RNP/Sm ELISA

The RNP/Sm ELISA contributes to the diagnosis of mixed connective tissue disease (MCTD) and related autoimmune diseases

Circulating antibodies to intra-cellular structures especially to nuclear antigens represent a characteristic feature of systemic autoimmune diseases. Antibodies to the ribonucleoprotein (RNP) complex in high titers are considered as a specific marker for mixed connective tissue disease (MCTD) found in 40-90% of MCTD patients. Due to the high disease specificity of anti-RNP/Sm they have been included as one of the disease criteria for the diagnosis of MCTD. The RNP/Sm complex consists of 3 known U1-specific RNPs (U1-68 kDa, U1-A, U1-C) and 9 Sm polypeptides (SmB, SmB´, SmN, SmD1, SmD2, SmD3, SmE, SmF, SmG). Apart from patients with MCTD anti-RNP/Sm antibodies can also be detected in samples from patients with related autoimmune disorders such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSc).

The RNP/Sm ELISA is intended for the semi-quantitative determination of antibodies specific for the RNP/Sm protein complex. The results of the RNP/Sm ELISA aid to the diagnosis of MCTD and related autoimmune disorders.

RNP/Sm ELISA REF 25011 Σ 96

ID	Target	RU	Interpretation
CDC 1	DNA	3.0	positive
CDC 2	SS-B/La	0.3	negative
CDC 3	RNP/Sm, SS-A/Ro, SS-B (La)	7.3	positive
CDC 4	U-1 RNP	7.5	positive
CDC 5	Sm	7.8	positive
CDC 6	Fibrillarin	0.5	negative
CDC 7	SS-A/Ro	0.2	negative
CDC 8	Centromere	0.2	negative
CDC 9	Scl-70	0.2	negative
CDC 10	Jo-1	0.1	negative
CDC 11	PM/Scl (PM 1)	0.3	negative
CDC 12	Rib-P	0.2	negative

Figure 1
Results of the CDC ANA reference sera. 12 reference serum samples, available from the "Center for Disease Control and Prevention (CDC)" were tested in the RNP/Sm ELISA (REF: 25011). Sample CDC 1, CDC 3, CDC 4 and CDC 5 were positive for anti-RNP/Sm antibodies.

RNP/Sm ELISA Specifications

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

Assay performance

- Good correlation to reference ELISA systems
- Excellent "lot to lot" correlation $R^2 > 0.95$
- Low intra- and inter-assay variation CV% < 8
- Excellent linearity over the entire range

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ID	Diagnose	RU	Interpretation	No. of competitiors with positive results for RNP/Sm
AMLI 1	HD	0.2	negative	0
AMLI 2	SLE	6.8	positive	21/21
AMLI 3	MCTD	1.9	positive	20/21
AMLI 4	SjS	0.2	negative	0
AMLI 5	SjS	0.2	negative	0
AMLI 6	Scl	0.2	negative	0
AMLI 7	PM	0.2	negative	0
AMLI 8	CREST	0.2	negative	0
AMLI 9	SLE	0.4	negative	0
AMLI 10	HD	0.2	negative	0

HD = healthy donor; SLE = systemic lupus erythematosus; MCTD = mixed connective tissue disease; SjS = Sjögren Syndrome; Scl = systemic sclerosis; CREST = (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia); PM = Polymoysitis

Figure 2
Results of the AMLI reference sera. 10 reference serum samples, available from the Association of Medical Laboratory Immunologists (AMLI) were tested in the RNP/Sm ELISA (REF: 25011). Samples AMLI 2 and AMLI 3 were tested positive in concordance to the findings of 21 reference laboratories.

Table 1
Prevalence of anti-RNP/Sm in different disease groups and healthy donors according to the literature

Group	% pos Literature	% pos RNP/Sm ELISA (REF:25011)
SLE	20 - 40	35,1
SSc (Scl)	2 - 14	6
SjS	< 10	8,6
Myositis	4 - 17	7
HD	0	0

HD = healthy donor; SLE = systemic lupus erythematosus; SjS = Sjögren Syndrome; SSc (Scl) = systemic sclerosis

RNP/Sm ELISA (2501 1)				
		neg	pos	
Reference	neg	0	0	0
	pos	3*	62	65
		3	62	65

Figure 3
Agreement to reference method. 65 serum samples from patients with connective tissue disease tested in the RNP/Sm ELISA (REF: 25011) and in a validated reference ELISA demonstrated a good agreement (96.0%) between the two assays.
*One sample was borderline by RNP/Sm ELISA (RU=1.0)

Literature

1. Tan EM: Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989, 44:93-151.
2. James K, Carpenter AB, Cook L, Marchand R, and Nakamura RM for the Association of Medical Laboratory Immunologists Standards Committee: Development of the Antinuclear and Anti-Cytoplasmic Antibody Consensus Panel by the Association of Medical Laboratory Immunologists. Clin Diagn Lab Immunol 2000, 7:436–443.
3. Mahler M, Waka A, Hiepe F, Fritzler MJ: Effect of dsDNA binding to SmD derived peptides on the clinical accuracy in the diagnosis of systemic lupus erythematosus. Arthritis Res Ther 2007, 9:R68.
4. Conrad K, Schößler W, Hiepe F: Autoantibodies in Systemic Autoimmune Diseases Pabst Science Publishers Lengerich, Berlin, Riga, Rom, Wien, Zagreb.
5. Ho KT, Reveille JD: The clinical relevance of autoantibodies in scleroderma. Arthritis Res Ther 2003, 5: 80-93.

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