



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. Some cANCA exhibit a flat homogenous cytoplasmic staining in IIF (mostly termed atypical cANCA) which is often directed against bactericidal/permeability increasing protein (BPI).

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 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)
ANA VarioProfile (IgG)
BP180-4X (IgG)
cardiolipin (IgA, IgG, IgM, IgAGM)
cyclic citrullinated peptide (CCP; IgG)
centromere protein B (IgG)
double-stranded DNA (dsDNA, nDNA; IgG)
ENA Pool* (IgG)
ENA PoolPlus (IgG)
ENA ProfilePlus 1 or 2 (IgG)
ENA SLE Profile 1 or 2 (IgG)
GAD
GAD/IA-2 Pool
glomerular basement membrane (GBM; IgG)
β2-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)
p53 (IgG)
parietal cells (PCA; IgG)
PM-Scl (PM-1; IgG)
phosphatidylserine (IgA, IgG, IgM, IgAGM)
proteinase 3 (IgG)
PR3 hn-hr (IgG)
PR3 capture (IgG)
rheumatoid factor (IgA, IgG, IgM)
ribosomal P-proteins (IgG)
Scl-70 (IgG)
single-stranded DNA (ssDNA; IgG)
SLA/LP (IgG)
Sm (IgG)
SS-A (Ro; IgG)
SS-B (La; IgG)
thyroglobulin (TG; IgG)
thyroid peroxidase (TPO; IgG)
tissue transglutaminase (endomy; IgA, IgG)
TSH receptor (TBLI; IgG)

Further autoimmune diagnostics:

circulating immune complexes (CIC)
gliadin (IgA, IgG)
Saccharomyces cerevisiae (IgA, IgG)

Infectious serology:

Adenovirus (IgA, IgG, IgM)
Borrelia (IgG, IgM)
Borrelia VisE (IgG)
Chlamydia pneumoniae (IgA, IgG, IgM)
Chlamydia trachomatis (IgA, IgG, IgM)
Cytomegalovirus (IgG, IgM)
Diphtheria toxoid (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)
Helicobacter pylori (IgA, IgG)
Helicobacter pylori CagA (IgA, IgG)
HSV-1 (glycoprotein C1; IgA, IgG, IgM)
HSV-2 (glycoprotein G2; IgA, IgG, IgM)
HSV-1/2 Pool (IgA, IgG, IgM)
Influenza virus type A (IgA, IgG, IgM)
Influenza virus type B (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)
RSV (IgA, IgG, IgM)
Rubella virus (IgG, IgM)
SARS-CoV (IgG)
TBE virus (IgG, IgM)
Tetanus toxoid (IgG)
Toxoplasma gondii (IgG, IgM)
Treponema pallidum (IgG, IgM)
Varicella zoster virus (IgG, IgM)
Yersinia enterocol. virulence fact. (IgA, IgG)

Allergology:

total IgE
Allercoat™ 6-ELISA (600 different allergens and allergen mixtures)

Serum proteins and tumour markers:

anti-p53
C-reactive protein (CRP; highly sensitive)

* Currently not available as IVD in the EU.

Made in Germany



EUROIMMUN Immunoassays

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:

ANA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B, dsDNA, nucleosomes, histones, ribosomal P-proteins

ANA Profile 3: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2

Anti-ENA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1

Myositis Profile: Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12, Ro-52

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

Neuronal Antigens Profile: amphiphysin, CV2/CRMP5, PNMMA2 (Ma-2), Ri, Yo, Hu

Anti-Ganglioside Profile 1: GM1, GD1b, GQ1b

Anti-Ganglioside Profile 2: GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b

ANCA Profiles: MPO, PR3, GBM

EUROLINE-WB:

liver-specific antigens (+ recomb. SLA/LP)
neuronal antigens (+ recomb. Hu, Yo, Ri)
HEp-2 cell antigens (+ SS-A and Ro-52, CENP B)
Myositis ag (Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12)

Infectious serology:

EUROLINE:

EBV Profile (IgG, IgM, VCA gp125, VCA p19 and EBNA-1, p22, EA-D)
TORCH Profile* (T. gond., rubella, CMV, HSV-1, -2)
Malaria Profile 1: Plasmodium falciparum HRP2 and MSP2, Plasmodium vivax MSP and CSP

Westernblot:

Borrelia burgdorferi (IgG, IgM)
Borrelia afzelii (IgG, IgM)
Borrelia garinii (IgG, IgM)
Epstein-Barr virus (IgG, IgM)
Helicobacter pylori (IgA, IgG)
Treponema pallidum (IgG, IgM)
Yersinia enterocol. virulence fact. (IgA, IgG)

EUROLINE-WB:

Anti-Borrelia (B. afzelii + rec. VlsE)
Anti-HSV (HSV-1 + HSV-2 gG2)
Treponema pallidum + cardiolipin

Allergy:

EUROASSAY:

Domestic Animal Profile (IgE)
Food Profile (IgE)
Inhalation Profile (IgE)
Insect/Venom Profile (IgE)
Latex Profile (IgE)
Latex plus Profile (with ficus and fruit; IgE)

EUROLINE:

Atopy Profile (IgE)
Food Profile (IgE)
Inhalation Profile (IgE)
Paediatric Inhalation Profile
Pollen-Food Cross Reaction 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)

* Currently not available as IVD in the EU.

Made in Germany

Version: 05/07
EA_1201_D_UK_B03

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

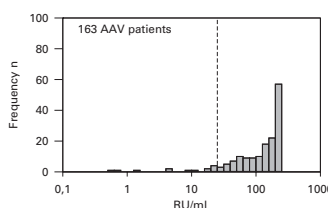
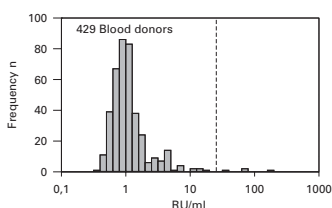
Linearity: The linearity of the Anti-PR3-hn-hr ELISA (IgG) was determined by assaying 4 serial dilutions of 6 serum samples. The linear regression was calculated, R^2 amounting to >0.95 in all samples. The Anti-PR3-hn-hr ELISA (IgG) is linear at least in the tested concentration range of 28 RU/ml to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

Serum	Intra-assay variation, n = 20		Inter-assay variation, n = 4 x 6	
	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	95 th	98 th	99 th
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



Important information for users of the EUROIMMUN Anti-PR3-hn-hr ELISA

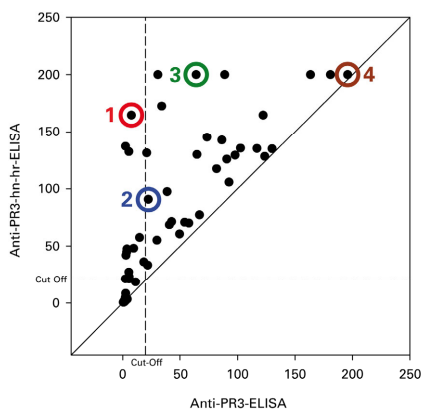
The Anti-PR3-hn-hr ELISA is a new milestone in the serological diagnosis of Wegener's granulomatosis. The antigen substrate used consists of two components: **human native proteinase 3 (PR3)** and **recombinant PR3 expressed in a human cell line** (source: Institute for Experimental Immunology, a facility of EUROIMMUN AG). The already potent recombinant PR3 is supplemented with native antigen in order to be able to present the complete antigen spectrum, since the recombinant antigen does not always possess all native antigen epitopes.

Special characteristics of the recombinant antigen component

- Human cells are used for the first time worldwide for the expression of PR3 in full-scale production. In human systems the posttranslational modifications that take place are authentic and true to species, in contrast to (heterological) insect cells or bacteria usually used. Therefore, the target antigen used for diagnostics conforms better to the natural one.
- In recombinant PR3 the proteolytic active centre is artificially switched off by the exchange of an amino acid.
 - Since the proteinase activity of the enzyme no longer interferes with cell metabolism, the cultured cells can accumulate PR3 in high concentrations – without this manipulation they die early.
 - The synthesised PR3 does not digest itself in an uncontrolled manner in the preparation and can be produced in large quantities.

Comparison of results

The new Anti-PR3-hn-hr ELISA is much more sensitive than conventional Anti-PR3 tests: in a panel from Damoiseaux et al. (Ann Rheum Dis, 2008 Mar 28, Epub ahead of print) a sensitivity of 95% was obtained for the Anti-PR3-hn-hr ELISA (conventional Anti-PR3 ELISA: 80%), with respect to the indirect immunofluorescence test and calculated for a specificity of 99%.



Serum	Measurement values for the concentration of Anti-PR3 AAb (IgG)	
	Conventional Anti-PR3 ELISA	Anti-PR3-hn-hr ELISA
1	8 RU/ml	165 RU/ml
2	22 RU/ml	91 RU/ml
3	64 RU/ml	200 RU/ml
4	196 RU/ml	200 RU/ml

Four examples

Consequences for the laboratory daily routine

If conventional Anti-PR3 tests are replaced by the new Anti-PR3-hn-hr ELISA, it must be expected that:

- some previously negative sera react positively (example 1),
- higher measurement values are generally obtained for antibody concentrations (sometimes many times higher, examples 2 and 3),
- in individual cases comparable values are obtained (example 4),
- measurement values do not always increase by the same ratio; the percentage increase in values can vary substantially from serum to serum.

We recommend that you inform your customers about the change of test in writing. We are happy to provide data sheets for this purpose. If you wish you can use the following text, in whole or in part: "We will in the future be using a new test system for the determination of antibodies against PR3 (EUROIMMUN Anti-PR3-hn-hr ELISA), which detects up to 15% more cases of Wegener's granulomatosis or ANCA-associated vasculitis. With this test a purely technically caused increase in measurement values for antibody concentrations can occur, which should not be interpreted as an increase in clinical activity. The increase can vary in strength from case to case".