



THREE MULTIPLEXED TESTS FOR EXTRACTABLE NUCLEAR ANTIGENS USING THE LUMINEX 100™

Rufus W. Burlingame¹, Andrea L. Piette¹, Carlos von Mühlen², K. Michael Pollard³ and Walter L. Binder¹

¹INOVA Diagnostics, Inc., San Diego, CA; ²PUC School of Medicine, Porto Alegre, Brazil; ³The Scripps Research Institute, La Jolla, CA

ABSTRACT

Objective: Develop convenient multiplexed immunoassays for the detection of autoantibodies to the extractable nuclear antigens Sm, RNP, SSA, SSB, Scl-70 and Jo-1. Compare the results obtained with this new technology to clinical diagnosis and the results found with standard ELISA methodology. Determine if the number of autoantibodies measured at one time affects the results.

Methods: A no-wash multiplexed assay that detects autoantibodies to up to 6 antigens in a single well was developed. Each of the 6 antigens is coated on a unique bead. The appropriate beads are then placed in a single well as either a 4-plex comprised of Sm, RNP, SSA and SSB, a 5-plex comprised of those 4 antigens plus Scl-70, or a 6-plex comprised of those 5 antigens plus Jo-1. The tests are called the QUANTA Plex™ ENA Profile 4, ENA Profile 5 and ENA Profile 6. Sera from normal blood donors, from patients with clinically defined rheumatic diseases, and from samples with high titers of specific antibodies were tested on all multiplex tests and the corresponding ELISAs. Samples with discrepant results were tested on Ouchterlony immunodiffusion, if possible.

Results: Control samples were obtained from 202 normal blood donors, 45 patients with rheumatoid arthritis and 27 sera with antibodies to known infectious organisms. There was between 98% and 100% agreement in results when the corresponding QUANTA Plex™ test and ELISA were compared. Clinical samples were compared from 61 patients with systemic lupus erythematosus, 165 with scleroderma and 33 with Sjögren's syndrome. There was between 93% and 97% agreement in results when the corresponding QUANTA Plex™ test and ELISA were compared. Thus, it is not surprising that the ELISA and QUANTA Plex tests had the same clinical sensitivity and specificity. Adding beads with Scl-70 and Jo-1 antigens to the QUANTA Plex ENA Profile 4 did not change the reactivity of any sera to the original 4 antigens. The average inter-assay variation for positive samples was between 6% and 9% for the 6 antigens. The cutoff between negative and positive on the QUANTA Plex™ tests was determined by non-parametric statistical techniques and ranged between 4 and 8 standard deviations above the average of the control group.

Conclusion: The 3 QUANTA Plex ENA Profile tests using the Luminex flow cytometer yield sensitivity, specificity and reproducibility nearly identical to ELISA. There are no measurable interactions among the different beads in the same well. Performing all assays for a given sera in only 1 well saves space and time.

METHODOLOGY

The INOVA QUANTA Plex™ ENA Profile 4, Profile 5 and Profile 6

The QUANTA Plex™ ENA Profiles are semi-quantitative immunoassays that detect autoantibodies against Sm, RNP, SS-A, SS-B, Scl-70 and Jo-1 in a single reaction well. The autoantibodies are markers for systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome, scleroderma and polymyositis, five of the most common autoimmune diseases. The assays also contain a control bead that ensures that no false negative results are reported. The ENA 4 measures antibodies to the first 4 antigens listed above, the ENA 5 includes those plus Scl-70, while the ENA 6 includes both Scl-70 and Jo-1.

Principles of the Assay

Each of the 6 auto-antigens and the control antigen is bound to a uniquely colored bead set. The 7 bead sets are mixed together for the ENA Profile 6 in each well of a 96 well plate. Diluted patient sample is incubated with the beads for 30 minutes, followed by a 30 minute incubation with an anti-IgG conjugate. The appropriate wells are then run on the Luminex 100™ and semi-quantitative results for each autoantibody reactivity are obtained.

References

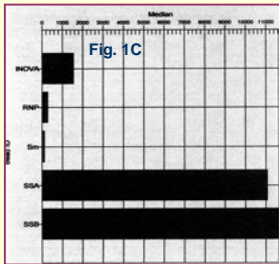
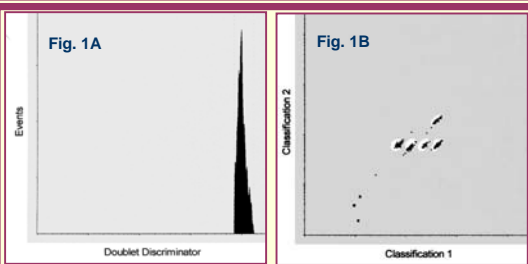
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INTRODUCTION

The Luminex 100™ Integrated System

The Luminex 100™ Integrated System consists of 100 uniquely identifiable latex bead sets, a dual laser flow cytometer, and software to process the accumulated data in real time. The bead sets are identified in the flow cytometer by the absolute amounts of two fluorochromes in each bead that are excited at the same wavelength but emit at widely separated wavelengths (1, 2).

The Luminex 100™ flow cytometer measures 4 parameters for each bead: side light scatter (Fig 1A); the absolute fluorescence of the 2 embedded dyes (Fig. 1B); and the fluorescence of the conjugate that is bound to the bead (Fig. 1C).



Patient Groups

Clinical Samples, N=259:

61 Systemic Lupus Erythematosus
165 Scleroderma
33 Sjögren's Syndrome

Control Samples, N=274:

202 Blood Donors
45 Rheumatoid Arthritis
27 Infectious Disease

Table 1. Agreement among ENA Profiles 4, 5 and 6

All Samples N=533	All Pos	All Neg	One Disagrees	Percent Agreement
Sm	40	482	11*	98%
RNP	52	473	8*	98%
SS-A	75	450	8*	98%
SS-B	31	494	8*	98%
Scl-70**	50	479	4*	99%

* These samples were all low positive on the QUANTA Plex™ test.
** Tested on ENA Profiles 5 and 6 only.

Table 2. Comparison of QUANTA Plex™ ENA Profile 6 and the corresponding ELISAs

Clinically Defined N=259	Both Pos	Both Neg	ELISA Pos QP Neg	ELISA Neg QP Pos	Percent Agreement
Sm	29	218	7	5	95%
RNP	50	199	7	3	96%
SS-A	71	181	3	4	97%
SS-B	29	222	3	5	97%
Scl-70	45	196	14	4	93%
Jo-1	2	249	7	1	97%

Controls N=274	Both Pos	Both Neg	ELISA Pos QP Neg	ELISA Neg QP Pos	Percent Agreement
Sm	1	268	1	4	98%
RNP	1	268	3	2	98%
SS-A	1	270	1	2	99%
SS-B	0	274	0	0	100%
Scl-70	2	270	2	0	99%
Jo-1	1	270	3	0	99%

Comparison of the Average and Standard Deviation of the Control Group

	ENA Profile 6			ELISA		
	Avg	S.D.	20/S.D.	Avg	S.D.	20/S.D.
Sm	3.0	5.1	3.9	4.6	2.8	7.2
RNP	2.5	3.9	5.1	5.7	3.0	6.6
SSA	3.2	3.6	5.5	4.5	3.6	5.6
SSB	1.9	2.4	8.2	3.9	1.6	12.4
Scl-70	4.7	3.8	5.2	7.7	5.8	3.4
Jo-1	1.6	3.3	6.1	6.0	8.6	2.3

Inter-Assay Variation for Sm

	Avg (LU)	St. Dev.	%C.V.
HPC	207	21	10%
Sm 23	217	19	9%
Sm 29	57	6	10%
H61	43	9	21%
H57	377	38	10%

Linear Regression Clinical Samples

$R^2 = 0.77$ for Sm
 $R^2 = 0.79$ for RNP
 $R^2 = 0.85$ for SS-A
 $R^2 = 0.80$ for SS-B
 $R^2 = 0.73$ for Scl-70
 $R^2 = 0.75$ for Jo-1

CONCLUSIONS

- The QUANTA Plex™ ENA Profiles are sensitive, specific and reproducible alternatives to ELISA.
- Autoantibodies reactive with Sm, RNP, SS-A, SS-B, Scl-70 and Jo-1 are measured in a single reaction well instead of in six separate wells, thus decreasing labor, space and time compared with ELISA.
- The presence of multiple antigens in the same reaction well does not cause interference in the measurement of the 6 different autoantibody specificities.
- The QUANTA Plex™ ENA Profile 4 is cleared by the FDA for *in vitro* diagnostic use.