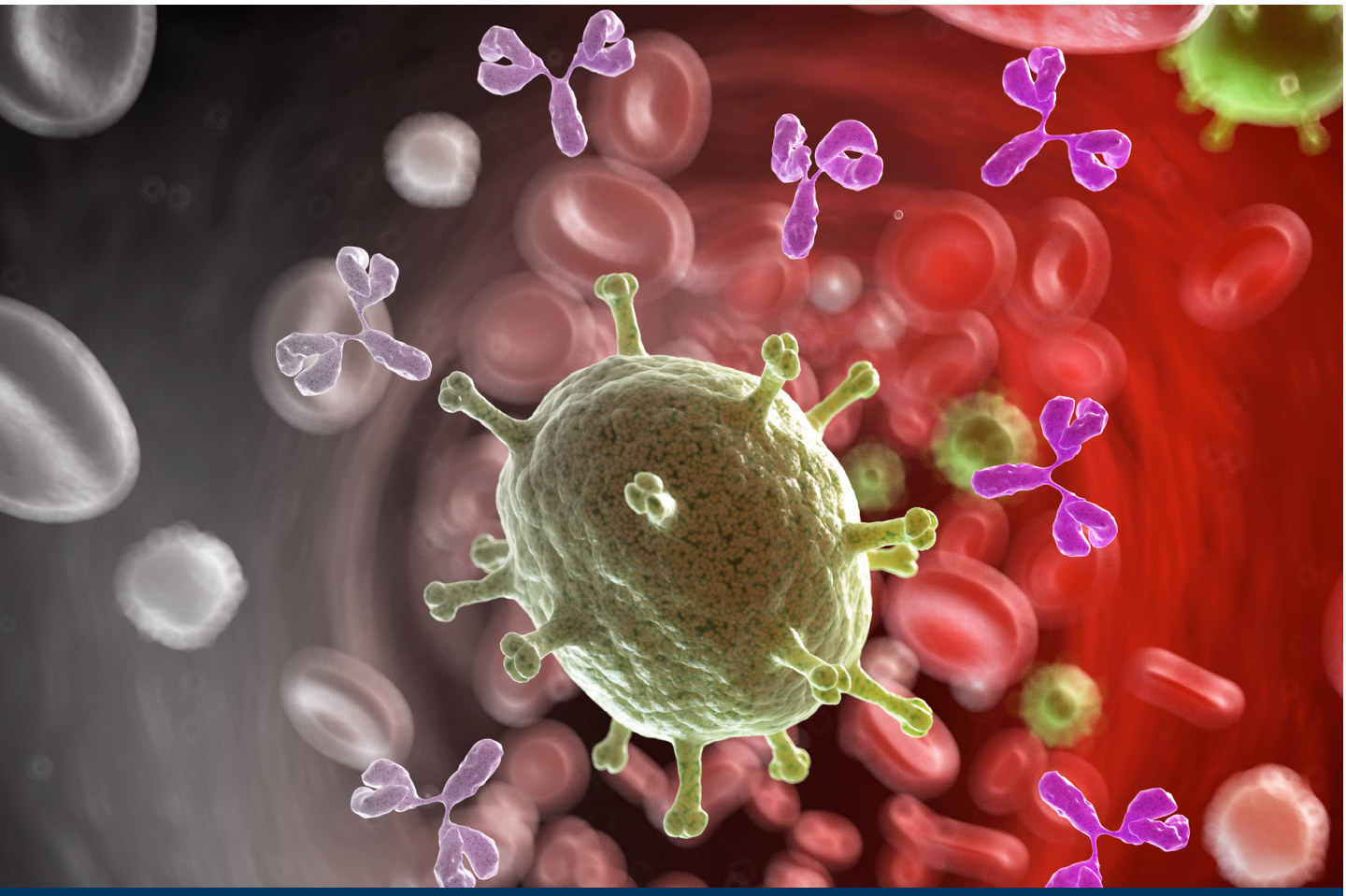


Myeloperoxidase (MPO) | Proteinase 3 (PR3)
Glomerular basal membrane (GBM)

Immunoenzymatic kit for the diagnosis of antibodies against neutrophil cytoplasm and GBM

IMMUNOBLOT kit is optimized and validated for detection of specific IgG antibodies in human serum or plasma

TestLine®

Introduction

Antineutrophil cytoplasmic antibodies (ANCA) are a group of antibodies directed against cytoplasm antigens of neutrophilic granulocytes and monocytes. ANCA examinations are considered basic tests in immunological laboratories. The determination of ANCA is of great importance, in particular in case of suspected acute vasculitis of small vessels, with severe pulmonary impairment or renal failure, but also in some non-vasculitic clinical syndromes such as inflammatory bowel diseases, e.g. ulcerative colitis.

The most common target antigens of ANCA-associated vasculitis are proteinase 3 or myeloperoxidase.

Antibodies against **proteinase 3** (PR3) are referred to as c-ANCA fluorescent subtype, namely cytoplasmic antibodies (granular cytoplasmic fluorescence). PR3 is a neutral serine proteinase 3, also known as Wegener's autoantigen. Antibodies against PR3 are a highly specific marker in diagnosing Wegener's granulomatosis.

Antibodies against **myeloperoxidase** (MPO) are referred to as p-ANCA subtype, since they form a perinuclear fluorescence pattern. This ANCA fluorescent subtype includes other antibodies such as antibodies against lactoferrin, cathepsin G, or elastase. However, in at least 60% of p-ANCA reactivity cases, the main antigen is MPO. Anti-MPO antibodies are primarily considered an important indicator for progressing nephritis; they are largely present in patients with severe renal impairment. They are also important for diagnosing Churg-Strauss syndrome and microscopic polyangiitis.

The presence or absence of antibodies against MPO and PR3 in combination with the positivity of antinuclear antibodies may be regarded as a differentiating marker between ANCA-associated vasculitis and SLE -induced vasculitis.

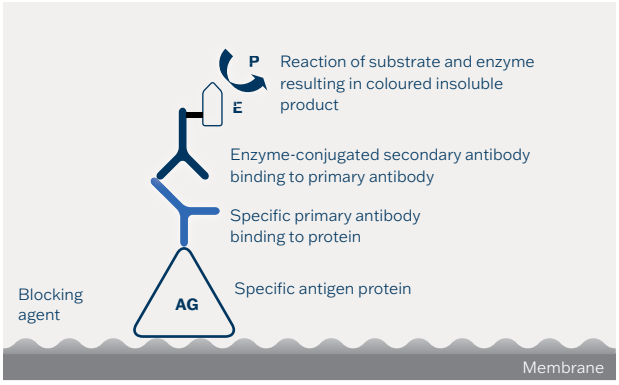
Anti-GBM antibodies (glomerular basal membrane; Goodpeasteure's antigen) are important for the diagnosis of glomerulonephritis, which may be accompanied by pulmonary haemorrhage (Goodpasteure's pulmo-renal syndrome). Due to clinical symptoms similar with systemic vasculitis, it is appropriate to concurrently carry out the tests for anti-GBM and ANCA antibodies.

In all cases, the activity and severity of the disease closely correlates with the concentration of antibodies. Thus, the information about the determined levels of antibodies can also be used in monitoring disease progression.

IMMUNOBLOT

Test Principle

Recombinant antigens are transferred to a nitrocellulose membrane



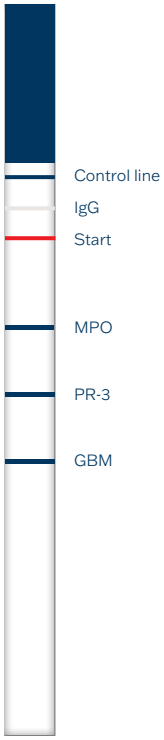
Comparison of Diagnostics Methods

Parameter	ELISA	BLOT (competitors)	IIF
MPO	90.3%	93.2%	75.6%
PR3	95.6%	85.7%	81.8%
GBM	90.9%	93.0%	

Test Characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
MPO	91.1%	91.9%
PR3	93.5%	96.7%
GBM	100.0%	95.5%

Antigens



BLOT-LINE ANCA

Myeloperoxidase (MPO)
– a highly specific sign for the diagnosis of rapidly progressive nephritis, necrotising glomerulonephritis; a positive response in 70-90% of patients with severe renal impairment. Churg-Strauss Syndrome (CSS), microscopic polyangiitis (MPA) and other vasculitis.

Proteinase 3 (PR3)
– a highly specific serological sign for the diagnosis of Wegener's granulomatosis. Microscopic polyarteritis, Churg-Strauss syndrome, mild systemic sclerosis, ulcerative colitis.

Basal membrane of glomeruli (Goodpasture's antigen, GBM)
– diagnostically significant antibodies in glomerulonephritis; Pulmo-renal Goodpasture's syndrome (rapidly progressive glomerulonephritis, in 2/3 of patients with pulmonary haemorrhage.

Clinical Application

- Detailed detection of the presence of antibodies against specific myeloperoxidase (MPO) antigens, proteinase 3 (PR3) and Glomerular basal membrane (GBM)
- Confirmation of controversial results
- Confirmatory test ELISA and IIF examination

Advantages

- Identical assay procedure
- Easy interpretation and reproducibility of results
- Sophisticated evaluation software
- High diagnostic efficiency
- Possibility of automation
- Customer support

Protocol Summary

Step		Test steps
	1.	Pipette Universal solution 2 ml
	2.	Strips soaking 10 min. at room temperature – Shaker
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (30 µl + 1,5 ml)
	5.	Pipette Controls and diluted samples 1.5 ml
	6.	Incubate 30 min. at room temperature – Shaker
	7.	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	8.	Pipette Conjugate 1.5 ml
	9.	Incubate 30 min. at room temperature – Shaker
	10.	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	11.	Pipette Substrate solution (BCIP/NBT) 1.5 ml
	12.	Incubate 15 min. at room temperature – Shaker
	13.	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. – Shaker
	14.	Sticking and evaluation of strips

User Comfort

- Ready-to-use components
- Colour-coded strips, interchangeable components
- Positive and Negative controls
- Control line on the strip
- Possibility of software evaluation

Ordering Information

IMMUNOBLOT

Cat. No	Product	No. of Tests
ANC3L20	BLOT-LINE ANCA-3	20

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Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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