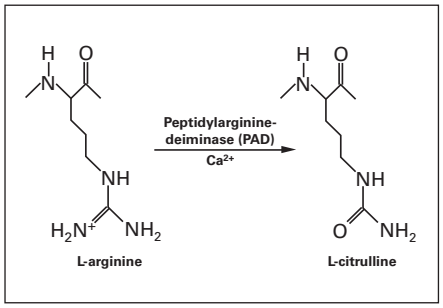
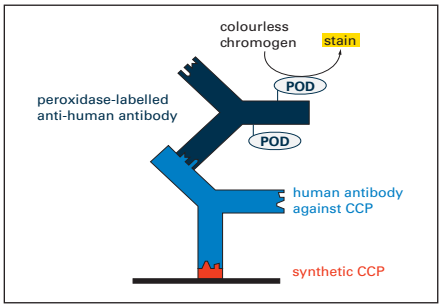
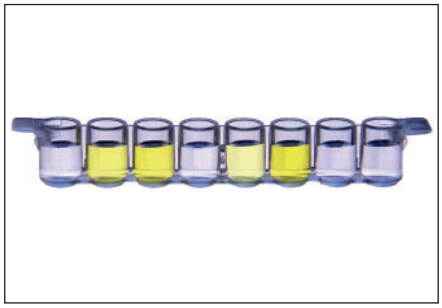
Antibodies against cyclic citrullinated peptides (CCP): An ELISA for specific diagnosis of rheumatoid arthritis



The amino acid citrulline



Principle of the anti-CCP ELISA



Anti-CCP ELISA

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting around 1% of the world population. It is characterised 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**[1] **(RA keratin, anti-perinuclear factor)** in their 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[2]. 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[3]. Antibodies against **cyclic citrullinated peptides (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[4]. Antibodies against CCP possess a **much higher specificity** than RF (anti-CCP: 97%, RF: 62%) **with the same sensitivity** (anti-CCP: 79%, RF: 78%)[5]. They can be detected in early stages of the disease in 79% of patients.

EUROIMMUN offers an innovative **microplate ELISA** for quantitative determination of autoantibodies against CCP. Diluted patient sera are incubated in wells coated with synthetic cyclic citrullinated peptides (second generation). Specific antibodies in the serum bind to the immobilised antigen and cause a photometric colour reaction by means of an enzyme-coupled secondary antibody. Five calibration sera ensure reliable measurement of antibody concentrations. The EUROIMMUN Anti-CCP ELISA is a highly specific and sensitive serological test system for the diagnosis of 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%)
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 (4.0%)
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%)**

1) Nogueira et al., Ann. Rheum. Dis. 60: 882 (2001)
2) Schellekens et al., J. Clin. Invest. 101: 273-281 (1998)
3) Schellekens et al., Arthritis Rheum. 43: 155-163 (2000)
4) Kroot et al., Arthritis Rheum. 43: 1831-1835 (2000)
5) Vasishta, Am. Clin. Lab. 21: 34-36 (2002)