

# AtheNA Provides An Innovative Autoimmune Testing Platform

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**M** measuring autoimmune antibodies accurately is important for making the diagnosis, and in some cases following the progress, of many autoimmune diseases. For the last 50 years the technology has changed dramatically from subjectively evaluating cell preps microscopically to now quantitatively measuring specific proteins. Autoantibodies have been measured with hemagglutination, gel diffusion techniques, radioimmunoassays, indirect immunofluorescence, and more recently with ELISA assays. All of these have had limiting problems with reproducibility and accuracy. The AtheNA Multi-Lyte® ANA technology provides the latest improvement in accurately measuring known disease-related autoantibodies.

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The AtheNA Multi-Lyte® ANA test system can measure multiple autoantibodies simultaneously using individual color-coded polystyrene beads. Each set of beads has a single color and a specific autoantigen covalently attached. A patient's serum is added to a mixture of multiplexed beads in suspension. The beads are then incubated with fluorescent anti-human IgG. The suspended beads are then analyzed with a Luminex dual laser flow cytometer that detects both the color of the bead and the amount of specific autoantibody that corresponds to each colored bead. Quantitation of the fluorescence is accomplished using Intra-Well Calibration Technology®. The bead sets contain one of the following autoantigens: SSA, SSB, U snRNP B/B', U1 snRNP A, U1 snRNP C, Scl-70, Jo-1, Centromere B, dsDNA, Histone H, Histone HLY, and a nuclear extract of Hep-2 cells. The bead mix also contains a set to measure nonspecific antibodies and four sets for assay calibration. Each cytometer analysis of the bead mix measures at least fifty beads of each color for a considerably

more accurate determination of each autoantibody than can be achieved with other techniques. The results give a semi-quantitative measurement of SSA, SSB, RNP, Scl-70, Jo-1, Centromere B, Histone, and Sm autoantibodies. The dsDNA measurement is a quantitative measure and the ANA measure is purely qualitative.

This FDA-approved technology has been compared to conventional testing, and the reproducibility and accuracy have been well established (data available on request at Zeus Scientific, Inc.). The detection of autoantibodies aids in the diagnosis of known autoimmune diseases (see Table 1), although no test is absolutely specific for a condition. Clearly, the more accurate the laboratory tests are, the more definitive the diagnosis will be. AtheNA testing has several advantages over earlier testing. The bead analysis is automated and totally objective. The ANA screen and the specific autoantibodies are measured at the same time. At least fifty assays (bead analyses) are performed for each test to improve accuracy. The binding of

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**Table I****Clinical Prevalence and Relevance of Autoantibodies**

Autoantibody	Associated disease	Frequency in patients with disease
SSA (Ro)	SLE	~30%
	Sjogren's Syndrome	~60%
	Neonatal Lupus	~100%
SSB (La)	Sjogren's Syndrome	~30%
	SLE	~30%
Sm	SLE	~25%
RNP	Mixed Connective Tissue Disease	~95%
	Systemic Sclerosis	~20%
	SLE	~40%
Scl-70	Scleroderma	~30%
Jo-1	Polymyositis	~30%
Centromere	CREST variant of Scleroderma	~80%
Histones	Drug-induced SLE	~90%
DsDNA	SLE	~50%

autoantibodies is done in solution and not on a column or surface, which eliminates a lot of false-positives. Efficiency, cost, and accuracy are features of AtheNA testing that make it more advantageous for the laboratory performing the tests. However, for the physician getting the results, the non-subjective interpretation, precision of the results, and the direct measurement of the clinically significant autoantibodies are an improvement over previously available tests. Results are given in U/ml for each of the measured tests and all tests utilize the same numeric scale. The anti-dsDNA is given in International Units/ml (IU/ml). These quantitative results are more accurate and reproducible than titers subjectively measured from older tests. There is little direct correlation between the quantitative results of AtheNA and the titers and patterns from older tests. If the AtheNA ANA screen is positive,

a profile of known autoantibodies can be generated for each patient from the same serum sample at the same time. Once a profile is known, then those specific autoantibodies can be followed with therapy and clinical changes.

Our experience with more than 1900 patients over three years has clearly shown the AtheNA results with the ANA screening to have far fewer false positive ANA results. Comparing 200 patients with both traditional FANA and AtheNA tests, the concordance for ANA testing was 85%. Virtually all of our fibromyalgia and nonspecific arthralgia patients with low to moderate positive FANA and ELISA tests are negative on AtheNA ANA testing. The concordance with traditional anti-dsDNA and the AtheNA anti-dsDNA was 90%. Comparisons with SSA and SSB showed a concordance of 97% with both tests. We are no longer relying on the subjective microscopic

analysis of our technicians to read patterns and titers. Getting quantitative results has been more useful than the older titers which fluctuated unpredictably. The AtheNA autoantibody tests have been stable over 3 years in our patients with unchanging clinical status. Our results (see Table 2) showed that ~80% of the patients tested in a typical Rheumatology Clinic have negative ANA screening AtheNA tests. About 3% were equivocal and ~17% had a positive ANA. Less than 1% of patients that had a positive ANA screening had a negative autoantibody panel. These patients tended to be well controlled SLE patients. When all of the negative ANA screening tests were analyzed for the full autoantibody profile, none of the tests was positive. This has given us great confidence in the AtheNA screening test for possible autoantibodies.

For those patients that did screen positive for the AtheNA ANA, the distribution of autoantibodies was typical of a Rheumatology practice (see Table 3).

**Table 2**

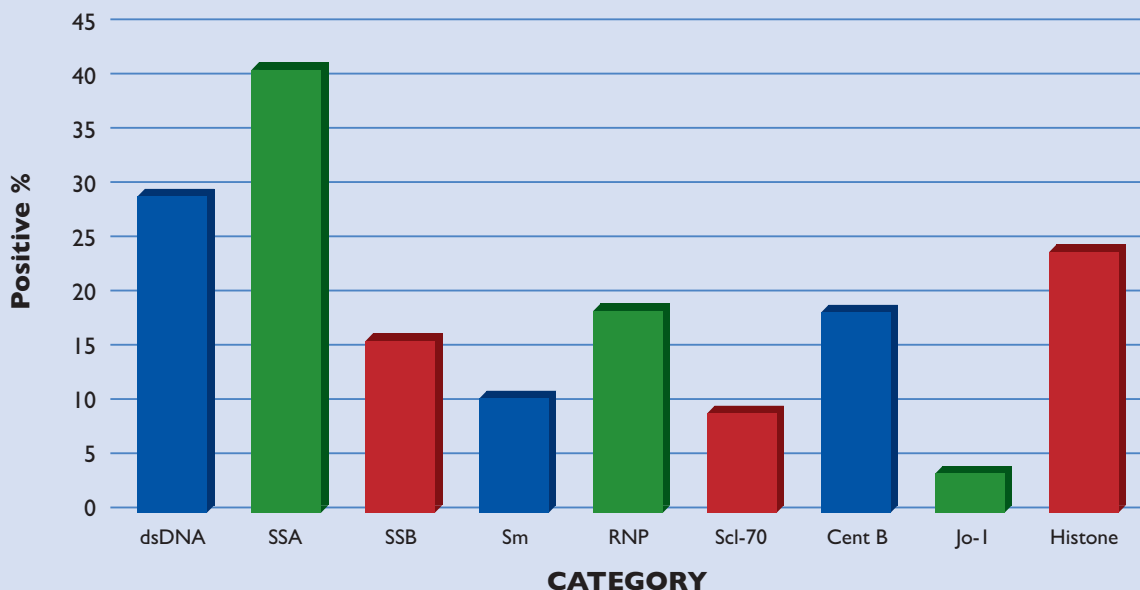
### AtheNA Results in Rheumatology Clinic

1938 Patients

Negative	80.3%
Equivocal	2.9%
Positive	16.8%
+ Screen – Panel	<1%
– Screen + Panel	0

**Table 3**

### Distribution of AtheNA Results



Many patients had more than one autoantibody, typical of the polyclonal nature of autoimmune disease. Over time the dsDNA levels tended to vary with disease activity, similar to results reported elsewhere. Now that the other auto-antibodies are being specifically measured, it will be interesting to see if they also vary with time and disease activity. The profile of a patient's autoantibody panel has been helpful to follow characteristics of their disease. A positive dsDNA or Sm often predicted renal and CNS disease. A positive SSA/SSB was often associated with sicca symptoms. Patients with SSA/SSB antibodies have even been identified before they developed obvious sicca symptoms and Sjogren's Disease. RNP antibodies were often seen in patients with

overlapping symptoms. These relationships have helped to classify patients with nonspecific connective tissue diseases and to further define patients with SLE.

In conclusion, the AtheNA Multi-Lyte® technology has improved the reliability and accuracy of ANA testing. For the laboratory it is more efficient, reproducible, and objective. For the physician it is more helpful to get a full autoantibody profile with non-subjective quantitation as this provides higher quality clinical data. The reproducibility, ease of use, and quick turn-around times of the tests have helped provide better clinical results for our patients. The AtheNA multiplexing platform for autoimmune testing is an exciting and positive step forward in clinical testing.