



LABORATORY ALLIANCE of Central New York, LLC

ANCA-Associated Vasculitis Testing:

**Anti-Neutrophil Cytoplasmic Antibodies
Myeloperoxidase Antibodies
Serine Protease-3 Antibodies**

Antineutrophil cytoplasmic antibodies (ANCA) can occur in patients with autoimmune vasculitis including Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), or organ-limited variants such as pauci-immune necrotizing glomerulonephritis. Detection of ANCA is a well-established diagnostic test for the evaluation of patients suspected of having autoimmune vasculitis. ANCA react with enzymes in the cytoplasmic granules of human neutrophils including proteinase 3 (PR3), myeloperoxidase (MPO), elastase, and cathepsin G. Antibodies to PR3 occur in patients with WG (both classical WG and WG with limited end-organ involvement) and produce a characteristic pattern of granular cytoplasmic fluorescence on ethanol-fixed neutrophils called the cANCA pattern. Antibodies to MPO occur predominately in patients with MPA and produce a pattern of perinuclear cytoplasmic fluorescence on ethanol-fixed neutrophils called the pANCA pattern.

Commonly requested tests for patients with suspected or known autoimmune vasculitis include Anti-Neutrophil Cytoplasmic Antibodies (ANCA), Myeloperoxidase Antibodies (MPO) and Serine Protease-3 Antibodies (PR3). These tests are available through our reference laboratory (ARUP).

ANCA testing is performed by immunofluorescence immunoassay (IFA). Both ethanol- and formalin-fixed slides are used, allowing differentiation of cANCA (granular cytoplasmic staining) and pANCA (perinuclear staining) patterns. Over 90% of patients with certain necrotizing systemic vasculitides show positive results for ANCA, while usually less than 5% of patients with collagen vascular disease or arthritis will be positive. If positive, further testing is performed to provide a semi-quantitative result and both titer and pattern of staining is reported.

MPO and PR3 testing is performed by multiplex bead immunoassay and reported semi-quantitatively in AU/mL (arbitrary units/mL). It is important to note that although results are numerical, they represent positivity relative to a cutoff (antibody index) rather than a true quantitative result. Approximately 90 percent of patients with a pANCA pattern by IFA have antibodies specific for MPO. Approximately 85 percent of patients with a cANCA pattern by IFA have antibodies specific for PR-3.



While each of these tests provides useful information in patients at risk of ANCA-associated vasculitis, the likelihood of false-positive results is greatly increased if such testing is not used in the appropriate clinical context. Several recent publications describing the clinical utility of ANCA testing for diagnosing as well as monitoring previously diagnosed vasculitis (controversial) are listed below.

The preferred testing for the workup of suspected vasculitis is a panel that includes ANCA, MPO, and PR-3 antibodies. Monitoring a patient with a history of vasculitis may be accomplished by choosing either MPO/PR3 panel or ANCA with reflex to MPO and PR3 if positive. The decision on which monitoring pathway to choose would be dependent on the results of the initial positive tests from the vasculitis screening 3-test panel.

The following table summarizes Laboratory Alliance's ANCA-associated vasculitis testing menu.

Test Name	Test Code	Includes	Suggested use
Vasculitis Profile	VASCAB	<ul style="list-style-type: none">• ANCA (reflexes to titer and pattern if positive)• MPO• PR3	Confirm diagnosis for patients with suspected autoimmune vasculitis Rule out autoimmune process for patients with collagen vascular disease or arthritis
ANCA IgG	ANCIGG	<ul style="list-style-type: none">• ANCA (reflexes to titer and pattern if positive)	Monitor patients with previously positive ANCA results
ANCA C P w/Reflex	ANCAB	<ul style="list-style-type: none">• ANCA (reflexes to titer, pattern, MPO and PR3 if positive)	Monitor patients with previously positive ANCA, MPO and/or PR3 results
MPO/PR-3 Antibodies	MPOPR3	<ul style="list-style-type: none">• MPO• PR3	Monitor patients with previously positive MPO or PR3 results

Specific test details are provided in the table below and in our Directory of Services.

Test Code:	VASCAB	ANCIGG	ANCAB	MPOPR3
Method:	See text above.			
Specimen Requirements:	One 5 mL gold (SST) top tube.			
Storage and Transport:	1 mL serum at 2-8°C. (Min: 0.5 mL)			
Stability:	Ambient: 2 days; Refrigerated: 2 weeks; Frozen: 1 year (avoid repeated freeze/thaw cycles)			
Testing Schedule: (at reference laboratory)	Mon-Sat	Mon-Sat	Mon - Sat	Daily
CPT Code:	83516 MPO; 83516 PR-3; 86255 ANCA; if positive, add 86256 ANCA titer	86255 ANCA; if positive, add 86256 ANCA titer	86255 ANCA; if positive add 86256 ANCA titer and 83516 x 2 (MPO, PR3)	83516 x 2 (MPO, PR3)
Billing Code:	5011423	5010201	5011116	5011122

Questions regarding these tests may be directed to Cheryl Haskins, MS, MT(ASCP)SC, Manager, Chemistry and Referral Testing, at 315-410-7014 or cherylhaskins@lacny.com.

References:

Tomasson G *et al.* Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis – a meta-analysis. *Rheumatology*. 2012;51:100-9.

Specks U. Controversies in ANCA testing. *Cleve Clin J Med* 2012;79:S7-S10

Flores-Suárez LF *et al.* Antineutrophil Cytoplasm Autoantibodies: Usefulness in Rheumatology. *Reumatol Clin*. 2012;08:351-7 - Vol. 08