# 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.

| 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%) |
| Systemic 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 hepatitides | 54 | 0 |
| Anti-HIV positive | 5 | 0 |
| Tuberculosis | 10 | 0 |
| **Specificity for RA** | **1154** | **21 (98.2%)** |

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 agains 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.

**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**

**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.

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# Test Characteristics
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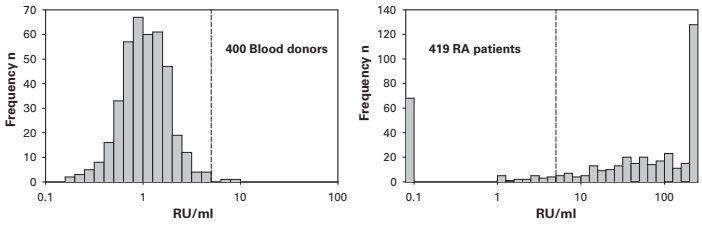
**Linearity:** The linearity of the ELISA was determined by assaying serial dilutions of 6 serum samples. The average concordance of dilution-factor-corrected results for the serum samples amounted to 103% (86%-125%). The Anti-CCP ELISA is linear in the tested concentration range (3 RU/ml to 196 RU/ml).

**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.

| | *Intra-assay variation, n=20* | | *Inter-assay variation, n=24* | |
|---|---|---|---|---|
| **Serum** | **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 (±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 | 95th | 98th | 99th |
| 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 RU/ml | 95.0 % | 81.4 % |
| 4.2 RU/ml | 98.0 % | 79.0 % |
| 8.0 RU/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%.

| RA (n=259) | | Euro-Diagnostica positive | Euro-Diagnostica negative |
|---|---|---|---|
| EUROIMMUN | 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):<br>Calibrator 1: 1 RU/ml<br>Calibrator 2: 5 RU/ml; cut-off value<br>Calibrator 3: 20 RU/ml<br>Calibrator 4: 100 RU/ml<br>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).<br>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 ≥ 620 nm). |
| Kit format | 12 x 8 break off reagent wells, kit includes all necessary reagents. |
| Order no. | EA 1505-9601 G |

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