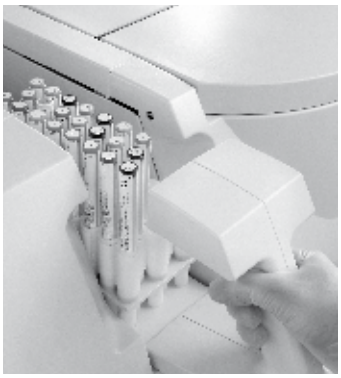
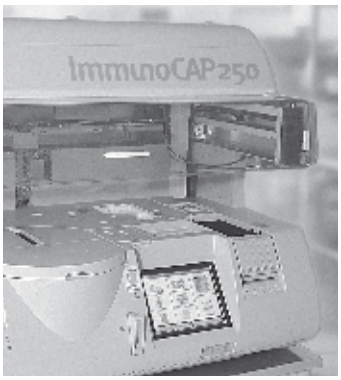
EliA™

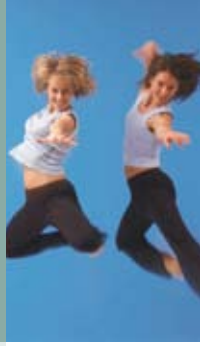




PR3, MPO, GBM

EliA™

Excellence in Autoimmunity



The EliA™ PR3, EliA™ MPO and EliA™ GBM Assays – surer diagnosis of ANCA associated vasculitides and Goodpasture syndrome

High Clinical Relevance

- high sensitivity supports diagnosis
- high specificity avoids wrong diagnostic decisions and inadequate treatment
- quantitative evaluation allows precise follow-up

High Technical Performance

- low variances and high reproducibility for consistent results
- high lot-to-lot consistency due to validated production procedures
- quantitative results expressed as U/ml based on a six-point standard curve

State-of-the-art antigens

- human recombinant GBM antigen from the baculovirus/insect cell system
- GBM antigen restricted to the relevant epitopes to avoid false positive results
- highly purified human PR3 and MPO maintaining enzymatic activity

Automation

- PR3, MPO and GBM in one run
- two ImmunoCAP instruments available for low to high throughput
- one stored standard curve to be used with all IgG analytes
- emergency samples can be run cost-efficiently

Easy Handling

- serum as well as plasma can be used
- automated sample dilution
- STAT function for immediate testing of high priority samples (ImmunoCAP™ 250)

ANCA Associated Vasculitis

PR3 and MPO ANCA are useful for both diagnosis and monitoring of vasculitides

A certain proportion of vasculitides is associated with anti-neutrophil cytoplasmic antibodies (ANCA), namely anti-PR3 and anti-MPO. Other ANCA have no clinical relevance for vasculitides. Tests for PR3 and MPO antibodies provide great help in both diagnosis and monitoring of this disease group including Wegener's granulomatosis (WG), microscopic polyangiitis (MPO), Churg-Strauss syndrome (CSS) and necrotizing crescentic glomerulonephritis (NCGN). Antibodies to PR3 are highly sensitive for Wegener's granulomatosis, while those to MPO show a lower prevalence in MPA, CSS or NCGN (Table 1).

Antibody specifity	Prevalence in			
	WG	MPA	CSS	NCGN
PR3	66 %	26 %	< 5 %	30 %
MPO	24 %	58 %	50 %	64 %

Table 1: *Prevalence of PR3 and MPO antibodies in ANCA associated vasculitides according to Kallenberg CGM (2007). Antineutrophil cytoplasmic autoantibodies with specficity for myeloperoxidase. In: Autoantibodies, 2nd edition: 95-103. Shoenfeld Y, Gershwin ME, Meroni PL eds. Elsevier B.V., London.*

Goodpasture Syndrome and anti-GBM Disease

GBM antibodies also occur in ANCA positive patients

Goodpasture syndrome and anti-GBM disease do not belong to the group of ANCA associated vasculitides but may exhibit similar clinical symptoms, particularly when it comes to kidney damage (GBM = glomerular basement membrane). Since vasculitis patients as well as Goodpasture syndrome and anti-GBM disease patients are often taken to hospital as emergencies, parallel testing of anti-PR3, anti-MPO and anti-GBM is important for differential diagnosis and rapid decisions on treatment. Anti-GBM assays exhibit a high clinical specificity, however, there is a certain proportion of ANCA positive patients (about 8-10 %) who also have GBM antibodies and differ in disease prognosis, depending on the anti-GBM level.

The Antigens

PR3 (proteinase 3) and MPO (myeloperoxidase) are enzymes involved in pathogen defence. They are both located in the azurophilic granules of neutrophil granulocytes. The antigens used for EliA™ PR3 and EliA™ MPO are both highly purified from human granulocytes.

Human recombinant GBM antigen for outstanding specificity

The antigen for GBM antibodies is the so-called NC1-domain of the α3-chain of collagen type IV, a constituent of the kidney's glomerular basement membrane. In order to avoid false positive results caused by antibodies to other collagen chains EliA™ GBM uses a truncated human recombinant α3-chain of collagen type IV containing the NC1-domain as antigen, which gives the assay an exceptionally high specificity.

High Clinical Relevance

EliA™ PR3 and EliA™ MPO combine high sensitivity with superb specificity

Both ANCA as well as GBM antibodies are assigned a high value in the diagnostic process of the associated diseases. Thus, the requirements for the respective immunoassays are particularly high in terms of clinical sensitivity and specificity. Our EliA™ PR3, MPO and GBM tests have been shown in independent, external clinical trials to fulfil these requirements to the highest degree.

In a study from Villalta et al. (2004) serum samples from patients with a clinical diagnosis of WG or MPA were tested with different methods for ANCA detection (Table 2). The diagnosis was made irrespective of the presence of ANCA positivity.

EliA™ PR3 as well as EliA™ MPO performed very well and exhibit a clinical sensitivity which is on the same level as indirect immunofluorescence (IIF). In terms of clinical specificity the EliA™ MPO test even has advantages compared to IIF. This data shows that EliA PR3 and MPO are excellent tests even for use as the only ANCA tests performed.

Method	WG Patients		MPA Patients		disease controls	
	pos. / total	% pos.	pos. / total	% pos.	pos. / total	% pos.
IIF cANCA	26/29	89.7	2/23	8.7	0/70	0.0
EliA™ PR3	25/29	86.2	1/23	4.3	2/70	2.8
IIF pANCA	1/29	3.4	17/23	73.9	4/40	5.7
EliA™ MPO	1/29	3.4	16/23	69.6	0/70	0.0

Table 2: *Clinical performance of EliA™ PR3 and EliA™ MPO compared to IFA on human granulocytes.*

The authors conclude that: *"the EliA MPO-ANCA and PR3-ANCA methods provide good diagnostic accuracy and excellent analytical accuracy, which, in association with the practicality of the automated EliA system, make this method a useful tool for the diagnosis of ANCA-associated vasculitides."*

EliA™ GBM provides extremely high predictive values

In GBM antibody detection clinical specificity is most important, as these antibodies are a diagnostic criterion for Goodpasture syndrome and anti-GBM disease. Using human recombinant GBM antigen EliA™ GBM perfectly fulfils this requirement. This could be demonstrated by an external clinical study by Radice et al. (2004), who tested anti-GBM disease sera from 17 patients and 32 sera from disease controls. EliA™ GBM exhibited an optimal 100 % clinical specificity. ROC analysis revealed that EliA™ GBM shows a better clinical performance than both IIF on kidney and an ELISA using purified GBM antigen, the diagnostic assay used in the study (Table 3).

test	sensitivity in %	specificity in %	positive predictive value	negative predictive value	area under the curve (ROC analysis)
diagnostic assay	100.0	90.6	94.4	100.0	0.952
EliA™ GBM	94.4	100.0	100.0	97.0	0.988

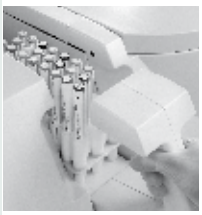
Table 3: *Clinical performance of EliA™ GBM compared to the ELISA used for diagnosis of the study cohort.*

The authors conclude that: *"the better performance, in terms of sensitivity/specificity, was reached by the fluoro-enzymatic-immunoassay EliA."*

References

Villalta D, Tonutti E, Tampoia M, Bizzaro N, Papisch W, Tozzoli R, Stella S (2004). Analytical and diagnostic accuracy of the EliA™ automated enzyme fluoroimmunoassay for antineutrophil cytoplasmic autoantibody detection. Clin Chem Lab Med 42(10):1161–1167

A. Radice, C. Corace, B. Bollini and R.A. Sinico (2004). Anti-glomerular basement membrane (GBM) antibodies in the diagnosis of Goodpasture syndrome: comparison of different methods. Poster presented at the 4th International Congress on Autoimmunity in Budapest, November 2004



The EliA™ System

Time for the Essentials

- completely automated (true walk-away, overnight runs)
- easy instrument management with flexible ImmunoCAP™ IDM software
- barcode-reader (optional for ImmunoCAP™ 100€)
- protocols, QC and raw data easily accessible
- optional host link
- detailed QC management
- integrated stock management system on the ImmunoCAP™ 250

Cost efficient and flexible

- autoimmunity and allergy on the same instrument
- different autoimmune tests in the same run (puzzle-kit-approach)
- no batching of samples necessary – small runs can be handled cost-effectively
- once-monthly calibration – curve control each run
- several ImmunoCAP™ instruments can be linked

A boost in service for your laboratory and your clinicians

- sample – result turnaround the same day
- STAT function on ImmunoCAP™ 250 for immediate testing of emergency samples
- overnight runs possible
- detailed documentation of results (patient or requester specific)
- ImmunoCAP™ 100€ – up to 46 determinations in less than 2.5 hours
- ImmunoCAP™ 250 – fully automated random access – up to 350 determinations per shift
- multiple methods in one run
- positive identification and traceability of samples and reagents on ImmunoCAP™ 250

EliA™

Technical Data

- **Products** — EliA™ PR3
EliA™ MPO
EliA™ GBM
- **Antigens** — EliA™ PR3: human purified proteinase 3
EliA™ MPO: human purified myeloperoxidase
EliA™ GBM: human recombinant α3 chain of collagen IV
- **Standardisation** — 6 point standard curve
- **Cut-off / measuring range**

	negative	equivocal	positive	measuring range
EliA™ PR3	< 7 U/ml	7–10 U/ml	> 10 U/ml	0.6 – (at least) 530 U/ml
EliA™ MPO	< 7 U/ml	7–10 U/ml	> 10 U/ml	0.7 – (at least) 600 U/ml
EliA™ GBM	< 7 U/ml	7–10 U/ml	> 10 U/ml	0.8 – (at least) 680 U/ml

- **Dilution** — EliA™ PR3, EliA™ MPO: 1:50 (automated)
EliA™ GBM: 1:100 (automated)
- **Sample Material** — Serum, Plasma (EDTA, heparin, citrate)
- **Normal Distribution**

	mean	95% percentile
EliA™ PR3	< 0.6 U/ml	< 0.6 U/ml
EliA™ MPO	< 0.7 U/ml	1.8 U/ml
EliA™ GBM	< 0.8 U/ml	0.9 U/ml

- **Reproducibility** — Intra-run CV* 2.5 – 13.7 %
Inter-run CV* 0.9 – 5.9 %

*for details see Directions For Use

Ordering Information

	Package size	*Article No.*
EliA™ PR3 Well	4 x 12 determinations	14-5512-01
EliA™ MPO Well	4 x 12 determinations	14-5513-01
EliA™ GBM Well	2 x 12 determinations	14-5514-01

For EliA™ specific reagents and general reagents please refer to the Phadia product catalogue.

Phadia

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