Anti-CEP-1 ELISA (IgG)

- **High specificity for RA, thus a supplementary serological parameter for securing a diagnosis**
- **Research into pathogenesis of RA and subtyping of the disease**
- **Potentially relevant for monitoring treatment responses and assessing therapy success**

Technical data

Antigen	Citrullinated α-enolase peptide 1 (CEP-1)
Calibration	Quantitative, in relative units per milliliter (RU/ml) Calibrator 1: 200 RU/ml Calibrator 2: 20 RU/ml Calibrator 3: 2 RU/ml Recommended upper threshold value for normal range (cut-off): 20 RU/ml
Sample dilution	Serum or plasma; 1 : 101 in sample buffer
Reagents	Ready for use, with the exception of the wash buffer (10x)
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 break-off wells. Kit includes all necessary reagents.
Order number	**EA 151b-9601 G**

Clinical significance

In suspected cases of rheumatoid arthritis (RA), mainly rheumatoid factors (RF) and autoantibodies against cyclic citrullinated peptides (CCP) are serologically determined alongside general inflammation parameters. RF are found in 70 to 90 % of sera from RA patients, but they also occur in patients with other autoimmune and infectious diseases. The detection of autoantibodies against CCP which is optimised for high specificity (97 %) and sensitivity (72 %) is on the other hand an established standard test. However, although available tests for the detection of autoantibodies against citrullinated protein/peptide antigens (ACPA) such as CCP allow the detection of ACPA, they do not deliver any information about the nature of the autoantibodies. To gain more information, the reactivity of the citrullinated proteins that actually occur in the inflamed joints of RA patients, for example fibrinogen/fibrin, vimentin, collagen type II, fibronectin and α-enolase, must be investigated. Citrullinated α-enolase was discovered as an autoantigen in RA in 2005. The epitope within the α-enolase "citrullinated α-enolase peptide 1" (CEP-1) which is responsible for the autoimmune reaction was described as the relevant autoantigen in 37 to 62 % of RA patients by Prof. Lundberg. Antibodies against this peptide show a high specificity, and are only found in the sera of 2 to 3 % of healthy blood donors or control patients.

Diagnostic application

Antibodies against CEP-1 are a supplementary parameter for diagnosis of RA and for research into the pathogenesis and subtyping of the disease. In collaboration with Prof. Venables (Kennedy Institute of Rheumatology, University of Oxford), a commercial CE-certified Anti-CEP-1 ELISA has been developed, for which EUROIMMUN has an exclusive license. Results from studies demonstrate a sensitivity of 50 % on average and a high specificity of 98 % for the test system. In 2009 it was shown in *Nature Genetics* that autoantibodies against CEP-1 are associated with a subtype of RA for which smoking is a main risk factor. Further, an association between anti-CEP-1 and Porphyromonas gingivalis infection, one of the main causes of periodontitis, has been reported. Since anti-CEP-1 represents an immune response to a relevant target antigen, the detection of these autoantibodies is better suited for gaining insight into the cause and pathogenesis of RA than CCP2-based test systems. Current studies are investigating whether the determination of autoantibodies against CEP-1 might also be suitable for prognosis about the response to treatment and for monitoring the disease and therapy course.

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Linearity

The linearity of the Anti-CEP-1 ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The coefficient of determination R^2 was >0.95 for all sera. The Anti-CEP-1 ELISA (IgG) is linear at least in the tested concentration range of 3 RU/ml to 200 RU/ml.

Reference range

The levels of anti-CEP-1 antibodies were determined in 300 samples from apparently healthy blood donors using the EUROIMMUN ELISA. The mean concentration of antibodies against CEP-1 was 4 RU/ml with values ranging from 0.9 to 53.6 RU/ml. With a cut-off of 20 RU/ml, 1.7 % of the blood donors were anti-CEP-1 positive.

Blood donors (n = 300)			
Percentile	97th	98th	99th
Cut-off	16.1 RU/ml	19.5 RU/ml	23.8 RU/ml

Reproducibility

To evaluate the test reproducibility the intra- and inter-assay coefficients of variation were determined using 3 sera. The intra-assay CVs are based on 20 measurements and the inter-assay CVs on 3 determinations repeated in 10 different test runs.

	Intra-assay variation, n=20		Inter-assay variation, n=3 x 10	
Serum	Mean value (RU/ml)	CV (%)	Mean value (RU/ml)	CV (%)
1	16.3	3.6	16.5	7.6
2	67.3	2.4	67.5	5.7
3	170.3	1.5	170.9	2.5

Sensitivity and specificity

110 patients with rheumatoid arthritis (RA), a control panel of 238 patients with other diseases (SLE, SS, SCL, FM, SpA, PsA or infectious diseases) and 500 apparently healthy blood donors were investigated with the EUROIMMUN Anti-CEP-1 ELISA (IgG). The sensitivity of the ELISA for RA was 43.6 % at a specificity of 97.6 %.

Panel (n = 848)	n	Anti-CEP-1 ELISA (IgG) positive
Rheumatoid arthritis (RA)	110	48
Sensitivity for RA	**110**	**48 (43.6 %)**
Systemic lupus erythematosus (SLE)	120	5
Sjögren's syndrome (SS)	30	1
Scleroderma (SCL)	30	2
Fibromyalgia (FM)	20	0
Axial spondyloarthritis (SpA)	20	1
Psoriatic arthritis (PsA)	2	0
Infectious diseases	16	1
Healthy blood donors	500	8
Specificity for RA	**738**	**18 (97.6 %)**

Literature

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