Anti-PR3-hn-hr ELISA (IgG)

Indication: Test system for the in vitro determination of antibodies against proteinase 3 in human serum or plasma for the diagnosis of the following disease: Wegener's granulomatosis.

Clinical significance: ANCA are autoantibodies directed against antigens found in cytoplasmic granules of neutrophils and monocytes. Several methods are used for the detection of ANCA. Standard technique is the indirect immunofluorescence (IIF) on ethanol-fixed granulocytes. At least two different staining patterns can be differentiated: a granular fluorescence which is distributed regularly over the entire cytoplasm of the granulocytes, leaving the cell nuclei free (cANCA: cytoplasmic pattern), and a predominantly smooth, partly fine granular fluorescence wrapped ribbon-like around the cell nuclei of the granulocytes (pANCA: perinuclear pattern). ANCA are typically found in Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) including renal limited vasculitis, and Churg-Strauss syndrome (CSS), which are all forms of small-vessel vasculitis. These three diseases are grouped together as ANCA-associated vasculitides (AAV) according to the widely accepted classification system introduced by the Chapel Hill Consensus Conference. Classical cANCA are present in most patients with WG (more than 90% in general WG with glomerulonephritis, 70% in limited WG without glomerular involvement) and in about 30% of patients with MPA. Classical cANCA are almost always directed against proteinase 3 (PR3) and very rarely against myeloperoxidase (MPO) or against PR3 and MPO simultaneously.

The most important clinical symptoms of ANCA-associated vasculitides are caused by poor blood supply to organs or formation of aneurysms and bleeding due to destruction of blood vessels. Wegener's granulomatosis is a febrile, chronic granulomatosis disease, mainly of the nasopharynx, lungs and kidney. Since cANCA have been investigated, the diagnosis of Wegener's granulomatosis has tripled. Due to the high specificity of cANCA the number of diagnosed early-stage and abortive cases of Wegener's granulomatosis increases steadily.

Application of the Anti-PR3-hn-hr ELISA: The reagent wells of the Anti-PR3-hn-hr ELISA are coated with a mixture of recombinant PR3 (based on human cDNA, expressed in human cells; Sun, Specks et al., 1998) and native PR3. Owing to this, the test has a significantly higher sensitivity (94%) at a very good specificity (99%) compared to other ELISA only using a native antigen (88% and 78%, respectively; study performed in cooperation with the ANCA reference centre University of Maastricht, Prof. Cohen-Tervaert). The significantly higher sensitivity of the PR3-hn-hr ELISA and its suitability for the diagnosis of relapses in patients under treatment has meanwhile been described in an independent publication (Damoiseaux et al. Ann Rheum. Dis. 2009, 68(2):228-233).The International Consensus Statement recommends screening for ANCA using IIF and the confirmation of IIF-positive sera with both Anti-PR3 and Anti-MPO ELISA since the combination of both test systems yields the highest specificity and sensitivity for the diagnosis of small vessel vasculitis.

Panels (source: ANCA reference centre University of Maastricht)		**n**	**Anti-PR3-hn-hr ELISA positive**	**Anti-PR3 Capture ELISA positive**	**Anti-PR3 ELISA positive**
AAV (cANCA-positive)	Biopsy-proven AAV	58	55 (95%)	53 (91%)	53 (91%)
	AAV outpatients	35	33 (94%)	32 (91%)	26 (74%)
	AAV relapses	23	23 (100%)	23 (100%)	18 (78%)
	Wegener's granulomatosis	47	43 (91%)	36 (77%)	30 (64%)
Sensitivity with respect to IIFT (cANCA)		163	154 (94%)	144 (88%)	127 (78%)
Non-ANCA-associated vasculitides (e.g. cryoglobulinemia, Henoch-Schönlein purpura, large vessel vasculitides)		55	0	2 (4%)	0
Rheumatoid arthritis		230	0	7 (3%)	0
Systemic lupus erythematosus		100	0	0	0
Sjögren's syndrome		200	1 (1%)	5 (3%)	2 (1%)
Blood donors, asymptomatic		429	4 (1%)	10 (2%)	3 (1%)
Specificity		1014	5 (99%)	24 (98%)	5 (99%)

EUROIMMUN Microplate ELISA

Autoantibody determination:
AMA M2-3E (IgG)
ANCA Profile (IgG)
ANA Screen (IgG)
ANA Screen 9 or 11 (IgG)
BP180-NC16A-4X (IgG)
BP230-CF (IgG)
cardiolipin (IgA, IgG, IgM, IgAGM)
circulating immune complexes (CIC)
cyclic citrullinated peptide (CCP; IgG)
centromere protein B (IgG)
desmoglein 1 (IgG)
desmoglein 3 (IgG)
double-stranded DNA (dsDNA, nDNA; IgG)
dsDNA-NcX (IgG)
ENA Pool (IgG)
ENA PoolPlus (IgG)
ENA ProfilePlus 1 or 2 (IgG)
ENA SLE Profile 1 or 2 (IgG)
Envoplakin (IgG)*
GAD
GAD/IA-2 Pool
glomerular basement membrane (GBM; IgG)
ß2-glycoprotein 1 (IgA, IgG, IgM, IgAGM)
histones (IgG)
IA-2
intrinsic factor (IgG)
Jo-1 (IgG)
liver cytosolic antigen type 1 (LC-1; IgG)
liver-kidney microsomes (LKM-1; IgG)
myeloperoxidase (MPO; IgG)
nRNP/Sm (IgG)
nucleosomes (IgG)
ovary (IgAGM)
parietal cells (PCA; IgG)
PM-Scl (PM-1; IgG)
phosphatidylserine (IgA, IgG, IgM, IgAGM)
PR3 hn-hr (IgG)
rheumatoid factor (IgA, IgG, IgM)
ribosomal P-proteins (IgG)
Sa (IgG)
Scl-70 (IgG)
single-stranded DNA (ssDNA; IgG)
SLA/LP (IgG)
Sm (IgG)
spermatozoa (IgAGM)
SS-A (Ro; IgG)
SS-B (La; IgG)
thyroglobulin (TG; IgG)
thyroid peroxidase (TPO; IgG)
tiss. transglutaminase (endomy.; IgA, IgG, IgAG)
TSH receptor (TBII; IgG)
TRAk Fast (IgG)
zona pellucida (IgAGM)

Further autoimmune diagnostics:
gliadin (GAF-3X; IgA, IgG)
Saccharomyces cerevisiae (IgA, IgG)

Infectious serology:
Adenovirus (IgA, IgG, IgM)
Bordetella pertussis (IgM)
Bordetella pertussis toxin (IgA, IgG)
Bordetella FHA (IgA, IgG)
Borrelia (IgG, IgM)
Borrelia VlsE (IgG)
Brucella abortus (IgA, IgG, IgM)
Campylobacter jejuni (IgA, IgG)
Chikungunya virus (IgG)
Chlamydia pneumoniae (IgA, IgG, IgM)
Chlamydia trachomatis (IgA, IgG, IgM)
Cytomegalovirus (IgG, IgM)
Dengue virus (IgA, IgG, IgM)
Diphtheria toxoid (IgG)
Echinococcus granulosus (IgG)
Epstein-Barr virus capsid ag (IgA, IgG, IgM)
Epstein-Barr virus early ag (IgA, IgG, IgM)
Epstein-Barr virus nuclear ag, EBNA-1 (IgG)
Hanta virus "Eurasia" + "America" (IgG, IgM)
Helicobacter pylori (IgA, IgG)
Helicobacter pylori CagA (IgA, IgG)
HSV-1 (glycoprotein C1; IgG, IgM)
HSV-2 (glycoprotein G2; IgG, IgM)
HSV-1/2 Pool (IgA, IgG, IgM)
Influenza virus type A (IgA, IgG, IgM)
Influenza virus type B (IgA, IgG, IgM)
Influenza Pool (IgA, IgG, IgM)
Legionella pneumophila (IgA, IgG, IgM)
Measles virus (IgG, IgM)
Mumps virus (IgG, IgM)
Mycoplasma pneumoniae (IgA, IgG, IgM)
Parainfluenza virus Pool (IgA, IgG, IgM)
Parvovirus B19 (IgG, IgM)
RSV (IgA, IgG, IgM)
Rubella virus (IgG, IgM)
TBE virus (IgG, IgM)
Tetanus toxoid (IgG)
Toxoplasma gondii (IgG, IgM)
Treponema pallidum (IgG, IgM)
Usutu virus (IgG)
Varicella zoster virus (IgG, IgM)
West Nile virus (IgG, IgM)
Yersinia enterocolitica (IgA, IgG)

Allergology:
total IgE
Allercoat™ 6-ELISA (650 different allergens and allergen mixtures)
Software EUROIMMUN Allercoat™

Bone metabolism marker:
25-OH-Vitamin-D

Saliva diagnostics:
alpha-amylase
cortisol
sIgA

Software/Automation:
EUROLabOffice
EUROIMMUN Analyzer I + I-2P

* Currently not available as IVD in the EU.

Made in Germany

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Test characteristics
Anti-PR3-hn-hr ELISA (IgG)

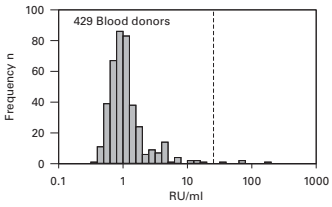
Linearity: The linearity of the Anti-PR3-hn-hr ELISA (IgG) was determined by assaying 4 serial dilutions of 6 serum samples. The linear regression was calculated, R^2 amounting to >0.95 in all samples. The Anti-PR3-hn-hr ELISA (IgG) is linear at least in the tested concentration range of 28 RU/ml to 197 RU/ml.

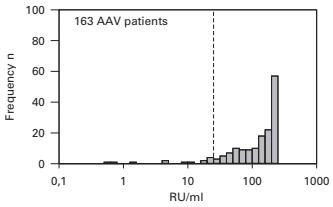
Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

	Intra-assay variation, n = 20		*Inter-assay variation, n = 4 x 6*	
Serum	**Mean value (RU/ml)**	**CV (%)**	**Mean value (RU/ml)**	**CV (%)**
1	55	4.1	47	11.2
2	89	2.6	85	4.3
3	108	1.8	106	4.2
4	152	2.8	159	3.9

Clinical sensitivity and specificity: Sera from 163 patients with ANCA-associated vasculitides (AAV; cANCA-positive), a control panel of 585 patients with other diseases and 429 healthy blood donors were analysed using the EUROIMMUN Anti-PR3-hn-hr ELISA (IgG). The sensitivity of ELISA for cANCA-positive AAV was 94%, at a specificity of 99%.

Reference range: Levels of anti-PR3 antibodies were investigated in 429 sera from healthy blood donors between 19 and 68 years of age (172 women, 257 men) using the EUROIMMUN ELISA. No differences with respect to age or gender were observed. The mean concentration of antibodies against PR3 was 2.2 RU/ml (± 9.6 RU/ml of standard deviation) and the values ranged from 0.1 to 171.7 RU/ml. With a cut-off of 20 RU/ml, 4 blood donors were anti-PR3 positive.





Blood donors, n = 429			
Percentile	95th	98th	99th
Cut-off	4.4 RU/ml	12.5 RU/ml	17.7 RU/ml

ROC analysis: In an analysis of 140 samples from patients with ANCA-associated vasculitides (cANCA-positive) and 1014 control samples the following characteristics were determined:

Cut-off	**Specificity**	**Sensitivity**
4.9 RU/ml	95%	96%
12.0 RU/ml	98%	95%
17.6 RU/ml	99%	94%

Technical data:

Antigen	Mixture of native proteinase 3 from human neutrophils and recombinant proteinase 3, based on human cDNA and expressed in human cells.
Calibration	Quantitative, in relative units per milliliter (RU/ml). Calibration serum 1: 200 RU/ml Calibration serum 2: 20 RU/ml; cut-off Calibration serum 3: 2 RU/ml
Sample dilution	Serum or plasma; 1:101 in sample buffer.
Reagents	Ready for use. Exception: wash buffer (10x). Colour-coded solutions, largely exchangeable with those of other EUROIMMUN ELISA.
Test procedure	30 min / 30 min / 15 min. Room temperature. Fully automatable.
Measurement	450 nm. Reference wavelength between 620 nm and 650 nm.
Kit format	96 single break-off wells, incl. all necessary reagents.
Order no.	EA 1201-9601-2 G

EUROIMMUN
Immunoblots

Autoantibody determination:

EUROASSAY:
flexible profiles of up to 7 antigens from:
ENA and related antigens: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, dsDNA, histones, nucleosomes, CENP B, PM-Scl, ribosomal P-proteins, AMA M2

liver antigens: LKM-1, LC-1, SLA/LP, AMA M2, M4, M9

ANCA antigens: MPO, PR3

thyroid antigens: TG, TPO

EUROLINE:

flexible profiles of up to 18 antigens from:
ENA and related antigens: nRNP/Sm, Sm, RNP70, RNPA, RNPC, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2, Mi-2, Ku

Systemic Sclerosis Profile: Scl-70, CENP A, CENP B, RP11, RP155, fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR, Ro-52

Myositis Profiles: Mi-2, Ku, PM-Scl100, PM-Scl75, SRP, Jo-1, PL-7, PL-12, OJ, EJ, Ro-52

Liver Profiles: AMA M2, 3E (BPO), Sp100, PML, gp210, LKM-1, LC-1, SLA/LP, Ro-52

Neuronal Antigen Profiles: amphiphysin, CV2, PNMA2 (Ma-2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin

Ganglioside Profiles: GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b

ANCA Profiles: MPO, PR3, GBM

EUROLINE-WB:

neuronal antigens (+ recomb. Hu, Yo, Ri)
HEp-2 cell antigens (+ SS-A and Ro-52, CENP B)

Infectious serology:

EUROLINE:

Bordetella pertussis (IgA, IgG)
Borrelia-RN-AT (IgG, IgM)
Borrelia-RN-AT-adv (IgM)
EBV Profile (IgG, IgM)
Hantavirus profiles (IgG, IgM)
Parvovirus B19 (IgG, IgM)
TORCH Profile* (IgG, IgM)

Westernblot:

Borrelia burgdorferi (IgG, IgM)
Borrelia afzelii (IgG, IgM)
Borrelia garinii (IgG, IgM)
Cytomegalovirus (IgG, IgM)*
Echinococcus granulosus (IgG)
Epstein-Barr virus (IgG, IgM)
Rubella virus (IgG)
Treponema pallidum (IgG, IgM)
Yersinia enterocolitica (IgA, IgG)

EUROLINE-WB:

Borrelia (IgG, IgM)
HSV-1/2 (IgG, IgM)
Helicobacter pylori (IgA, IgG)
Treponema pallidum + cardiolipin (IgG, IgM)

Allergology:

EUROASSAY:

Food Profile (IgE)
Inhalation Profile (IgE)
Pediatric/Atopy Profile (IgE)
Insect Venom Profile (IgE)

EUROLINE:

Atopy Profile (IgE; also region-specific profiles)
Food Profile (IgE; also region-specific profiles)
Inhalation Profile (IgE; also region-specific profiles)
Paediatric Profile (IgE)
Pollen–Food Cross Reaction Profile (IgE)
Insect Venom Profile (IgE)

Software/Automation:

EUROLineScan
camera system EUROBlotCamera
scanner system EUROBlotScanner
incubation processor EUROBlotMaster

EUROIMMUN
Radioimmunoassays

Autoantibody determination:

thyroid peroxidase (TPO; IgG)
thyroglobulin (TG; IgG)
TSH receptor (IgG)
acetylcholine receptor (ACHR; IgG)
glutamic acid decarboxylase (GAD; IgG)
insulin (IAA; IgG)
P/Q calcium channel* (VGCC; IgG)
tyrosine phosphatase (IA2; IgG)
dsDNA (IgA/IgG/IgM)

Antigen determination:

thyroglobulin (TG)

Hormone determination:

free triiodothyronine (FT3)
free thyroxine (FT4)
thyrotropin (TSH)
calcitonin

* Currently not available as IVD in the EU.

Made in Germany

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