



# IMPROVEMENTS IN THE CLINICAL SENSITIVITY OF ANA ELISA TESTING

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## ABSTRACT

**Objective:** Develop an anti-nuclear antibody (ANA) ELISA that has high sensitivity for all samples that are ANA positive by immunofluorescence on HEp-2 cells, with an emphasis on detecting patients with clinically defined diseases. It has been well documented that commonly used ANA ELISAs fail to detect certain clinically important antibodies. The current work aims to correct those deficiencies.

**Methods:** Optimal antigenic extracts of HEp-2 cells and nucleol were obtained by testing numerous biochemical preparations for reactivity by ELISA with appropriate sera. In order to discover which other antigens were clinically important and should be added to the extracts, sera from patients with systemic lupus erythematosus (SLE), scleroderma and Sjögren's Syndrome (SS) that were negative on the extracts were examined in detail to discover their antigenic specificity.

**Results:** A nucleolar extract that preserves reactivity with fibrillarin, the most diagnostically important nucleolar autoantibody in scleroderma, was obtained. A HEp-2 extract that reacts with a high proportion of ANA positive sera with no known specificity was also developed. In examining clinically defined sera it was found that 5%-10% of centromere positive sera recognized only CENP-A or CENP-B, while the other 80%-90% recognized both centromere antigens. Approximately 1%-2% of patients with SLE, scleroderma and SS have anti-SS-A 52kd as their only known autoantibody. Including the optimized extracts, both CENP-A and CENP-B, and SS-A 52kd in the ANA ELISA yields a highly sensitive screening test with specificities equal to or better than standard immunofluorescent techniques.

**Conclusion:** By optimizing the extracts and antigens included in the ANA ELISA, we have obtained a sensitivity on clinically important samples that is equal to immunofluorescence on HEp-2 cells. These reactivities correct the deficiencies noted in other ANA ELISAs without compromising high specificity.

## INTRODUCTION

The presence of anti-nuclear antibodies (ANA) in human serum is one of the hallmarks of the systemic autoimmune diseases<sup>1</sup>. Typically, ANA have been detected by an indirect immunofluorescence assay (IFA) using mouse kidney sections or HEp-2 cells as the substrate.

Recently, an ANA ELISA screen was developed<sup>2</sup>. The advantages of the ANA ELISA over the IFA include ease of performance, no observer or equipment bias, as well as semi-quantitative results. Until now, the disadvantages included an incomplete range of sensitivity to a few clinically defined antigens as well as a lack of sensitivity to unknown nuclear and cytoplasmic antigens. The 3<sup>rd</sup> generation QUANTA Lite<sup>TM</sup> ANA ELISA has overcome these deficiencies.

The inclusion of both SS-A 52kd and SS-A 60kd provides complete SS-A autoantibody detection<sup>3</sup>. Similarly, the use of both centromere autoantigens CENP-A and CENP-B allows full detection of centromere IFA positive sera<sup>4</sup>. And finally, comprehensive nucleolar and HEp-2 cell extracts are included. These extracts add fibrillarin, Scl-70, ribosomal-P, and chromatin, among others, to the already extensive list of defined autoantigens<sup>5</sup>. In addition, these extracts dramatically increase sensitivity to ANA positive sera with unknown specificity.

## METHODOLOGY

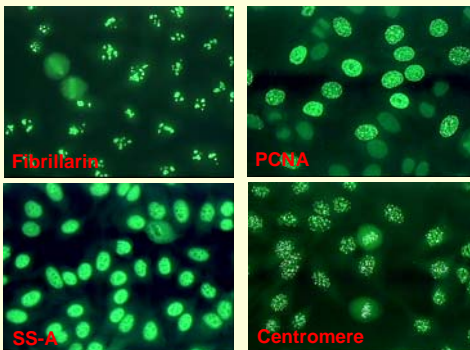
### QUANTA Lite<sup>TM</sup> ANA ELISA

Highly purified individual antigens plus nucleolar and HEp-2 cell extracts containing many defined and undefined autoantigens are bound to the surface of a microwell plate. Patient samples are run at a 1:41 dilution in sample diluent compatible with conventional IFA techniques. Ready-to-use reagents and 30 minute room temperature incubations allow quick sample evaluation using pre-diluted controls to calculate units of reactivity.

### NOVA Lite<sup>TM</sup> HEp-2 ANA IFA

Patient samples were run at 1:41 in either PBS or ANA Sample Diluent. Samples were only titrated if discrepents were seen. All slides were read blinded.

## QUANTA Lite<sup>TM</sup> ANA ELISA positive sera on NOVA Lite<sup>TM</sup> HEp-2 ANA Slides.



## Comparison of patients with SRD on the QUANTA Lite<sup>TM</sup> ANA ELISA and NOVA Lite<sup>TM</sup> HEp-2 cells.

SRD (N=179)	INOVA ANA ELISA		
	Positive	Negative	
IFA on HEp-2 cells	153	20*	
	Negative	2**	4

\* 15 SLE patients showed weak (1+ or less) ANA staining at 1:41. 3 SLE and 2 scleroderma patients showed a variety of low intensity staining at 1:41, including cytoplasmic and nonspecific cellular staining.  
\*\* 2 SLE patients were 27 and 34 units.

## Comparison of healthy volunteers on the QUANTA Lite<sup>TM</sup> ANA ELISA and NOVA Lite<sup>TM</sup> HEp-2 cells.

Normals (N=296)	INOVA ANA ELISA		
	Positive	Negative	
IFA on HEp-2 cells	38	41*	
	Negative	51**	166

\* 34 of these samples showed weak (1+ or less) ANA staining at 1:41. 4 of these samples showed only cytoplasmic staining at 1:41.  
\*\* 44 of these samples were 36 units or less, while the remainder were no more than 45 units.

## Clinical significance of including both CENP-A and CENP-B in the QUANTA Lite<sup>TM</sup> ANA ELISA.

Centromere sera (N=269)*	CENP-A	CENP-B	CENP-A/B
Positive	9	27	231

\* Defined as centromere positive by IFA on HEp-2 cells. 2 of these samples were neither CENP-A or CENP-B positive by ELISA.

## References

1. Von Müllen CA and Tan EM: Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin Arthritis Rheum* 24:323-358 (1995).
2. Jaskowski TD et al: Screening for antinuclear antibodies by enzyme immunoassay. *Am J Clin Pathol* 105:468-473 (1996).
3. McCaulliffe DP et al: Recombinant 52 kDa Ro(SSA) ELISA detects autoantibodies in Sjögren's syndrome sera that go undetected by conventional serologic assays. *J Rheumatol* 24:860-866 (1997).
4. Russo K et al: Circulating anticentromere CENP-A and CENP-B antibodies in patients with diffuse and limited systemic sclerosis, systemic lupus erythematosus, and rheumatoid arthritis. *J Rheumatol* 27:142-148 (2000).
5. Arnett FC et al: Autoantibodies to fibrillarin in systemic sclerosis. *Arthritis Rheum* 33:1151-1160 (1996).

## Performance Characteristics with Sensitivity and Specificity

Patient Group	Total Number	ANA ELISA Positives	%
Clinically defined patients*	179	153	86%
Antigenically defined**	135	133	99%
Unknown ANA positive†	153	92	60%
Healthy volunteers	296	89	30%

\* These included patients with systemic lupus erythematosus (SLE), Sjögren's Syndrome (SS), and scleroderma.

\*\* Between 4 and 9 samples known to be positive for SS-A, SS-B, Sm, RNP, Scl-70, Jo-1, Chromatin, ribosomal-P and M2 were tested. Also tested were samples positive for P80 colin and PCNA as well as monospecific samples positive for fibrillarin, SS-A 52kd, and CENP-A and/or -B.

† These included clinically and antigenically undefined samples which were positive by IFA on HEp-2 cells. Most had weak to moderate staining of unusual and/or unknown pattern.

**Sensitivity: 92.9%      Specificity: 76.5%**

## CONCLUSIONS

- The QUANTA Lite<sup>TM</sup> ANA ELISA is a sensitive and specific immunoassay without the labor intensive and subjective determinations experienced with traditional IFA techniques.
- The inclusion of both CENP-A and CENP-B ensures virtually complete detection of all centromere positive sera.
- Similarly, inclusion of both SS-A 52kd and SS-A 60kd provides thorough SS-A autoantibody detection.
- Optimized nucleolar and HEp-2 cell extracts add fibrillarin to the extensive list of defined autoantigens. These extracts also vastly increase reactivity to many unknown ANA positive sera.