classification criteria incorporate serological ANCA testing along with clinical symptoms, imaging, and biopsy results to determine a score which allows for the classification of the various ANCA-associated vasculitides.

Reference Values

<1:4 (Negative)

Interpretation

Positive results for proteinase 3 (PR3) antineutrophil cytoplasmic antibodies (ANCA) by antigen-specific immunoassay and cANCA by indirect immunofluorescence are consistent with the diagnosis of granulomatosus with polyangiitis, in patients with the appropriate clinical presentation.

Positive results for myeloperoxidase-ANCA by antigen-specific immunoassay and pANCA by indirect immunofluorescence are consistent with the diagnosis of microscopic polyangiitis or eosinophilic granulomatosus with polyangiitis, in patients with the appropriate clinical presentation.

Cautions

A positive result for proteinase 3 (PR3)- anti-neutrophil cytoplasmic antibody (ANCA), myeloperoxidase (MPO)-ANCA, or ANCA by IIF is not diagnostic for any ANCA-associated vasculitis and must be interpreted in the clinical context of the patient.

Negative results for PR3-ANCA, MPO-ANCA, and ANCA by indirect immunofluorescence (IIF) do not exclude the possibility of ANCA-associated vasculitis.

For monitoring disease activity, suggest ordering PR3 / Proteinase 3 Antibodies, Serum or MPO / Myeloperoxidase Antibodies, IgG, Serum

Antibodies specific for antigens other than PR3 and MPO may lead to nuclear, perinuclear or cytoplasmic staining on ethanol-fixed neutrophils. A positive or indeterminate pANCA or cANCA by IIF in the absence of a detectable PR3-ANCA or MPO-ANCA by antigen-specific immunoassay may indicate the presence of an antibody of unidentified specificity.

If ordered as a stand-alone test, all positive ANCA by IIF results should be confirmed for PR3-ANCA MPO-ANCA by solid-phase immunoassays.

Clinical Reference

1. Kitching AR, Anders HJ, Basu N, et al. ANCA-associated vasculitis. Nat Rev Dis Primers. 2020:6(1):71
2. Ramponi G, Folci M, De Santis M, et al. The biology, pathogenetic role, clinical implications, and open issues of serum anti-neutrophil cytoplasmic antibodies. Autoimmun Rev. 2021:20(3):102759
3. Bossuyt X, Cohen Tervaert W, Arimura Y, et al. Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. Nat Rev Rheumatol. 2017:13(11):683-692
4. Suppiah R, Robson JC, Grayson PC, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for microscopic polyangiitis. Ann Rheum Dis. 2022;81(3):321-326. doi:10.1136/annrheumdis-2021-221796
5. Robson JC, Grayson PC, Ponte C, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for granulomatosis with polyangiitis. Ann Rheum Dis. 2022;81(3):315-320. doi:10.1136/annrheumdis-2021-221795
6. Grayson PC, Ponte C, Suppiah R, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for eosinophilic granulomatosis with polyangiitis. Ann Rheum Dis.

2022;81(3):309-314. doi:10.1136/annrheumdis-2021-221794

Performance

Method Description
Antibodies to cytoplasmic antigens in neutrophils are detected by an indirect immunofluorescent technique. Commercial and in-house slides prepared from human neutrophils are used as a substrate. IgG antibodies in serum specimens are detected after incubation of serum with the commercial and in-house slides by the addition of a fluorescein isothiocyanate-labeled antihuman IgG reagent. All patient specimens are initially screened at 1:4 and 1:8 dilutions.(Unpublished Mayo Method)

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 was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
86036 x2
86037-Titer (if appropriate)

LOINC® Information

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Test Definition: ANCA

Cytoplasmic Neutrophil Antibodies, Serum

Test ID	Test Order Name	Order LOINC® Value
ANCA	Cytoplasmic Neutrophilic Ab, S	87427-1

Result ID	Test Result Name	Result LOINC® Value
3114	c-ANCA	In Process
3119	p-ANCA	17357-5