



# Anti-Neutrophil Cytoplasmic Antibody (ANCA)

Autoimmune IFA





# ANCA

## Anti-Neutrophil Cytoplasmic Antibody

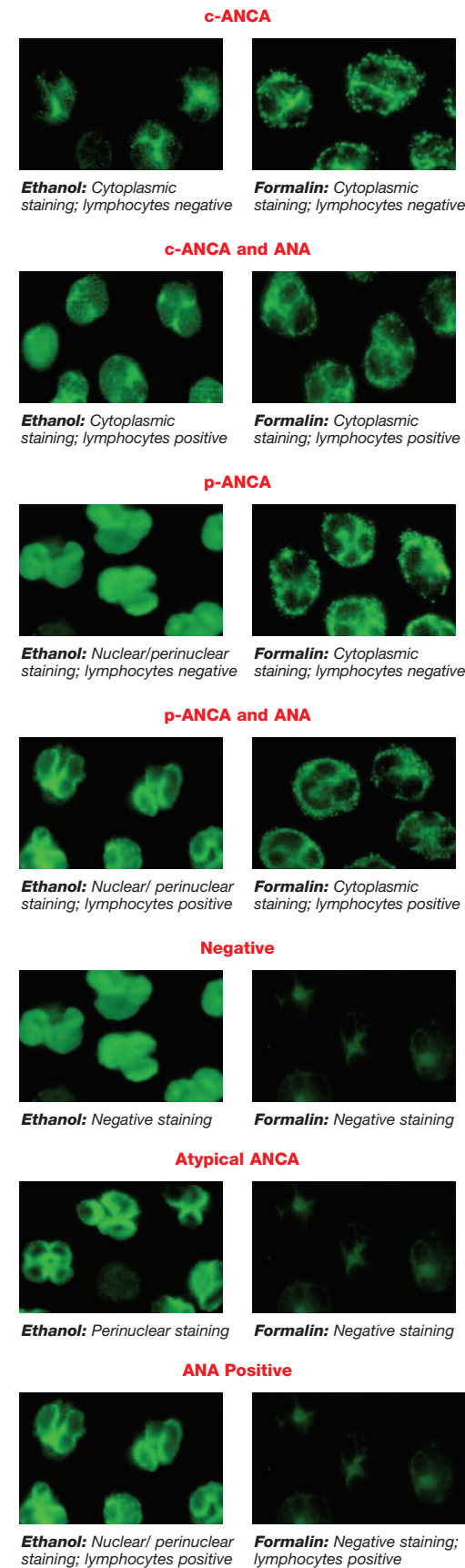
Vasculitis is a term used to describe inflammation of the blood vessels caused by white blood cells invading the associated tissue. Vasculitis can affect very small (capillaries), medium-size (arterioles or venules) or large blood vessels (arteries and veins). The clinical implication of vasculitis will vary greatly with the size and type of vessel affected. For example, an inflamed capillary will often break producing a tiny area of bleeding in the tissue. In contrast, a large vessel may narrow or close causing tissues receiving blood from that vessel to die.

### Anti-Neutrophil Cytoplasmic Antibodies (ANCA)

Anti-neutrophil cytoplasmic antibodies (ANCA) are autoantibodies associated with certain forms of vasculitis, including Wegener's granulomatosis, micropolyarteritis, glomerulonephritis, other vasculitides and inflammatory bowel disorders. The two major antigens associated with ANCA are proteinase 3 (PR3) and myeloperoxidase (MPO), which are found in the primary and secondary granules of neutrophils. Using IFA testing, ANCA are typically classified as either c-ANCA (often PR3), p-ANCA (most commonly MPO), atypical or x-ANCA. The clinical significance of these antibodies vary.<sup>1</sup> c-ANCA are found primarily in patients with Wegener's granulomatosis, whereas p-ANCA are most often associated with microscopic polyarteritis, necrotizing glomerulonephritis and Churg-Strauss syndrome.<sup>2-5</sup> Atypical or x-ANCA are often associated with irritable bowel syndrome.<sup>6-9</sup>

### Product Highlights

- Combination testing on a single slide
- Large, easy-to-read cells
- Identical assay protocol and reagents
- High quality, consistent products

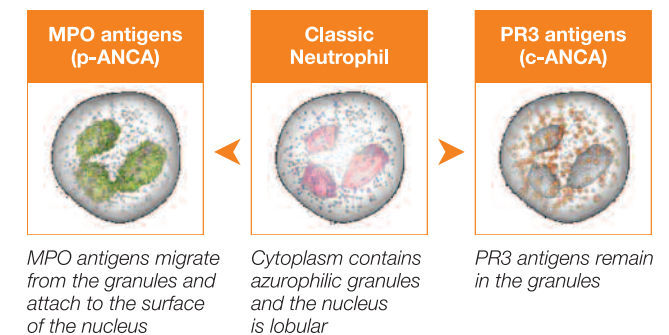


## Anti-Neutrophil Cytoplasmic Antibody Testing

ANCA testing aids physicians in the diagnosis of necrotizing vasculitides and irritable bowel syndrome. As treatments will significantly vary, diagnosis and differentiation of antibody type is essential.

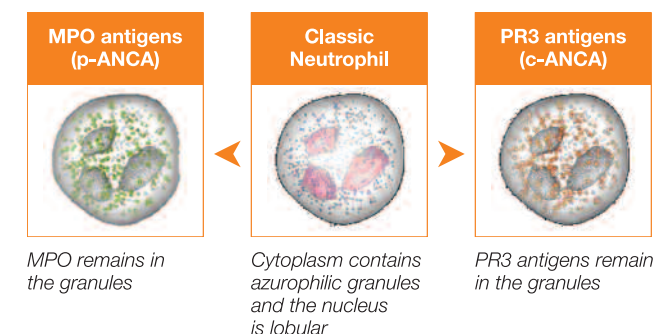
The Bio-Rad ANCA test system is made up of three types of immunofluorescent antibody tests (ethanol, formalin and combination slides) for the detection and semi-quantitation of ANCA in human serum. In ethanol fixed slides, MPO (p-ANCA) antigens migrate from the azurophilic granules and attach to the surface of the nucleus whereas the PR3 (c-ANCA) antigens remain in the granules. ANCA positive samples will demonstrate either nuclear (p-ANCA) or cytoplasmic (c-ANCA) staining dependent on antibody specificity.

### Ethanol Fixation



In formalin fixed slides, all ANCA antigens remain associated with the azurophilic granules causing both c-ANCA and p-ANCA to produce cytoplasmic staining. Because anti-nuclear antibody positive samples may demonstrate a positive p-ANCA result, formalin fixed slides are typically used to confirm p-ANCA patterns. In combination slides, both fixation types are used allowing simultaneous testing of patients.

### Formalin Fixation



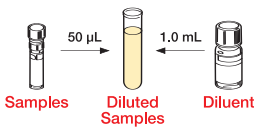


# Anti-Neutrophil Cytoplasmic Antibody (ANCA)

**Test Method** – The indirect immunofluorescence staining procedure is illustrated in the following figures

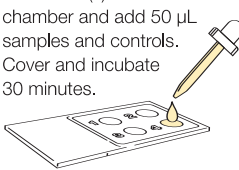
**1** Bring all reagents to room temperature (18 – 26°C).

**2** Make 1:20 working solutions by diluting 50 µL of patient's sera in 1.0 mL diluent.

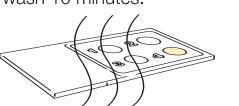


**3** Let pouch equilibrate to room temperature, then remove slide(s) from pouch.

**4** Remove slides from pouch. Place slide(s) into moisture chamber and add 50 µL samples and controls. Cover and incubate 30 minutes.




**5** Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.



**6** Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.


**7** Apply Conjugate ~50 µL to each well. Cover and incubate 30 minutes.



**8** Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.

**9** Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.

**10** Mount cover slip and read under fluorescent microscope.



## Ordering Information

### REFERENCES

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- Gigase P., DeClerck L. S., Van Cotthem K. A. et al. Anti-Neutrophil cytoplasmic antibodies in inflammatory bowel disease with special attention for IgA-class antibodies. *Dig Dis and Sci* 42:2171-2174, 1997.
- Shanahan F. Neutrophil autoantibodies in inflammatory bowel disease: Are they important? *Gastroenterol* 107:586-589, 1994.
- Shanahan F. and Bernstein C. N. ANCA's weigh in on colitis. *Gastroenterol* 105:946-947, 1993.

Catalog No.	Description
29403	Complete ANCA Kit (10 x 6 well ethanol fixed slides) (includes slides, conjugate, mounting media, PBS, positive/negative c-ANCA control, cover slips, sample diluent and counterstain) .....60 tests
29418	ANCA Formalin Fixed Slides (5 x 6 wells) .....30 tests
29417	ANCA Ethanol Fixed Slides (5 x 12 wells) .....60 tests
29416	ANCA Ethanol Fixed Slides (5 x 6 wells) .....30 tests
29419	ANCA Combination Ethanol/Formalin Fixed Slides (5 x 6 wells each) .....30 ethanol + 30 formalin tests
29412	PBS .....2 vials
29413	Cover Slips .....12 per box
29407	ANCA Sample Diluent .....60 mL
29400	c-ANCA Positive Control .....0.5 mL
29401	p-ANCA Positive Control .....0.5 mL
130*/29408	ANCA Negative Control .....0.5 mL
29411	ANCA Anti-human IgG FITC Conjugate .....5 mL
29409	Evans Blue .....1 mL
29410	ANCA Mounting Media .....5 mL
29414	6-Well Blotters .....12 per box
29415	12-Well Blotters .....12 per box

\* Bio-Rad QSD third party negative control



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