TPO/Tg Plus Test System

REF A51101

IVD

CE

Rx Only

INTENDED USE

The ZEUS **AtheNA Multi-Lyte®** TPO/Tg Plus Test System is intended for the quantitative detection of IgG class antibody to 2 separate Thyroid Antigens; Thyroid Peroxidase (TPO) and Thyroglobulin (Tg), in human serum. The test system is intended to be used as an aid in the diagnosis of various autoimmune thyroid diseases. This test is for *In Vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Thyroid antibodies are a characteristic finding in patients with Hashimoto's and Graves' diseases (1). The presence of thyroid antibodies in the sera of 80% of patients with these two diseases led to the recommendation that some type of thyroid antibody testing be a feature of the work-up of any patient with a goiter (1). Although thyroid antibodies are predominantly associated with Hashimoto's or Graves' diseases, they may be found in the sera of patients with other diseases such as myxedma, granulomatous thyroiditis, nontoxic nodular goiter, and thyroid carcinoma (1). Thyroid antibodies are also found in most cases of lymphocytic thyroiditis in children (2), and rarely in patients with pernicious anemia and Sjögren's Syndrome (3 - 4).

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** TPO/Tg Plus Test System is designed to detect IgG class antibodies in human sera to TPO and Tg. 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 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 beads 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 antigens: Thyroid Peroxidase (TPO) and Thyroglobulin (Tg). 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	+

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

CONTROL	-

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

DIL	SPE

5. 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

6. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, clear-capped bottle containing 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 **AtheNA Multi-Lyte** 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 (5).
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

2°C – 8°C	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 Control, Negative Control, SAVe Diluent®
2°C – 25°C	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. Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera (6, 7).
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 (8).

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 and Positive Control with each run. The Negative Control should be tested in well A1 and Positive Control in well B1. 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	
C	Patient 1	
D	Patient 2	
E	Patient 3	
F	Patient 4	
G	Patient 5	
H	Patient 6	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Positive Control, 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 TPO/Tg 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 Positive Control (in well B1).
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 and 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. The Positive Control must be positive for both TPO and Tg and must meet the lot specific ranges for these 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. **Do not report the patient results.**
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. If a specimen is repeatedly invalid, it must be tested using an alternate methodology since it is incompatible with the **AtheNA Multi-Lyte®** Plus Test System.
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 **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. Good laboratory practice recommends the use of positive and negative controls to assure functionality of reagents and proper performance of the assay procedure. Quality control requirements must be performed in conformance with local, state and/or federal regulations or accreditation requirements and the user's laboratory standard Quality Control procedures. It is recommended that the user refer to CLSI EP12-A and 42 CFR 493.1256 for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations**
   a. Assay Calibration: The ZEUS **AtheNA Multi-Lyte** TPO/Tg 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** TPO/Tg 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 **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. **Cutoff Determination:** The cut off for each assay was established using a negative population for each marker. The **AtheNA Multi-Lyte** results were determined for this population, and the cut off was set at approximately the mean plus three times the standard deviation. Based upon the results of this testing, the manufacturer has established the following guidelines for interpretation of patient samples.
   b. **Individual anti-Thyroid Analyte Interpretation**: Specimen unit values for each of the multiplexed analytes are interpreted as follows:

	Unit Value
Negative Specimens	< 100 IU/mL
Positive Specimens	> 120 IU/mL
Equivocal Specimens	100 to 120 IU/mL

LIMITATIONS OF THE ASSAY

1. The ZEUS **AtheNA Multi-Lyte** TPO/Tg 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. 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

The clinical investigation included 150 specimens from normal blood donors. It is presumed that this group should in large part be disease free and therefore display a low incidence of both TPO and Tg autoantibody. The investigation also included 300 specimens from patients all diagnosed with an autoantibody disorder associated with anti-thyroid antibody. With respect to the TPO assay, in the normal blood donor group, three of the specimens were invalid on the assay, reducing the total to 147 normal specimens. Of the 147 remaining specimens, 137/147 (93.2%) were negative, 3/147 (2.0%) were equivocal and 7/147 (4.8%) were positive. The numerical results of this population ranged from a low of 4 IU/mL to a high of 1183 IU/mL with a mean result of 53 IU/mL and a median result of 15 IU/mL. In the clinical specimens (those diagnosed with a thyroid autoimmune disorder), 0/300 (0%) were negative, 0/300 (0%) were equivocal and 300/300 (100%) were positive. The numerical results of this population ranged from a low of 643 IU/mL to a high of 1207 IU/mL with a mean result of 972 IU/mL and a median result of 978 IU/mL. With respect to the Tg assay, in the normal blood donor group, three of the specimens were invalid on the assay, reducing the total to 147 normal specimens. Of the 147 remaining specimens, 133/147 (90.5%) were negative, 3/147 (2.0%) were equivocal and 11/147 (7.5%) were positive. The numerical results of this population ranged from a low of 15 IU/mL to a high of 976 IU/mL with a mean result of 78 IU/mL and a median result of 44 IU/mL. In the clinical specimens (those diagnosed with a thyroid autoimmune disorder), 0/300 (0%) were negative, 0/300 (0%) were equivocal and 300/300 (100%) were positive. The numerical results of this population ranged from a low of 428 IU/mL to a high of 1968 IU/mL with a mean result of 1153 IU/mL and a median result of 1188 IU/mL. Both the TPO and Tg assays have been calibrated to standards provided by the World Health Organization (WHO). TPO has been calibrated to WHO 66/387 and Tg has been calibrated to WHO 65/93. Using those standards, each with an expected outcome of 1000 IU/mL, the **AtheNA Multi-Lyte** TPO assay yielded a result of 1080 IU/mL and the **AtheNA Multi-Lyte** Tg assay yielded a result of 1126 IU/mL.

PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

An in-house comparative study was performed to demonstrate the equivalence of the ZEUS **AtheNA Multi-Lyte** TPO/Tg Plus Test System to commercially available ELISA test systems. Performance was evaluated using 750 specimens; 150 normal donor sera, 300 specimens previously sent to a lab for routine thyroid autoantibody testing, and 300 disease-state specimens from clinically diagnosed patients with thyroid autoimmune disorders. The results of the investigation have been summarized in Tables 1 and 2 below.

Table 1: Performance of the ZEUS AtheNA Multi-Lyte TPO Plus Test System Relative to the TPO IgG ELISA

		ELISA Results			
		Positive	Negative	Equivocal**	Total:
AtheNA Results	Positive	531	31	33	595
	Negative	0	143	0	143
	Equivocal**	0	7	0	7
	Total:	531	181	33	745*

Relative Sensitivity = 531/531 = 100% Relative Specificity = 143/174 = 82.2% Relative Agreement = 674/705 = 95.6%

* Five samples were invalid by AtheNA. Their results were excluded from calculations of relative sensitivity, specificity and agreement.

** Equivocal samples were excluded from calculations of relative sensitivity, specificity and agreement. The notable differences in the number of equivocal samples between ELISA and AtheNA is attributed to the difference in AtheNA and ELISA methodologies. AtheNA exhibits greater signal-to-noise performance relative to ELISA and can therefore better discriminate negative from positive specimens while minimizing the number of specimens falling into the equivocal zone.

Clinical Sensitivity of the ZEUS AtheNA Multi-Lyte TPO Plus Test System

Clinical sensitivity of the ZEUS **AtheNA Multi-Lyte** TPO Plus Test System was evaluated using 300 clinically defined serum samples from patients diagnosed with an autoimmune thyroid disorder. Using this group, all three hundred were positive for TPO IgG antibody. The clinical sensitivity of the AtheNA Multi-Lyte TPO IgG test system was therefore determined to be 300/300 or 100%.

Clinical Specificity of ZEUS AtheNA Multi-Lyte TPO Plus Test System

Clinical specificity of the ZEUS **AtheNA Multi-Lyte** TPO Plus Test System was evaluated using 150 normal blood donors since it was presumed that such a group should be free of autoimmue disease. Three of these specimens yielded invalid results by AtheNA leaving 147 specimens. Of the remaining 147 specimens, 7/147 were AtheNA positive, 3/147 were AtheNA equivocal and 137/147 were AtheNA negative. The clinical specificity of the ZEUS **AtheNA Multi-Lyte** TPO Plus Test System was therefore determined to be 137/147 or 93.2%. Expressed as a 95% confidence interval, the clinical specificity was determined to be 89.1 to 97.3%.

Table 2: Performance of the ZEUS AtheNA Multi-Lyte Tg Plus Test System Relative to the Tg IgG ELISA

		ELISA Results			
		Positive	Negative	Equivocal**	Total:
AtheNA Results	Positive	575	9	23	607
	Negative	0	135	0	135
	Equivocal**	0	3	0	3
	Total:	575	150	23	745*

Relative Sensitivity = 575/575 = 100% Relative Specificity = 135/144 = 93.8% Relative Agreement = 710/719 = 98.8%

* Five samples were invalid by AtheNA. Their results were excluded from calculations of relative sensitivity, specificity and agreement.

** Equivocal samples were excluded from calculations of relative sensitivity, specificity and agreement

Clinical Sensitivity of the ZEUS AtheNA Multi-Lyte Tg Plus Test System

Clinical sensitivity of the ZEUS **AtheNA Multi-Lyte** Tg Plus Test System was evaluated using 300 clinically defined serum samples from patients diagnosed with an autoimmune thyroid disorder. Using this group, all three hundred were positive for Tg IgG antibody. The clinical sensitivity of the ZEUS **AtheNA Multi-Lyte** Tg Plus Test System was therefore determined to be 300/300 or 100%.

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

Clinical specificity of the ZEUS **AtheNA Multi-Lyte** Tg Plus Test System was evaluated using 150 normal blood donors since it was presumed that such a group should be free of autoimmue disease. Three of these specimens yielded invalid results by AtheNA leaving 147 specimens. Of the remaining 147 specimens, 11/147 were AtheNA positive, 2/147 were AtheNA equivocal and 134/147 were AtheNA negative. The clinical specificity of the ZEUS **AtheNA Multi-Lyte** Tg Plus Test System was therefore determined to be 134/147 or 91.2%. Expressed as a 95% confidence interval, the clinical specificity was determined to be 86.6 to 95.7%

2. **Reproducibility**

An in-house evaluation of both intra-assay and inter-assay reproducibility was conducted. Six specimens were tested. On each day of testing, each sample was diluted twice and then loaded for four replicates resulting in a total of eight wells of each of the six samples. This protocol was followed for three days. These results were then used to calculate mean IU/mL values, standard deviations, and percent CV. Specimens were selected in such a way that resulted in two of them being clearly negative, two being clearly positive and two were selected that were weakly positive. The results of this study have been summarized in Table 3 and 4 below:

Table 3: ZEUS AtheNA Multi-Lyte TPO Plus Precision Study

Sample ID	Characteristic	Day 1 Results		Day 2 Results		Day 3 Results	
		Dilution 1	Dilution 2	Dilution 1	Dilution 2	Dilution 1	Dilution 2
1	Strong Positive	1229	1196	1202	1254	1224	1252
		1085	1223	1098	1164	1191	1118
		1170	1218	1189	1093	1191	1206
		1201	1174	1314	1136	1343	1174
2		1185	1134	1103	1122	1138	1117
		1126	1160	1140	1150	1175	1122
		1083	1079	1050	1087	1185	1164
		1149	1181	1055	1008	1060	11067
3	Positive	259	267	351	328	312	346
		249	266	261	295	362	360
		295	259	309	207	327	297
		297	289	313	296	314	310
4		266	296	310	235	317	346
		293	276	263	235	309	311
		270	269	283	253	295	322
		318	276	247	260	242	303
5	Negative	4	5	6	7	7	6
		4	5	6	6	5	6
		4	5	8	6	5	5
		3	4	6	7	6	7
6		6	5	6	7	8	8
		5	4	6	7	5	7
		5	5	6	4	6	6
		6	5	7	6	8	7

Sample ID	Calculation	Inter-Assay Precision			Inter-Assay Precision
		Day 1	Day 2	Day 3	
1	Mean	1187	1180	1212	193
	StD	46.53724	77.75603	65.57643	63.32340
	%CV	3.9	6.6	5.4	5.3
2	Mean	1137	1089	1129	1118
	StD	40.21882	49.05955	46.83405	48.40290
	%CV	3.5	4.5	4.2	4.3
3	Mean	273	295	329	299
	StD	18.39206	44.15233	24.61126	37.82509
	%CV	6.7	15.0	7.5	12.7
4	Mean	283	261	306	283
	StD	17.91249	25.35885	29.84693	30.24439
	%CV	6.3	9.7	9.8	10.7
5	Mean	4	7	6	6
	StD	0.707107	0.755929	0.834523	1.21509
	%CV	16.6	11.6	14.2	21.9
6	Mean	5	6	7	6
	StD	0.64087	0.991031	1.125992	1.16018
	%CV	12.5	16.2	16.4	19.2

Table 3: ZEUS AtheNA Multi-Lyte Tg Plus Precision Study

Sample ID	Characteristic	Day 1 Results		Day 2 Results		Day 3 Results	
		Dilution 1	Dilution 2	Dilution 1	Dilution 2	Dilution 1	Dilution 2
1	Strong Positive	2936	2685	2725	2864	3006	2931
		2557	2874	2798	2621	2543	2721
		2762	2921	2894	2644	2909	3109
		2958	2831	2938	2686	3155	2629
2		3007	2807	2714	2950	2935	2832
		2659	2869	2912	2931	2949	2852
		2808	2955	2741	2690	2942	2837
		2860	2894	2700	2598	2739	2700
3	Positive	294	328	378	342	201	233
		299	324	327	288	226	217
		291	286	331	270	204	207
		324	296	330	312	206	242
4		248	267	304	312	327	324
		287	262	282	263	290	340
		269	301	319	266	329	344
		283	267	184	268	326	307
5	Negative	21	20	18	19	23	24
		20	19	20	16	22	17
		20	18	19	16	19	24
		17	23	16	18	18	23
6		18	18	20	15	19	23
		19	20	18	17	19	20
		20	16	19	11	25	21
		14	18	16	18	19	14

Sample ID	Calculation	Inter-Assay Precision			Inter-Assay Precision
		Day 1	Day 2	Day 3	
1	Mean	2816	2771	2875	282
	StD	139.5575	119.7411	223.3076	165.43184
	%CV	5.0	4.3	7.8	5.9
2	Mean	2857	2780	2848	2828
	StD	105.3768	132.3545	93.18453	112.31470
	%CV	3.7	4.8	3.3	4.0
3	Mean	305	322	217	282
	StD	17.09428	33.09186	15.15633	52.08605
	%CV	5.6	10.3	7.0	18.5
4	Mean	273	275	323	290
	StD	16.53568	4.67736	17.46783	36.04323
	%CV	6.1	15.5	5.4	12.4
5	Mean	20	17	21	19
	StD	1.832251	1.846812	2.815772	2.66995
	%CV	9.3	10.6	13.3	13.7
6	Mean	18	17	20	18
	StD	2.03101	2.748376	3.251373	2.88424
	%CV	11.4	16.0	16.3	15.7

3. **Cross Reactivity and Interfering Substances**

The ZEUS **AtheNA Multi-Lyte** TPO/Tg Plus Test System was evaluated for potential cross reactivity to other antibodies and interference from serum components. For this study, a total of 39 specimens were evaluated. Nineteen of the specimens were positive for various autoimmune and infectious disease antibodies. Of the nineteen evaluated, one was reactive on the ZEUS **AtheNA Multi-Lyte** Tg Plus assay. The same sample was not reactive by ELISA. There were a total of 20 specimens evaluated that contained potentially interfering substances. These 20 specimens contained either abnormal levels of hemolysis, (n=5), bilirubin (n=5), above normal IgG concentration (n=5) or above normal lipid levels (n=5). Five of the specimens were positive using the ZEUS **AtheNA Multi-Lyte** TPO Plus Test System. Four of those five were also positive on the TPO ELISA. Four of the specimens were positive using the ZEUS **AtheNA Multi-Lyte** Tg Plus Test System. Two of the four were also positive on the Tg ELISA.

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