ANA-III Plus Test System

REF A22001

IVD

CE

Rx Only

INTENDED USE

The ZEUS **AtheNA Multi-Lyte®** ANA-III Plus Test System is intended for the semi-quantitative detection of IgG class antibody to nine separate analytes (SSA-52, SSA-60, SSB, Sm, RNP, Scl-70, Jo-1, Centromere B, and Ribosomal P) in human serum, the quantitative detection of IgG class antibody to dsDNA in human serum. The test system is intended to be used as an aid in the diagnosis of various systemic autoimmune disorders. This test is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Normally, one does not generate antibodies to self antigens. A key function of the immune system is to distinguish foreign antigens (such as those from infectious agents) from self tissues. Immune disorders exist when one's immune system generates potentially destructive antibodies to self antigens (autoantibodies). Most autoimmune disorders can be classified as either non-organ specific (systemic) or organ specific.

In non-organ specific or systemic autoimmune disease, tissue injury and inflammation occur in multiple sites in organs without relation to their antigenic makeup and are usually initiated by tissue deposition of circulating immune complexes. These immune complexes are formed by autoantibody responses to soluble cellular antigens of nuclear or less commonly cytoplasmic origin. Some of the more common examples of systemic autoimmune disease are systemic lupus erythematosus (SLE), rheumatoid arthritis, scleroderma (and CREST), polymyositis, mixed connective tissue disease (MCTD), drug-induced SLE and Sjögren's Syndrome.

The ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System is designed to aid in the diagnosis of many of the systemic autoimmune disorders. It may assist in the detection and identification of many autoantibodies to a number of nuclear and cytoplasmic cellular constituents. The table below shows the relationship between autoantibody and disease state for some of the most common systemic autoimmune disorders.

Autoantibody	Disease Association(s):
SSA-52, SSA-60	SLE, Sjögrens Syndrome
SSB	Sjögrens Syndrome
Sm	SLE
RNP	Mixed Connective Tissue Disease (MCTD)
Scl-70	Scleroderma
Jo-1	Myositis
Centromere B	Scleroderma CREST variant (calcinosis), Raynaud's, esophageal dysmotility, sclerodactyly and telangiectasia
Ribosomal P	Systemic lupus erythematosus
dsDNA	SLE

Although the exact etiology of autoimmune diseases is unknown, and the specific role played by autoantibodies in the onset of various autoimmune diseases is obscure, the association and frequency of detection of these antibodies, particularly those of the IgG class, by the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System, offers an efficient test procedure for the laboratory workup of patients with suspected systemic autoimmune diseases.

Until recently, autoantibodies were tested individually by indirect immunofluorescence, Ouchterlony gel diffusion, hemagglutination, radioimmunoassay, or enzyme-linked immunosorbent assay (ELISA). Unlike several other systems, the **AtheNA Multi-Lyte** System is capable of evaluating several different analytes simultaneously in a multiplexed immunoassay format. When used according to the instructions below, one may evaluate a patient sample for a multitude of autoantigens at the same time in a single well.

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System is designed to detect IgG class antibodies in human sera to a variety of common nuclear antigens. The test procedure involves two incubation steps:

1. Test sera (properly diluted) are incubated in a vessel containing a multiplexed mixture Bead Suspension. The Bead Suspension contains a mixture of distinguishable sets of polystyrene microspheres (beads); each set conjugated with a different antigen. If present in patient sera, specific antibodies will bind to the immobilized antigen on one or more of the bead sets. The microspheres are rinsed to remove non-reactive serum proteins.
2. Phycoerythrin-conjugated goat anti-human IgG is added to the vessel and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The Bead Suspension is then analyzed by the **AtheNA Multi-Lyte** instrument. The bead set(s) are sorted (identified) and the amount of reporter molecule (PE conjugate) is determined for each bead set. Using the *Intra-Well Calibration Technology®*, internal calibration bead sets are used to convert raw fluorescence into outcome (units).

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Bead Suspension, Controls, Conjugate and SAVe Diluent®.**

SOLN	BEAD

1. Bead Suspension: Contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with the following autoantigens: SSA-52, SSA-60, SSB, Sm, U snRNP B/B', U1 snRNP 68, U1 snRNP A, U1 snRNP C, Scl-70, Jo-1, Centromere B, dsDNA, and Ribosomal P. The Bead Suspension also contains one bead set designed to detect non-specific antibodies in the patient sample (if present) and four separate bead sets used for assay calibration. One, amber bottle containing 5.5mL. Ready to use.

CONJ

2. Conjugate: Phycoerythrin conjugated goat anti-human IgG (γ chain specific). One, amber bottle containing 15mL. Ready to use.

CONTROL	+	1

3. Positive Control 1 (Human Serum): One, red-capped vial containing 0.2mL.

CONTROL	+	2

4. Positive Control 2 (Human Serum): One, white-capped vial containing 0.2mL.

CONTROL	+	3

5. Positive Control 3 (Human Serum): One, blue-capped vial containing 0.2mL.

CONTROL	-

6. Negative Control (Human Serum): One, green-capped vial containing 0.2mL.

DIL	SPE

7. SAVe Diluent®: One, green-capped bottle containing 50mL of phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**

WASHBUF	10X

8. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, clear-capped bottle containing 50mL of 10X concentrated phosphate-buffered-saline.

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS AtheNA Multi-Lyte Test Systems: Wash Buffer and SAVe Diluent®**
2. **Test System also contains:**
   a. **Component Label containing lot specific information inside the Test System box.**

b. **Calibration CD containing lot specific kit calibration values required for specimen analysis and assay quality control, and Package Inserts.**
c. **One 96-well dilution plate**.
d. **One 96-well filter plate.**

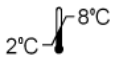
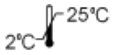
PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The ZEUS **AtheNA Multi-Lyte** Test System Bead Suspension does not contain viable organisms. However, the reagent should be considered **potentially biohazardous materials** and handled accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (1).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Bead Suspension, Controls, and Conjugate contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
9. Dilution or adulteration of these reagents may generate erroneous results.
10. Do not use reagents from other sources or manufacturers.
11. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
12. Avoid microbial contamination of reagents. Incorrect results may occur.
13. Cross contamination of reagents and/or samples could cause erroneous results.
14. Avoid splashing or generation of aerosols.
15. Do not expose reagents to strong light during storage or incubation. The Bead Suspension and Conjugate are light sensitive reagents. Both have been packaged in light protective containers. Normal exposures experienced during the course of performing the assay will not affect assay performance. Do not expose these reagents to strong sources of visible light unnecessarily.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
18. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
19. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes capable of accurately delivering 10 - 200µL.
2. Multichannel pipette capable of accurately delivering 10 - 200µL.
3. Reagent reservoirs for multichannel pipettes.
4. Serological pipettes.
5. Disposable pipette tips.
6. Paper towels.
7. Laboratory timer to monitor incubation steps.
8. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).
9. **AtheNA Multi-Lyte** System (Luminex® Instrument) with Sheath Fluid (Product Number 40-50035).
10. Distilled or deionized water.
11. Vortex.
12. Small Bath Sonicator.
13. Plate shaker capable of shaking at 800 RPM (optional for mixing).
14. Vacuum aspirator and vacuum manifold for washing the microspheres.

STORAGE CONDITIONS

	Bead Suspension: Remove only the required amount to analyze the specimens to be tested and return the unused portion to storage.
	Conjugate: DO NOT FREEZE.
	Unopened Test System, Positive Controls, Negative Control, SAVe Diluent®
	Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. Wash Buffer (10X): 2 - 25°C

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (2, 3). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (4).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).

2. Determine the total number of Controls and samples to be tested. It is necessary to include the Negative Control and the three Positive Controls with each run. The Negative Control should be tested in well A1, Positive Control 1 in well B1, Positive Control 2 in well C1 and Positive Control 3 in well D1. Each Control and sample requires one microwell for processing.
   a. To optimize read times, the Bead Suspension must be thoroughly mixed just prior to use. The most effective for re-suspension is to first vortex for approximately 30 seconds followed by sonication for approximately 30 seconds in a small bath sonicator.
   b. For proper performance, it is important that the contents of the assay are thoroughly mixed. Suitable means of mixing include mixing the plate on a plate shaker for approximately 30 seconds at approximately 800 RPMs or to set a pipettor to roughly ½ of the volume in the plate and repeatedly aspirate and expel (pump up and down) the contents of the well for a minimum of 5 cycles.

EXAMPLE PLATE SET-UP		
	1	2
A	Negative Control	Etc.
B	Positive Control 1	
C	Positive Control 2	
D	Positive Control 3	
E	Patient 1	
F	Patient 2	
G	Patient 3	
H	Patient 4	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Positive Controls, and each patient serum. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.** For proper performance, it is important that the sample dilutions are thoroughly mixed according to 2b above.
4. After determining the total number of wells to process, use a multichannel or a repeating pipette to dispense 50µL of the Bead Suspension into each of the wells of the filtration plate.
5. Transfer 10µL of each diluted sample (1:21) and Control from the dilution plate to the filtration plate. For proper performance, it is important that the sample dilution and Bead Suspension are thoroughly mixed according to 2b above.
6. Incubate the plate at room temperature (20 - 25°C) for 30 ± 10 minutes.
7. After the incubation, rinse the Beads by vacuum filtration using the supplied Wash Buffer diluted to the 1X concentration.
   a. Place the filtration plate on the vacuum manifold and remove the solution, leaving the beads behind.
   b. Turn off the vacuum and add 200µL of 1X Wash Buffer.
   c. Apply the vacuum and remove the solution.
   d. Repeat steps 7b and 7c for a total of three rinses.
8. Following the final wash, gently blot the bottom of the filter plate and allow the plate to air dry for 3 - 5 minutes before proceeding to the next step.
9. Add 150µL of the Conjugate to each well, at the same rate and same order as the specimens. For proper performance, it is important that the Conjugate and Bead Suspension are thoroughly mixed according to 2b above. As an option, while mixing the Conjugate one may transfer the mixture to empty wells of a polystyrene reaction plate.
10. Incubate the plate at room temperature (20 - 25°C) for 30 ± 10 minutes.
11. Set the **AtheNA Multi-Lyte** instrument to analyze the reactions by selecting the ANA-III Plus template. Refer to the operators manual for details regarding the operation of the **AtheNA Multi-Lyte** instrument. Results may be read from the filter plate or a reaction plate. **NOTE: For proper specimen analysis, it is important that the instrument is set-up, calibrated and maintained according to the manufacturer's instructions.** Please review the instrument manual for instrument preparation prior to reading the assay results.
12. The plate should be read within 60 minutes after the completion of the Conjugate incubation. One may decide to shake the plate for approximately 15 seconds prior to reading. This optional step may reduce the amount of time required to read the plate.

Step	Abbreviated Assay Procedure
1	Dilute specimens 1:21 in SAVe Diluent®. Mix well.
2	Combine 50µL of Bead Suspension and 10µL of diluted specimen in an empty well. Mix well.
3	Incubate at room temperature for 30 ± 10 minutes.
4	Rinse the microspheres 3 times with 200µL of 1X Wash Buffer.
5	Gently blot the bottom of the plate and air dry for 3 - 5 minutes.
6	Add 150µL of Conjugate to each well. Mix well.
7	Transfer to a reaction plate (optional).
8	Incubate at room temperature for 30 ± 10 minutes
9	Shake plate (optional).
10	Read results within 60 minutes.

QUALITY CONTROL

1. Each time the assay is run it is necessary to include the Negative Control (in well A1) and the three Positive Controls (in wells B1 through D1).
2. Run validity is determined through the performance of the Positive and Negative Controls. These criteria are analyzed automatically through *Intra-Well Calibration Technology*.
   a. The Negative Control and the three Positive Controls must all be negative on the non-specific or control antigen bead.
   b. The Negative Control must be negative for each and every analyte included in the Bead Suspension.
   c. Each Positive Control must be positive for a predetermined group of analytes included in the Bead Suspension. Each Positive Control must result in a positive ANA qualitative outcome. In addition to the qualitative outcome, each Positive Control must meet the predetermined ranges for activity. As a group, each analyte-specific bead set is controlled with the group of three Positive Controls.These ranges are encoded within the Calibration CD.
   d. If any of the above criteria are not met, the entire run will be considered invalid and should be repeated.
3. Specimen validity is based upon the characteristics of the calibration beads and their interactions with the patient sera. There are various parameters monitored automatically through *Intra-Well Calibration Technology*. If any of the criteria are found to be out of specification, the patient's results are considered invalid and should be repeated. Should this occur, the data report will indicate the particular specimen which has been invalidated as well as a trouble shooting code.
4. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. External Controls must be representative of normal human serum since ZEUS **AtheNA Multi-Lyte's** calibration system is partially based upon the characteristics of the serum sample. If the specimen formulation is artificial (not human serum), erroneous results may occur.
5. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations**
   a. Assay Calibration: The ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System utilizes *Intra-Well Calibration Technology*. *Intra-Well Calibration Technology*

includes a multi-point standard curve within the Bead Suspension. With *Intra-Well Calibration Technology*, each well of the assay is calibrated internally without any user intervention. The standard curve is designed to self-adjust based upon the unique characteristics of the patient or Control serum. Calibrator values are assigned to the internal standards by ZEUS, are lot specific and are encoded within the lot specific Calibration CD.

b. Analyte Cutoff Values: Each analyte of the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System has an assigned cutoff value. Cutoff values are determined by ZEUS for each test system lot, and are encoded within the lot specific Calibration CD.

c. Through *Intra-Well Calibration Technology*, all calculations are performed automatically when using the ZEUS **AtheNA Multi-Lyte** system. *Intra-Well Calibration Technology* performs a regression analysis of the internal standards and then adjusts the calculated unit values based upon an additional standard and the characteristics of the serum sample.

2. **Interpretations:**

a. **Individual ANA Analyte Interpretation**: The ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System is capable of generating up to ten individual assay results for each patient specimen tested. Individual test results that may be reported are: SSA-52, SSA-60, SSB, Sm, RNP, Scl-70, Jo-1, Centromere B, Ribosomal P, and dsDNA. Specimen unit values for each of the multiplexed analytes are interpreted as follows:

Negative Specimens	< 100
Positive Specimens	> 120
Equivocal Specimens	100 – 120

Unit values reported are IU/mL for dsDNA and AU/mL for the remaining analytes, as indicated on the ZEUS **AtheNA Multi-Lyte** Test System results printout.

b. **Qualitative ANA Interpretation**: The ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System is capable of generating a qualitative ANA determination. The qualitative ANA determination is based upon the individual determinations listed in 2a above. The qualitative ANA interpretation is determined as follows:

i. Specimens that are positive (>120) for one or more of the ten analytes are considered ANA positive.

ii. Specimens that are negative (< 100) for all ten analytes are considered negative for ANA.

iii. Specimens that are equivocal (100 - 120) for one or more analytes, and are negative for the remainder of the analytes are considered equivocal or borderline for ANA. Borderline specimens may be repeated in duplicate or evaluated using an alternative serologic procedure to determine their ANA reactivity.

LIMITATIONS OF THE ASSAY

1. The ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Positive ANA may be found in apparently healthy people. It is therefore imperative that the results be interpreted in light of the patient's clinical picture by a medical authority.
3. SLE patients undergoing steroid therapy may have negative test results.
4. Many commonly prescribed drugs may induce ANA.
5. Hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Additionally, specimens with abnormal IgG concentrations may interfere with the outcome of this assay. Use of these types of specimens should be avoided.

EXPECTED RESULTS

1. **SSB, Sm, RNP, Scl-70, Jo-1, DNA and Centromere Assays**

The clinical investigation of the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System included a total of 546 specimens from three major categories; normal blood donors (n=161), specimens previously characterized for autoantibody reactivity (n=350) and clinical/patient samples (n=35) from those individuals who were seeing a physician as a result of a rheumatologic disorder. The clinical samples included five each of the following disease states: Crest Syndrome, Drug-Induced Lupus, Mixed Connective Tissue Disease, Myositis, Scleroderma, Systemic Lupus and Sjogren's Syndrome. The percentage positive, negative and equivocal for each assay for each specimen group appears below in Table 1.

Table 1: Expected Outcome for SSB, Sm, RNP, Scl-70, Jo-1, DNA and Centromere

Number of Positive, Negative and Equivocal Specimens Results per Group									
	Normals (n=161)			**Characterized (n=350)**			**Clinical (n=35)**		
	Positive	**Negative**	**Equivocal**	**Positive**	**Negative**	**Equivocal**	**Positive**	**Negative**	**Equivocal**
SSB	0	160	1	83	263	4	6	29	0
Sm	0	161	0	56	290	4	4	31	0
RNP	2	158	1	83	264	3	7	28	0
Scl-70	1	160	0	42	305	3	3	31	1
Jo-1	0	160	1	45	304	1	5	30	0
dsDNA	0	161	0	48	297	5	1	34	0
Centromere	2	158	1	33	313	4	4	31	0
Percentatge of Population Size Outcome									
	Normals (n=161)			**Characterized (n=350)**			**Clinical (n=35)**		
	% Positive	**% Negative**	**% Equivocal**	**% Positive**	**% Negative**	**% Equivocal**	**% Positive**	**% Negative**	**% Equivocal**
SSB	0.0	99.4	0.6	23.7	75.1	1.1	17.1	82.9	0.0
Sm	0.0	100.00	0.0	16.0	82.9	1.1	11.4	88.6	0.0
RNP	1.2	98.1	0.6	23.7	75.4	0.9	20.0	80.0	0.0
Scl-70	0.6	99.4	0.0	12.0	87.1	0.9	8.6	88.6	2.9
Jo-1	0.0	99.4	0.6	12.9	86.9	0.3	14.3	85.7	0.0
dsDNA	0.0	100.0	0.0	13.7	84.9	1.4	2.9	97.1	0.0
Centromere	1.2	98.1	0.6	9.4	89.4	1.1	11.4	88.6	0.0

2. **SSA 52, SSA 60 and Ribosomal P Assays**

The clinical investigation of the **AtheNA Multi-Lyte** ANA-III Plus Test System included specimens from two major categories; clinical/patient samples from those individuals who were seeing a physician as a result of a rheumatologic disorder and routine specimens submitted to the laboratory for ANA testing. The clinical/patient samples included patients from the following disease states: Crest Syndrome, Drug-Induced Lupus, Mixed Connective Tissue Disease, Myositis, Scleroderma, Systemic Lupus and Sjogren's Syndrome. The percentage positive, negative and equivocal for each assay for each specimen group appears below in Table 2.

Table 2: Expected Outcome for SSA 52, SSA 60 and Ribosomal P

Number of Positive, Negative and Equivocal Specimens Results per Group								
	Clinical				Routine			
	Number Tested	Positive	Negative	Equivocal	Number Tested	Positive	Negative	Equivocal
SSA 52	26	14	10	2	53	2	51	0
SSA 60	26	15	10	1	53	2	50	1
Ribosomal P	22	6	16	0	55	0	55	0

Percentatge of Population Size Outcome						
	Characterized (n=350)			Clinical (n=35)		
	% Positive	% Negative	% Equivocal	% Positive	% Negative	% Equivocal
SSA 52	53.8	38.5	7.7	3.8	96.2	0.0
SSA 60	57.7	38.5	3.8	3.8	94.3	1.9
Ribosomal P	27.3	72.7	0.0	0.00	100.0	0.0

PERFORMANCE CHARACTERISTICS

1. **SSB, Sm, RNP, Scl-70, Jo-1, DNA and Centromere Assays**

a. **Comparative Study**

A comparative study was conducted to demonstrate the performance characteristics of the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System, relative to the ZEUS **AtheNA Multi-Lyte** ANA Test System. A total of 546 specimens were tested. The specimen types and numbers tested are described in Table 3.

Table 3: Specimen Types included in the Comparative Study

Type	Quantity	Description
Normal Blood Donor	161	Random specimens purchased from various commercial sources.
Characterized Samples	350	These specimens were acquired from numerous commercial sources. They were previously characterized regarding their autoantibody reactivity (or lack of) using a multitude of other methodologies. Positive specimens were more than likely acquired from diseased patients.
Clinical Samples	5 – Crest Syndrome 5 – Drug Induced Lupus 5 – MCTD 5 – Myositis 5 – SLE 5 – Scleroderma 5 – Sjogren's Syndrome	These specimens were purchased from a commercial source as having been diagnosed patients according to the categories listed.

Each of the above specimens were tested on both the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System and the ZEUS **AtheNA Multi-Lyte** ANA Test System. The result of this side by side study were used to calculate the relative sensitivity, relative specificity and relative agreement for each assay of the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System compared to the reference assay. The results of this comparative study have been summarized in Table 4 below:

Table 4: Performance of ZEUS AtheNA Multi-Lyte ANA-III Plus Test System Relative to the ZEUS AtheNA Multi-Lyte ANA Test System

Marker	Relative Sensitivity (%)	Relative Specificity (%)	Relative Agreement (%)
SSB	100	95.2	95.7
Sm	83.3	98.7	97.0
RNP	98.1	93.7	94.2
Scl-70	95.2	98.8	98.5
Jo-1	100	99.4	99.4
dsDNA	88.6	97.8	97.2
Centromere	96.2	97.5	97.4

Clinical Specificity of the ZEUS AtheNA Multi-Lyte ANA-III Plus Test System

Clinical specificity of the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System was evaluated using 161 normal blood donors since it was presumed that such a group should be free of autoimmune disease. Of the 161 specimens tested, seven were positive for one or more markers, three were equivocal for one or more markers (and not positive for any markers) and 151 were negative for all 10 assay results. For the individual test systems, the clinical specificity of the ZEUS **AtheNA Multi-Lyte** ANA-III Plus tests ranged from 98.1% to 100%.

Clinical Sensitivity of the ZEUS AtheNA Multi-Lyte ANA-III Plus Test System.

The comparative study include 35 clinically characterized (diagnosed) patient specimens. Of the 35 specimens tested, 26 (74.3%) were positive for one or more markers in the **AtheNA Multi-Lyte** ANA-III Plus Test System. The outcome of the 35 samples is shown in Table 5 below.

Table 5: Results for the 35 Clinical Specimens

Disease	SSB	Sm	RNP	Scl-70	J0-1	DNA	Cent
Crest1	8	1	9	7	6	24	270
Crest2	3	5	9	4	8	15	394
Crest3	3	1	3	2	6	12	4
Crest4	3	3	4	3	6	11	352
Crest5	2	14	13	6	5	15	511
DIL1	3	4	13	10	20	44	13
DIL2	10	4	13	12	23	30	12
DIL3	189	5	7	10	8	16	9
DIL4	10	89	50	9	28	30	19
DIL5	5	3	6	3	5	31	5
MCTD 1	3	24	853	8	8	28	10
MCTD 2	443	49	329	9	25	40	20
MCTD 3	6	14	188	6	12	26	12
MCTD 4	4	164	915	5	9	20	10
MCTD 5	3	51	69	10	7	177	10

Table 5 (continued): Results for the 35 Clinical Specimens

Disease	SSB	Sm	RNP	Scl-70	J0-1	DNA	Cent
Myositis 1	35	2	11	5	772	19	11
Myositis 2	11	4	60	9	1024	22	31
Myositis 3	2	4	5	7	1293	18	7
Myositis 4	3	10	9	9	1048	18	3
Myositis 5	4	4	6	5	946	13	8
Scleroderma 1	3	3	2	103	5	17	7
Scleroderma 2	2	2	4	145	5	20	9
Scleroderma 3	5	2	3	152	6	13	8
Scleroderma 4	2	3	4	88	3	12	6
Scleroderma 5	2	4	65	549	14	41	28
Lupus 1	39	301	432	4	4	21	5
Lupus 2	4	284	216	18	3	51	4
Lupus 3	44	9	657	4	5	36	11
Lupus 4	2	275	23	11	5	12	3
Lupus 5	3	22	23	10	6	12	3
Sjogrens 1	713	1	7	3	8	17	11
Sjogrens 2	186	2	1	6	21	10	3
Sjogrens 3	786	2	13	5	12	18	12
Sjogrens 4	732	5	8	11	8	17	9
Sjogrens 5	9	4	10	8	25	17	16

b. **Reproducibility**

A precision study was conducted to evaluate the precision of the ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System. The study was conducted as follows: Eight specimens were tested. Each of the eight specimens was diluted two times and each dilution plated four times for a total of eight replicates for each specimen. This protocol was performed three times, resulting in 24 results for each of the eight specimens. The 24 results for each specimen were used to calculate the mean result, the standard deviation and the percent CV. The results of this study are depicted in Table 6 below:

Table 6: Precision

Sample ID	All Runs	SSB	Sm	RNP	Scl-70	Jo-1	dsDNA	CentB
Sample 1	Mean	4	3	7	4	9	17	10
	StD	0.88	0.97	1.28	0.90	1.85	2.61	1.71
	%CV	22.1	28.7	17.3	21.8	21.3	15.4	17.8
Sample 2	Mean	726	555	190	9	10	20	11
	StD	61.61	55.10	28.25	1.75	1.83	4.39	2.07
	%CV	8.5	9.9	14.9	19.7	18.0	21.8	18.6
Sample 3	Mean	494	35	71	631	1525	663	566
	StD	49.53	5.93	10.02	48.35	113.01	107.69	59.80
	%CV	10.0	16.8	14.2	7.7	7.4	16.3	10.6
Sample 4	Mean	5	29	863	10	20	79	30
	StD	1.18	7.06	146.16	2.98	5.51	21.75	5.28
	%CV	23.6	24.8	16.9	31.3	27.4	27.6	17.6
Sample 5	Mean	8	3	6	4	8	13	6
	StD	1.63	0.83	1.25	1.01	2.51	2.74	1.28
	%CV	21.3	29.8	22.6	26.3	30.9	21.0	21.7
Sample 6	Mean	14	14	212	598	1505	78	784
	StD	4.62	4.52	47.89	72.60	178.50	15.03	106.30
	%CV	32.6	31.4	22.6	12.1	11.9	19.2	13.6
Sample 7	Mean	16	29	732	8	1551	57	22
	StD	3.21	3.97	83.25	1.67	120.45	9.71	4.02
	%CV	20.2	13.7	11.4	20.6	7.8	17.0	18.1
Sample 8	Mean	687	303	84	37	19	223	19
	StD	52.09	35.08	13.28	5.96	3.22	35.94	3.40
	%CV	7.6	11.6	15.8	15.9	16.9	16.1	18.2

c. **Interfering Substances**

The ZEUS **AtheNA Multi-Lyte** ANA-III Plus Test System was evaluated for potential interference from serum components. For this study, a total of 20 specimens were evaluated both by ZEUS **AtheNA Multi-Lyte** and by ELISA. These 20 specimens contained either abnormal levels of triglycerides, (n=5), above normal IgG levels (n=5), above normal bilirubin concentration (n=5) or above normal hemolysis levels (n=5). Of the twenty samples tested, one of the 20 showed a positive result.

Table 7: Summary of Interfering Substances

Sample ID	Qualitative Outcome	SSB	Sm	RNP	Scl-70	Jo-1	dsDNA	CentB
Triglyceride 1	Negative	5	7	10	31	14	22	8
Triglyceride 2	Negative	6	5	8	14	15	23	9
Triglyceride 3	Negative	4	7	10	16	16	18	8
Triglyceride 4	Negative	8	6	13	14	17	21	10
Triglyceride 5	Negative	5	7	10	21	15	19	8
Hemglobin 1	Negative	8	8	24	79	30	18	15
Hemglobin 2	Negative	9	9	17	23	25	25	11
Hemglobin 3	Negative	7	7	15	75	21	19	7
Hemglobin 4	Negative	54	20	30	25	46	66	18
Hemglobin 5	Negative	6	7	10	13	17	24	8
IgG+ 1	Negative	22	8	36	46	95	47	21
IgG+ 2	Negative	11	6	35	18	45	40	17
IgG+ 3	Negative	11	7	21	16	20	38	18
IgG+ 4	Positive	11	7	15	175	20	46	19
IgG+ 5	Negative	9	5	15	40	19	54	19
Bilirubin 1	Negative	5	8	19	57	18	34	18
Bilirubin 2	Negative	6	15	33	81	26	27	19
Bilirubin 3	Negative	5	5	8	19	18	16	8
Bilirubin 4	Negative	5	4	5	13	12	16	10
Bilirubin 5	Negative	6	6	10	17	18	24	18

2. **SSA 52, SSA 60 and Ribosomal P Assays**

a. **Comparative Study**

A comparative study was conducted to demonstrate the performance characteristics of the **AtheNA Multi-Lyte** ANA-III Plus Test System, relative to commercial ELISA Test Systems. Each of the specimens were tested on both the **AtheNA Multi-Lyte** ANA-III Plus Test System and the respective ELISA test system. The result of this side by side study were used to calculate the relative sensitivity, relative specificity and relative agreement for each assay of the **AtheNA Multi-Lyte** ANA-III Plus Test System compared to the reference assay. The results of this comparative study are summarized in Table 8.

Table 8: Performance of AtheNA Multi-Lyte ANA-III Plus Test System Relative to ELISA

Marker	Relative Sensitivity (%)	Relative Specificity (%)	Relative Agreement (%)
SSA 52	100	95.3	96.0
SSA 60	100	96.7	97.4
Ribosomal P	54.6	100	93.5

b. **Reproducibility**

A precision study was conducted to evaluate the precision of the **AtheNA Multi-Lyte** ANA-III Plus Test System. The study was conducted as follows: six specimens were tested. Each of the six specimens were diluted two times and each dilution plated four times for a total of eight replicates for each specimen. This protocol was performed three times, resulting in 24 results for each of the eight specimens. The 24 results for each specimen were used to calculate the mean result, the standard deviation and the percent CV. The results of this study are depicted in Table 9.

Table 9: Precision

		Intra-Assay Precision Summary									Inter-Assay Precision Summary		
		Ribosomal P			SSA 52			SSA 60					
Sample ID		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Ribo P	SSA 52	SSA 60
Sample 1	Mean	692.6	658.0	719.9	344.3	348.6	334.4	503.8	505.4	493.1	690.2	342.4	500.8
	StD	38.6	37.8	31.5	13.5	10.8	15.6	15.1	20.5	24.0	43.1	14.2	20.1
	%CV	5.6	5.7	4.4	3.9	3.1	4.7	3.0	4.1	4.9	6.2	4.2	4.0
Sample 2	Mean	913.9	904.8	914.9	289.5	304.8	301.4	499.3	494.9	498.0	911.2	298.5	497.4
	StD	32.3	34.2	53.8	6.6	6.6	14.9	30.3	33.2	16.6	39.7	11.8	26.5
	%CV	3.5	3.8	5.9	2.3	2.2	4.9	6.1	6.7	3.3	4.4	3.9	5.3
Sample 3	Mean	2.0	4.3	1.5	32.4	27.9	27.3	26.9	57.3	30.4	2.6	29.2	38.2
	StD	1.3	2.0	1.6	1.8	1.9	1.8	2.5	2.8	1.7	2.0	2.9	14.0
	%CV	65.5	46.6	106.9	5.7	6.8	6.4	9.4	4.9	5.5	77.3	10.0	36.8
Sample 4	Mean	3.5	5.0	1.8	35.3	30.3	30.3	24.5	54.8	27.8	3.4	31.9	35.7
	StD	2.1	2.3	3.5	2.0	2.1	1.8	1.8	3.6	3.1	2.9	3.0	14.1
	%CV	59.1	45.4	197.4	5.6	6.8	6.1	7.2	6.5	11.2	84.5	9.6	39.6
Sample 5	Mean	280.5	267.3	303.4	134.8	136.9	128.4	126.5	162.6	149.4	283.7	133.3	145.9
	StD	16.2	16.2	25.2	4.8	9.2	5.0	6.4	10.9	10.7	24.2	7.3	18.1
	%CV	5.8	6.1	8.3	3.5	6.7	3.9	5.1	6.7	7.2	8.5	5.5	12.4
Sample 6	Mean	250.8	249.1	276.6	156.4	160.1	156.4	126.9	169.5	156.4	258.8	157.6	150.9
	StD	9.7	17.6	21.6	7.0	7.2	7.2	2.7	8.9	6.6	20.7	7.0	19.3
	%CV	3.9	7.1	7.8	4.5	4.5	4.6	2.1	5.2	4.2	8.0	4.5	12.8

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