

# ANCA Associated Diseases

## Serine Proteinase 3 (PR3)

### ANCA Diagnostics

The emergence of sophisticated anti-neutrophilic cytoplasmic antibodies (ANCA) diagnostics was first described in the 1985 Lancet article of van der Woude and coworkers showing that the detection of ANCA was indeed related to Wegener's granulomatosis (ref. 1).

Two ANCA patterns may be seen with indirect immunofluorescence of ethanol-fixed neutrophils: a cytoplasmic pattern (c-ANCA) and the artifactual perinuclear pattern (p-ANCA). The major antigen for c-ANCA is a 29-kDa serine protease (ref. 2), proteinase 3 (PR3). PR3 and the major antigen of p-ANCA, myeloperoxidase (MPO), are present within the azurophilic granules of neutrophilic granulocytes. Some clinical overlap has been seen, but the two patterns have different disease associations. The c-ANCA pattern has been predominantly associated with Wegener's granulomatosis, whereas p-ANCA has been associated with microscopic polyarteritis, other vasculitides, idiopathic necrotizing and crescentic glomerulonephritis (c.f. DIARECT newsletter 2/2007).

Over the last two decades ANCA have become the established tool for the diagnosis of systemic vasculitides. The major role for ANCA testing is in diagnosing renal insufficiency of unknown origin, where a positive test indicates whether the patient will benefit from immunosuppressive treatment or not (ref. 3). A negative test result almost completely rules out the presence of systemic vasculitis.

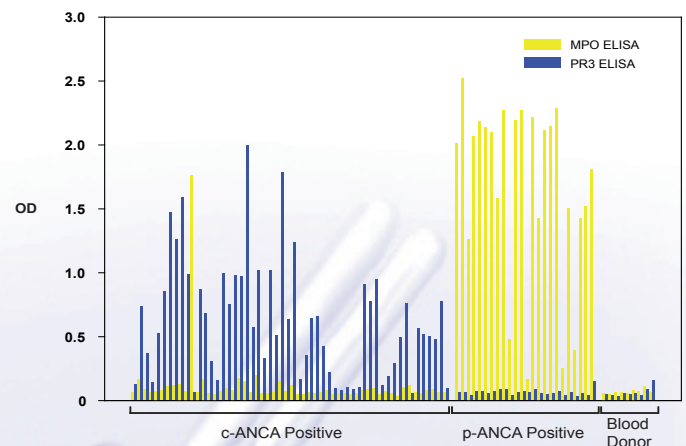
In this clinical setting the major autoantigens are proteinase 3 and myeloperoxidase. For a long time the best test for antibodies to these antigens has been ELISA. Nevertheless, in recent years several methods have been published as an alternative to or optimization of conventional direct ELISA methods.

PR3 and MPO ANCA recognize conformational epitopes that may be hidden or even destroyed during the coating process. One of the alternative approaches to avoid this problem has been the development of capture and so-called anchor techniques in order to increase assay sensitivity without loss of specificity (ref. 4, 5). Furthermore, a novel ELISA format based on a mixture of human native and recombinant PR3 has been claimed to significantly improve the diagnostic potential for ANCA-associated vasculitis (ref. 6). It remains to be seen which antigen or antigen mixtures and which assay design (capture, anchor etc.) will

stand the test of time.

Therefore, we at DIARECT have focused on establishing a manufacturing process for native PR3 from human peripheral blood polymorphonuclear cells. In collaboration with our partner in Luxembourg, Prof. R.-L. Humbel (LLIP, Luxembourg), this new PR3 antigen has been stringently tested with respect to specificity, sensitivity, and signal-to-noise ratio.

When sera classified according to their immunofluorescence pattern are tested in ELISAs employing PR3 and MPO antigens, there is virtually no overlap (see figure below). One remarkable finding - the MPO abs positive c-ANCA serum - documents a type of diagnostic anomaly which is characteristic for sera from Churg-Strauss-syndrome patients (R.-L. Humbel, personal communication). Also present are sera from Morbus Wegener patients under treatment, in which the antiPR3 titer has been markedly reduced.



### References

1. van der Woude, F.J., Rasmussen, N., Lobatto, S. et al. (1985) Lancet 1/425-9
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3. Segelmark, M., Westman, K., Wieslander, J. (2000) Clin. Exp. Rheumatol. 18, 629-35
4. Westman, K.W.A., Selga, D., Bygren, P., et al. (1998) 53, 1230-6
5. Hellmich, B., Csernok, E., Fredenhagen, G., Gross, W.L. (2007). Clin. Exp. Rheumatol. 25 (Suppl. 44), 6-10
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### Ordering Information

18500	Myeloperoxidase (MPO; non recombinant)	0.1 mg
18501	Myeloperoxidase (MPO; non recombinant)	1.0 mg
18600	Proteinase 3 (PR3; non recombinant)	0.1 mg
18601	Proteinase 3 (PR3; non recombinant)	1.0 mg

In some countries the use of recombinant antigens in diagnostic tests may be protected by certain patents. DIARECT is not responsible for the determination of these issues and suggests clarification prior to use.

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