CENP-A ELISA

REF 25024

Background

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 dys-motility, 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.

Intended use

The CENP-A ELISA is intended for the semi-quantitative determination of anti-bodies 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.

General features

- Synthetic CENP-A peptide antigen
- Significantly enhanced specificity (compared to CENP-B)
- CE labeled
- User-friendly
- Colored reagents
- Ready to use reagents (except washing buffer)
- Breakapart microtiter strips

Technical information

- 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

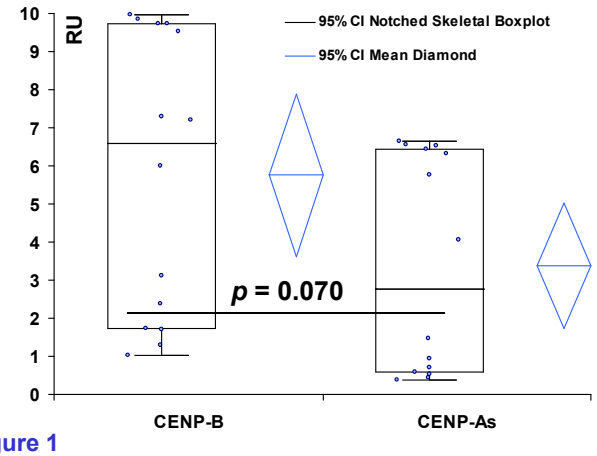

Figure 1
Comparative Boxplot analysis. Sera from patients with definite SLE and anti-centromere antibodies (ACA) identified by indirect immunofluorescence were tested for ACA by ELISA with recombinant CENP-B and synthetic CENP-A. Significantly lower reactivity was found with CENP-A demonstrating the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

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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.

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
- Excellent linearity over the entire range

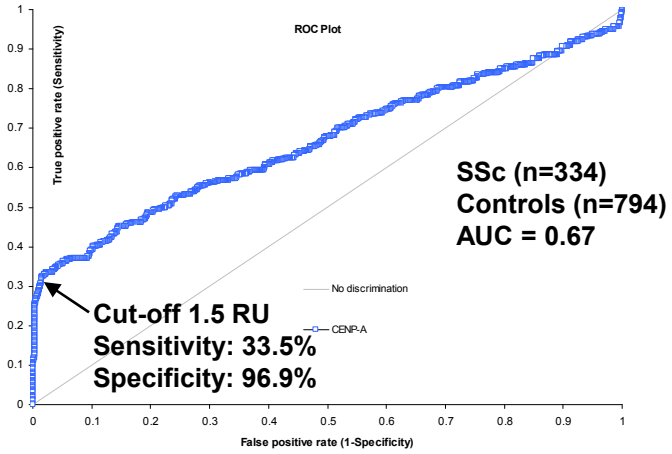

Figure 3
Receiver operating characteristics (ROC) analysis of the CENP-A ELISA. The results of 334 patients with systemic sclerosis and 794 controls were analyzed by ROC analysis. The ROC curve shows a good discrimination between SSc patients and controls (Area under the curve AUC = 0.67; sensitivity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

Literature

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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.
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