



## Anti-Neutrophil Cytoplasmic Antibodies in Systemic Vasculitis

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ON JULY 10, 2000, Pathology Associates Medical Laboratories began offering the Anti-Neutrophil Cytoplasmic Antibody (ANCA) Panel comprising the following tests: Anti-nuclear antibodies (ANA) with titer and pattern interpretation, ANCA IFA titer with pattern interpretation, and two tests that measure the specific autoantibodies, anti-proteinase 3 (PR3 Ab) and anti-myeloperoxidase (MPO Ab). In some cases the PR3 and MPO assays may be more sensitive than the conventional IFA technique.

Anti-neutrophil cytoplasmic antibody (ANCA) tests are used primarily in the diagnosis and evaluation of patients with Wegener's granulomatosis, a systemic necrotizing vasculitis associated with inflammation and destruction of blood vessels in various organs, especially the lung and kidney. It may also be useful in the diagnosis of other primary systemic small vessel vasculitides.

For optimal testing in new patients, ANCA testing should include a combination of indirect immunofluorescence (IFA) to detect proteins found in the primary and secondary granules of ethanol-fixed normal peripheral blood neutrophils, plus enzyme-linked immunosorbent assays (EIAs) that detect ANCA specific for proteinase 3 (PR3) or myeloperoxidase (MPO). The ANA assay is also included in the ANCA panel since the presence of anti-nuclear antibodies can sometimes interfere with interpretation of the ANCA IFA pattern. There are three characteristic staining patterns for ANCA:

- **Cytoplasmic.** These antibodies are designated C-ANCA and the target autoantigen is proteinase 3 (PR3). C-ANCA is found in 85% of patients with active Wegener's granulomatosis. Patients with inactive disease may have lower rates of positivity.
- **Peri-nuclear.** These antibodies are designated P-ANCA and the major target autoantigen is myeloperoxidase (MPO). P-ANCA is not specific for a single disease entity, but is associated with disease groups which show common clinical and histological features. P-ANCA occurs in vasculitis, glomerulonephritis, Churg-Strauss syndrome, polyarteritis nodosa, systemic lupus erythematosus, rheumatoid arthritis and associated inflammatory disorders.
- **Atypical P-ANCA.** The antigen(s) associated with atypical P-ANCA are poorly characterized and can only be detected by immunofluorescence. Atypical P-ANCA is associated with inflammatory bowel disease.

The results of ANCA tests should be interpreted in the context of other clinical, laboratory and histopathologic data in establishing a diagnosis of Wegener's granulomatosis or other systemic vasculitis.

### References

- Gross, W. L., 1995. Rheumatic Diseases Clinics of North America. 21:4.
- Savige, J., et al., 1999. International consensus Statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am J Clin Pathol.* 111:507-513.
- Werner, M., 1996. Antineutrophilic cytoplasmic antibody (ANCA) testing. Technical Bulletin.
- Proceedings of the 6<sup>th</sup> International ANCA Workshop, Paris, France. June 28 - July 1, 1995. 101 (Suppl. 1)
- Bradwell, A. R., et al., 1999. *Advanced Atlas of Autoantibody Patterns*. The Binding Site Inc., San Diego, CA.

### Indications for ANCA Testing

The following clinical conditions suggest a diagnosis of Wegener's granulomatosis or microscopic polyangiitis and, in the absence of any other obvious cause, are indicators for ANCA testing:

- ▶ **Glomerulonephritis, especially rapidly progressive disease.**
- ▶ **Pulmonary hemorrhage, especially pulmonary renal syndrome.**
- ▶ **Cutaneous vasculitis with systemic features.**
- ▶ **Multiple lung nodules.**
- ▶ **Chronic destructive disease of upper airways.**
- ▶ **Long-standing sinusitis or otitis.**
- ▶ **Subglottic tracheal stenosis.**
- ▶ **Mononeuritis multiplex or other peripheral neuropathy.**
- ▶ **Retro-orbital mass.**
- ▶ **Undiagnosed inflammatory disease.**

For more information, please contact Client Services.

## Association of C-ANCA and P-ANCA Antibodies in Disease

DISEASE	Clinical Sensitivity (% Positivity in Disease)	
	C-ANCA	P-ANCA
Wegener's granulomatosis	85	5 (up to 25% with active disease)
Microscopic polyangiitis	30	60
Necrotizing crescentic glomerulonephritis (NCGN)	30	60
Churg-Strauss syndrome	10	20
Polyarteritis nodosa	5	15
Ulcerative colitis		60
Crohn's disease		30
Chronic active hepatitis		80
Cystic fibrosis		90
Systemic lupus erythematosus		20
Rheumatoid arthritis		30
Primary sclerosing cholangitis		65
Primary biliary cirrhosis		Up to 30

► For your convenience, the ANCA tests may be ordered as a panel with the order code **ANCAD**.

*ANCA panel components may also be ordered separately with the order codes ANA, ANCASR, MPO and PR3AB for ANA, ANCA Screen, Myeloperoxidase Antibody, and Proteinase 3 Antibody respectively.*

## Test Information

**DESCRIPTION ANCA PANEL**

**METHOD** IFA / EIA

**ORDER CODE** ANCAD

**CPT CODES** 86038, 86256, 83520×2

**SPECIMEN** 2 mL serum (red top tube). Separate serum from cells ASAP and put in separate plastic tube. Store and transport refrigerated.

**COMMENTS** *Minimum amount:* 1 mL.

*Unacceptable conditions:* plasma, hemolyzed, lipemic, or contaminated samples, serum containing fluorescing drugs or subjected to repeated freeze/thaw cycles, and other body fluids.

*Stability:* 2 days refrigerated, 1 month frozen.

**SCHEDULE** Tuesday and Thursday evenings

**TURNAROUND** 2-6 days

<b>RANGES</b>	ANA	Negative: LT 1:40
	ANA Pattern	
	ANCA Titer, IFA	LT 1:20 Negative
	ANCA Pattern	
	Myeloperoxidase Ab	LT 9.0 U/mL
	Proteinase 3 Ab	LT 3.5 U/mL

**DESCRIPTION ANCA SCREEN, IFA**

**METHOD** IFA

**ORDER CODE** ANCASR

**CPT CODE** 86256

**SPECIMEN** 0.5 mL serum (red top tube). Separate serum from cells ASAP and put in separate plastic tube. Store and transport refrigerated.

**COMMENTS** *Minimum amount:* 0.3 mL.

*Unacceptable conditions:* plasma, hemolyzed, lipemic, or contaminated samples, serum containing fluorescing drugs or subjected to repeated freeze/thaw cycles, and other body fluids.

*Stability:* 2 days refrigerated, 1 month frozen.

**SCHEDULE** Tuesday and Thursday evenings

**TURNAROUND** 2-6 days

<b>RANGES</b>	ANCA Titer, IFA	LT 1:20 Negative
	ANCA Pattern	

**DESCRIPTION MYELOPEROXIDASE ANTIBODY**

**METHOD** EIA

**ORDER CODE** MPO

**CPT CODE** 83520

**SPECIMEN** 1 mL serum (red top tube). Separate serum from cells ASAP and put in separate plastic tube. Store and transport refrigerated.

**COMMENTS** *Minimum amount:* 1 mL.

*Unacceptable conditions:* plasma, hemolyzed, lipemic, or contaminated samples, serum containing fluorescing drugs or subjected to repeated freeze/thaw cycles, and other body fluids.

*Stability:* 48 hours at room temperature, 2 weeks refrigerated, 1 year frozen.

**SCHEDULE** Tuesday and Thursday evenings

**TURNAROUND** 2-6 days

<b>RANGES</b>	Myeloperoxidase Ab	LT 9.0	U/mL
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**DESCRIPTION PROTEINASE 3 ANTIBODY**

**METHOD** EIA

**ORDER CODE** PR3AB

**CPT CODE** 83520

**SPECIMEN** 1 mL serum (red top tube). Separate serum from cells ASAP and put in separate plastic tube. Store and transport refrigerated.

**COMMENTS** *Minimum amount:* 0.5 mL.

*Unacceptable conditions:* plasma, hemolyzed, lipemic, or contaminated samples, serum containing fluorescing drugs or subjected to repeated freeze/thaw cycles, and other body fluids.

*Stability:* 48 hours at room temperature, 2 weeks refrigerated, 1 year frozen.

**SCHEDULE** Tuesday and Thursday evenings

**TURNAROUND** 2-6 days

<b>RANGES</b>	Proteinase 3 Ab	LT 3.5	U/mL
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your local representative.*