

Multiplexed Diagnostic Analysis of Auto-Antibodies in Patients' Sera

Summary

Label-free single assay multiplexed analysis of patients' auto-antibodies against 5 SLE antigens is compared against commercial ELISA results from 5 single-plex (single marker) kits, one for each auto-antigen (Sm, RNP, SSA, SSb, and SCL-70).

Background

The detection of Anti-Nuclear Antibodies (ANA's) has long been an important tool in the diagnosis of systemic autoimmune diseases. The antigens used in their detection are purified by the saline extraction of human or animal nuclei, which is why they are called Extractable Nuclear Antigens (ENA's). The most commonly

	LFIRE				
	RNP	SSB	SCL70	SSA	Sm
Patient 1	Red	Green	Green	Red	Red
Patient 2	Green	Red	Green	Green	Green
Patient 3	Red	Red	Green	Green	Green
Patient 4	Green	Red	Green	Green	Green
Patient 5	Green	Green	Red	Red	Red
Patient 6	Red	Green	Green	Green	Green
Patient 7	Red	Green	Green	Green	Green
Patient 8	Red	Red	Green	Red	Green
Patient 9	Green	Green	Red	Green	Green
Patient 10	Green	Green	Green	Green	Green
Patient 11	Green	Green	Red	Green	Red
Patient 12	Green	Green	Green	Red	Red
Patient 13	Green	Green	Green	Green	Green
Patient 14	Red	Green	Green	Red	Green
Patient 15	Red	Green	Green	Green	Red
Patient 16	Green	Green	Red	Green	Green

measured ENA markers used for diagnosis of Systemic Lupus Erythematosus (SLE) are anti-SS-A/Ro, anti-SS-B/La, anti-Sm, anti-Sm/RNP, anti-Jo-1 and anti-Scl-70.

Method and Protocol

Multiplexed protein arrays were prepared with purified SSA, SSB, RNP, Sm, and SCL-70 antigens and were assayed using LFIRE™ to analyze 30 patient sera (14 negatives and 16 that were positive by single marker ELISA kits for one or more auto-antibodies). The results for the positive sera are given below. Green indicates negative and red, positive.

The concordance between LFIRE and the commercial ELISA's was greater than 99% since there were only two differences among the 150 total results. Both differences were near the cutoff for ELISA between positive and negative.

	ELISA				
	RNP	SSB	SCL70	SSA	Sm
Patient 1	Red	Green	Green	Red	Red
Patient 2	Green	Red	Green	Green	Green
Patient 3	Red	Red	Green	Green	Green
Patient 4	Green	Red	Green	Green	Green
Patient 5	Green	Green	Red	Red	Red
Patient 6	Red	Green	Green	Green	Green
Patient 7	Red	Green	Green	Green	Green
Patient 8	Red	Red	Green	Red	Green
Patient 9	Green	Green	Red	Red	Green
Patient 10	Green	Green	Green	Green	Green
Patient 11	Green	Green	Red	Green	Red
Patient 12	Green	Green	Green	Red	Red
Patient 13	Green	Green	Green	Green	Green
Patient 14	Red	Green	Green	Red	Green
Patient 15	Red	Green	Green	Green	Red
Patient 16	Green	Green	Red	Green	Green

ELISA	LFIRE™	
	+	-
+	26	1
-	0	123

Concordance > 99%