CENP-A ELISA

The CENP-A ELISA contributes to the diagnosis of the CREST Syndrom and systemic sclerosis (SSc)

Anti-centromere antibodies (ACA) are found in 20-50% of patients with systemic sclerosis (SSc) and especially in patients with the CREST Syndrom (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia). The precise prevalence of ACA depends on the detection system and on the selection of the patient cohort. Furthermore, ACA are found in patients with systemic lupus erythematosus (SLE), primary billiary cirrhosis (PBC) and rheumatoid arthritis (RA), however with lower prevalence. The CENP-A ELISA is the first assay for the detection of ACA which uses a highly specific peptide sequence (patent pending) from the N-terminal part of CENP-A and therefore results in a significantly higher specificity compared to common ACA assays.

The CENP-A ELISA is intended for the semi-quantitative determination of antibodies specific for the CENP-A protein. The results of the CENP-A ELISA aid to the diagnosis of systemic sclerosis and related autoimmune disorders and should be used as prognostic marker for the disease progression.

CENP-A ELISA REF 25024 96

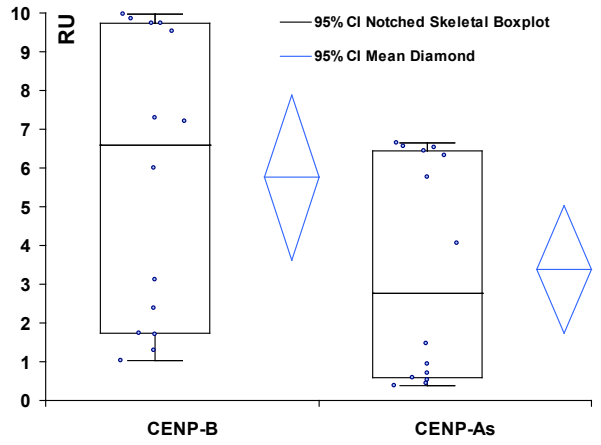

Figure 1
Comparative Boxplot analysis. Sera from patients with definite SLE and anti-centromere antibodies (ACA) identified by indirect immunofluorescence were tested for ACA by ELISA with recombinant CENP-B and synthetic CENP-A. Significantly lower reactivity was found with CENP-A demonstrating the higher specificity of the CENP-A antigen.

CENP-A ELISA Specifications

- Synthetic CENP-A peptide antigen
- Colored reagents
- Assay time: < 1.5 h at RT (30 min /30 min /15 min)
- 3 µL serum or plasma per test
- Detection system: HRP/TMB ($OD_{450\ nm\ /620\ nm}$)
- Wide measuring range
- Low detection limit

Assay performance

- Improved differentiation between SSc patients and controls (high specificity)
- Excellent "lot to lot" correlation $R^2 > 0.95$
- Low intra- and inter-assay variation CV%<10
- Excellent linearity over the entire range

Dr. Fooke-Achterrath Laboratorien GmbH - Habichtweg 16 - 41468 Neuss - Germany
Phone: + 49 2131 2984-0 - Fax: + 49 2131 2984-184 - email: information@fooke-labs.de

Table 1
Prevalence of anti-CENP-A antibodies in SSc and controls

	n	No. (%) > 1.0 RU	No. (%) > 1.5 RU	Mean RU	Median RU	Min RU	Max RU
SSc	334	141 (42.2)	112 (33.5)	2.51	0.81	0.0	12.7
Controls	791	105 (13.2)	25 (3.1)	0.63	0.55	0.0	7.5
SLE	214	38 (17.8)	9 (4.2)	0.69	0.55	0.1	7.1
RA	40	8 (15.7)	3 (5.9%)	0.54	0.48	0.1	1.2
SjS	44	4 (10)	0 (0)	0.69	0.64	0.2	1.6
MCTD	18	8 (18.2)	2 (4.5)	0.74	0.69	0.2	1.4
overlaps	16	17 (39.5%)	5 (11.6)	0.37	0.29	0.1	1.0
PM	43	5 (11.1)	0 (0.0)	0.93	0.85	0.2	1.9
DM	23	2 (8.7)	1 (4.3)	0.72	0.57	0.2	3.1
PBC	54	0 (0.0)	0 (0.0)	0.72	0.59	0.2	2.0
WG	3	4 (2.3)	1 (0.6)	0.21	0.20	0.1	0.3
Others	80	5 (6.3%)	3/80 (4.6%)	0.44	0.22	0.1	7.5
CFS	36	4 (11.1%)	0/36 (0%)	0.80	0.76	0.5	1.2
CD	48	9 (18.8%)	0/48 (0.0)	0.73	0.68	0.0	1.3
HD	175	4 (2.3%)	1/175 (0.6%)	0.49	0.46	0.1	2.8

SSc = Systemic sclerosis, SLE = systemic lupus erythematosus, RA = rheumatoid arthritis, SjS = Sjögren Syndrome, MCTD = Mixed connective tissue disease, PM = polymyositis, DM = dermatomyositis, PBC = primary billiary cirrhosis, WG = Wegener's granulomatosis, CFS = „chronic fatigue syndrome, CD = Crohn's disease, HD = healthy donors, * all controls combined

n = 99, kappa = 0.73	IIF		
CENP-A ELISA	pos	neg	Total
pos	19	6	25
neg	4	70	74
Total	23	76	99

n = 265, kappa = 0.86	CENP-B ELISA		
CENP-A ELISA	pos	neg	Total
pos	48	8	56
neg	4	205	209
Total	52	213	265

n = 100, kappa = 0.81	LIA (CENP-B)		
CENP-A ELISA	pos	neg	Total
pos	20	5	25
neg	2	73	75
Total	22	78	100

IIF = indirect immunofluorescence; LIA = line immunoassay

Figure 2
Comparison of the CENP-A ELISA with other methods. The results of the CENP-A ELISA were qualitatively compared to the results of the indirect immunofluorescence (IIF), of an ELISA based on recombinant CENP-B and of a line immunoassay. According to the kappa method good to excellent agreements were observed.

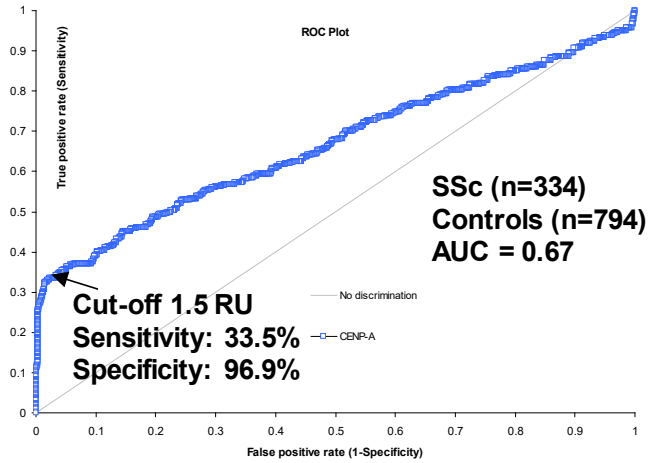

Figure 3
Receiver operating characteristics (ROC) analysis of the CENP-A ELISA. The results of 334 patients with systemic sclerosis and 794 controls were analyzed by ROC analysis. The ROC curve shows a good discrimination between SSc patients and controls (Area under the curve AUC = 0.67; sensitivity 33.5% and specificity 96.9%)

Literature

1. Tan EM: Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989, 44:93-151.
2. Fritzler MJ, Kinsella TD: The CREST syndrome: a distinct serologic entity with anticentromere antibodies. Am J Med 1980, 69:520-526.
3. Earnshaw WC, Machlin PS, Bordwell BJ, Rothfield NF, Cleveland DW: Analysis of anticentromere autoantibodies using cloned autoantigen CENP-B. Proc Natl Acad Sci U S A, 1987, 84:4979-4983.
4. Mahler M, Raijmakers R, Fritzler MJ: Challenges and Controversies in Autoantibodies Associated with Systemic Rheumatic Diseases. Current Rheumatology Reviews 2007, 3:67-78.
5. Mahler M, Schulte-Pelkum J, van Liempt M, Wither JE, Fooke M, Fritzler MJ: Anti-Cenp-A and Cenp-B Antibodies in SLE. 7th International Congress on Autoimmunity, Ljubljana 2010, Abstract 788.

DR FOOKE

Dr. Fooke-Achterrath Laboratorien GmbH - Habichtweg 16 - 41468 Neuss - Germany
Phone: + 49 2131 2984-0 - Fax: + 49 2131 2984-184 - email: information@fooke-labs.de