Ro60 (SS-A) ELISA

The Ro60 ELISA contributes to the diagnosis of the Sjögren Syndrome (SjS) and related autoimmune diseases

Circulating antibodies to intra-cellular structures especially to nuclear antigens represent a characteristic feature of systemic autoimmune diseases. One of those antigens, the Ro60 is frequently the target of autoantibodies in patients with Sjögren Syndrome (SjS), systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD) and systemic sclerosis (SSc). The Ro60, together with Ro52 is also termed as SS-A antigen (Sjögren Syndrome antigen-A). Anti-Ro60 antibodies are frequently accompanied by anti-La antibodies. The coexistence of anti-Ro60 and anti-Ro52 antibodies is disease dependent and reaches significantly higher degrees in SjS or SLE patients. Moreover, anti-Ro60 antibodies seem to play an important role for neonatal lupus and congenital heart block, especially those directed against certain Ro60 epitopes. Defined epitopes seem to correlate with complications during pregnancy.

The Ro60 ELISA is intended for the semi-quantitative determination of antibodies specific for the Ro60 protein. The results of the Ro60 ELISA aid to the diagnosis of SjS and related autoimmune disorders and should be used as prognostic marker for the disease progression.

Ro60 ELISA Specifications

- Recombinant 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

Ro60 ELISA REF 25007 96

ID	Target	ELISA (RU)	Interpretation
CDC 1	DNA	0.6	negative
CDC 2	SS-B/La	2.4	positive
CDC 3	RNP/Sm, SS-A/Ro, SS-B (La)	3.6	positive
CDC 4	U-1 RNP	0.5	negative
CDC 5	Sm	0.4	negative
CDC 6	Fibrillarin	0.3	negative
CDC 7	SS-A/Ro	4.4	positive
CDC 8	Centromere	0.2	negative
CDC 9	Scl-70	0.4	negative
CDC 10	Jo-1	0.2	negative
CDC 11	PM/Scl (PM 1)	0.4	negative
CDC 12	Rib-P	0.4	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 Ro60 ELISA (REF: 25007). Sample CDC 2, CDC 3, CDC 7 were positive for Ro60 antibodies.

Assay performance

- Good correlation to reference methods
- Excellent "lot to lot" correlation R2 > 0.97
- Low intra- and inter-assay variation CV% < 10
- Excellent sensitivity due to novel recombinant Ro60 antigen
- Excellent linearity over the entire range

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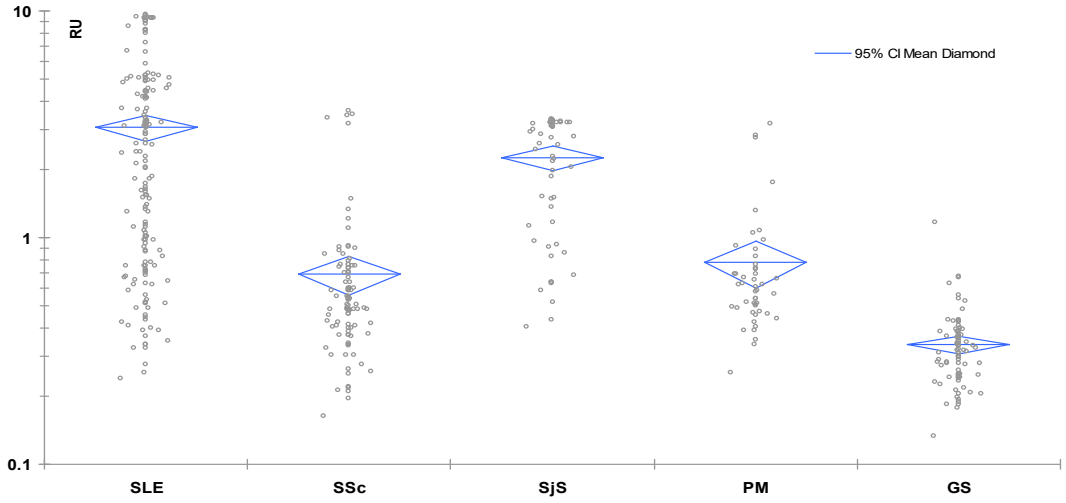

Figure 2
Anti-Ro60 reactivity in different cohorts of connective tissue diseases and healthy donors. Serum samples from patients with systemic lupus erythematosus (SLE, n=168), systemic sclerosis (SSc, n=100), Sjögren Syndrome (SjS, n=57), Polymyositis (PM, n=48) and healthy donors (HD, n=85) were tested for anti-Ro60 antibodies by Ro60 ELISA (REF: 25007). Highest prevalence and titers of anti-Ro60 antibodies were found in patients with SLE and SjS.

Table 1
Prevalence of anti-Ro60 antibodies in different disease cohorts and healthy donors

	% pos Ro60 ELISA (REF:25007)	% pos literature
SLE (n=168)	58.9	40-60
SSc (Scl, n=100)	5	~5
SjS (n=57)	68.4	~70
Myositis	8.3	0-14
HD	0	0

		Ro60 ELISA (25007)		
		pos	neg	
Reference	pos	96	1	97
	neg	30	61	91
		126	62	188

Figure 3
Agreement with reference method. 188 samples from patients with SLE (n=131) and Sjögren Syndrome (n=57) were tested by Ro60 ELISA (REF: 25007) and by a validated, FDA approved reference system (multiplex assay). The results show good concordance (84%) between both methods. The sensitivity of the ELISA is significantly higher compared to the multiplex assay at 100% specificity against unrelated disease controls and healthy donors.

Literature

1. Tan EM: Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989, 44:93-151.
2. Mahler M, Raijmakers R, Fritzler MJ: Challenges and Controversies in Autoantibodies Associated with Systemic Rheumatic Diseases. Curr Rhreumatol Rev 2007, 12:67-78.
3. Schulte-Pelkum J, Fritzler M, Mahler M: Latest update on the Ro/SS-A autoantibody system. Autoimmun Rev 2009, 8: 632-637.

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