



Anti-CCP ELISA (IgG)



Indication: Test system for the in vitro determination of antibodies against CCP in human serum or plasma for the diagnosis of the following disease: rheumatoid arthritis.

Clinical significance: Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting around 1% of the world population. It is characterized by inflammation of the synovial membrane, which spreads symmetrically from the small to the large joints. Initial symptoms include painful swelling of finger joints with morning stiffness in the joints. Early diagnosis and immediate commencement of suitable therapy is necessary to keep the disease under control.

The most commonly performed serological test in suspected RA cases was until now the determination of **rheumatoid factors (RF)**. These are antibodies (predominantly of class IgM) which react with gamma globulins and occur in 60-80% of RA patients. RF are a sensitive but not very specific marker for RA, since they also occur in healthy individuals and in patients with various infections or other autoimmune diseases (systemic lupus erythematosus, Sjögren's syndrome, scleroderma and others).

40-60% of RA patients also exhibit autoantibodies against epidermal **filaggrin (RA keratin, anti-perinuclear factor)** in the serum. Filaggrin is a protein of the epidermis, which links keratin filaments to one another. Autoantibodies against filaggrin are detected by indirect immunofluorescence: the antigen substrate rat oesophagus shows staining of the stratum corneum (RA keratin) on the luminal side; anti-perinuclear factors (APF) are apparent in the cytoplasmic inclusion bodies of human epithelial cells of the oral mucosa.

In recent years it has been shown that the rare amino acid citrulline, which is present in filaggrin, is a substantial component of the antigenic epitope. Enzyme immunoassays which use synthetic citrullinated peptide as the target antigen offer a useful alternative to indirect immunofluorescence. A direct comparison study demonstrated that the sensitivity can be increased from 49% to 68% by using cyclic citrullinated peptide instead of linear citrullinated peptide as an ELISA substrate. Antibodies against **cyclic citrullinated peptide (CCP)** are a new and highly specific marker for RA.

Antibodies against CCP are predominantly of class IgG and have a specificity of 98% for RA. They are observed very early in the disease course and have a high predictive value: patients with anti-CCP antibodies develop significantly more radiologically detectable joint damage than anti-CCP negative patients. Antibodies against CCP possess a **much higher specificity** than RF (anti-CCP: 97%, RF: 62%) **with the same sensitivity** (anti-CCP: 79%, RF: 78%). They can be detected in early stages of the disease in 79% of patients.

Application of the Anti-CCP ELISA: Autoantibodies against cyclic citrullinated peptide (CCP) are a new, highly specific and sensitive marker for rheumatoid arthritis. Because of their high specificity they are superior to rheumatoid factors for RA diagnostics. The peptide used as the antigen in the Anti-CCP ELISA contains the antigenic target structure citrulline, as does epidermal filaggrin. The ELISA provides a useful alternative to indirect immunofluorescence (RA keratin, anti-perinuclear factor).

The Anti-CCP ELISA is suitable for the early diagnosis of RA, which is crucial for the immediate commencement of appropriate therapy and prevention of damage. Moreover, it allows reliable differentiation of RA from other rheumatic diseases such as systemic lupus erythematosus, Sjögren's syndrome or polymyositis/dermatomyositis.

Panel	n	Anti-CCP positive
Sensitivity for RA	419	329 (78.5%)
Asymptomatic blood donors	400	2 (0.5%)
Psoriatic arthritis	28	0
Other arthritides	35	3 (8.6%)
System. lupus erythematosus	108	3 (2.8%)
Sjögren's syndrome	106	2 (1.9%)
Scleroderma	98	3 (3.1%)
Autoimmune thyroiditis	159	4 (2.5%)
Wegener's granulomatosis	25	1 (4%)
Anti-parvovirus B19-positive	126	3 (2.4%)
Viral hepatitis	54	0
Anti-HIV positive	5	0
Tuberculosis	10	0
Specificity for RA	1154	21 (98.2%)

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 (TBI; 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 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, CENP B, dsDNA, nucleosomes, 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, PNMA2 (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 (+ recom. SLA/LP)
neuronal antigens (+ recom. 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 HRP-2 and MSP-2, Plasmodium vivax MSP and CSP

Westemblot:

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: 06/07
EA_1505_D_UK_A05

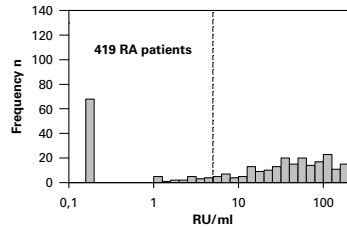
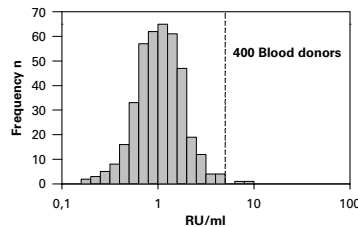
Test characteristics Anti-CCP ELISA (IgG)

Linearity: The linearity of the ELISA was determined by assaying serial dilutions of 6 serum samples. The average concordance of dilution-factor-corrected results for the serum samples amounted to 103% (86%–125%). The Anti-CCP ELISA is linear in the tested concentration range (3 RU/ml to 196 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 = 24	
	Mean value (RU/ml)	CV (%)	Mean value (RU/ml)	CV (%)
1	18	5.9	19	6.3
2	20	4.0	23	6.5
3	26	3.6	35	7.2
4	52	3.4	63	6.8

Reference range: Levels of anti-CCP antibodies were analysed in 400 sera from healthy blood donors of between 18 and 68 years of age (149 women, 251 men) using the EUROIMMUN ELISA. No differences with respect to age or gender were observed. The mean concentration of antibodies against CCP was 1.2 RU/ml (\pm 0.8 RU/ml of standard deviation) and the values ranged from 0.2 to 8.0 RU/ml. With a cut-off of 5 RU/ml, 0.5% of the blood donors were anti-CCP positive.



n = 400 Blood donors			
Percentile	95%	98%	99%
Cut-off	2.6 RU/ml	3.3 RU/ml	4.2 RU/ml

ROC analysis: In the analysis of 419 RA patients samples, 744 control samples and 400 blood donors the following characteristics were determined:

Cut-off	Specificity	Sensitivity
2.6 RE/ml	95.0%	81.4%
4.2 RE/ml	98.0%	79.0%
8.0 RE/ml	99.0%	75.4%

Correlation of the EUROIMMUN and Euro-Diagnostica Anti-CCP ELISA: The antibody concentration was determined in 259 sera from patients with RA using the EUROIMMUN and Euro-Diagnostica Anti-CCP ELISA. The qualitative results of both ELISA correlated in 97%.

EURO-IMMUN	RA (n = 259)		Euro-Diagnostica	
	positive	negative	positive	negative
positive	213	4		
negative	3	39		

Technical data:

Antigen	Synthetic cyclic citrullinated peptides (CCP) containing modified arginine residues.
Calibration	Quantitative, in relative units per ml (RU/ml): Calibrator 1: 1 RU/ml Calibrator 2: 5 RU/ml; cut-off value Calibrator 3: 20 RU/ml Calibrator 4: 100 RU/ml Calibrator 5: 200 RU/ml
Sample dilution	Serum or plasma; 1:101 in dilution buffer.
Reagents	Ready-to-use, with the exception of the wash buffer (10x). Colour-coded solutions, in most cases exchangeable with those in other EUROIMMUN ELISA kits.
Test procedure	60 min / 30 min / 30 min. Room temperature. Fully automatable.
Measurement	450 nm (Reference wavelength \geq 620 nm).
Kit format	12 x 8 Reagent wells, kit includes all necessary reagents.
Order no.	EA 1505-9601 G