TPO/Tg Plus Test System

A Multiplexed, Microparticle-Based Immunoassay for detection of IgG Antibody to TPO and Tg Antigens

Product Number: A51101

INTENDED USE

The Zeus Scientific, Inc. **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 *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 AtheNA Multi-Lyte TPO/Tg Plus Test System.

The Zeus Scientific, Inc. **AtheNA Multi-Lyte** anti-Thyroid 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 of the bead suspension. The multiplexed bead suspension contains a mixture of distinguishable sets of polystyrene microspheres; 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 (γ chain specific) 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).

KIT COMPONENTS

Reactive Reagents: (All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% w/v.)

1. Multiplexed bead suspension. Ready to use, 5.5 mL bottle. The suspension contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with the following antigens: Thyroid Peroxidase (TPO) and Thyroglobulin (Tg). The bead mix 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.
2. Phycoerythrin conjugated goat anti-human IgG (γ chain specific). Ready to use, 15 mL amber bottle.
3. Human positive serum control. One, 0.2mL vial.
4. Human negative serum control. One, 0.2mL vial.
5. Sample diluent. One 50 mL bottle containing phosphate-buffered-saline. Ready to use. NOTE, the sample diluent will change color in the presence of serum.
6. Wash Buffer Concentrate: Dilute 1 part concentrate + 9 parts deionized or distilled water. One 50 mL bottle containing 10X concentrate of phosphate buffered saline.

Non-reactive Components:

1. One, 96-well filtration plate for rinsing the microspheres
2. Data Labels: One label is adhered to the inside lid of the kit box and a second label is inside the kit box.
3. Package Insert providing instructions for use
4. Calibration CD. A compact disc that includes all lot-specific kit calibration values required for specimen analysis and assay quality control.

PRECAUTIONS

Safety Precautions:

1. The Zeus Scientific, Inc. **AtheNA Multi-Lyte** Plus assay is for *in vitro* diagnostic use. Normal precautions exercised in handling laboratory reagents should be followed. 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.
2. The **AtheNA Multi-Lyte** Plus Test System conjugated micro-spheres do not contain viable organisms. However, the reagent should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
3. 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, these products should be handled 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).
4. The sample diluent, controls, bead suspension 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.

Procedural Precautions:

1. For *in vitro* diagnostic use only.
2. Dilution or adulteration of these reagents may generate erroneous results.
3. Reagents from other sources or manufacturers should not be used.
4. 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.
5. To optimize read times the bead suspension must be thoroughly mixed just prior to use. The most effective means to resuspend the beads is to first vortex the bead suspension for approximately 30 seconds followed by sonication of the bead suspension for approximately 30 seconds in a small bath sonicator.
6. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
7. Avoid microbial contamination of reagents. Incorrect results may occur.
8. Cross contamination of reagents and/or samples could cause erroneous results
9. Strict 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.
10. Avoid splashing or generation of aerosols.
11. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
12. 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 kit.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C.
2. Multiplex bead suspension: Store at 2-8°C. Remove only the required amount of solution to analyze the specimens to be tested and return the unused portion to storage at 2-8°C.
3. Phycoerythrin conjugated goat anti-human antibody: Store at 2-8°C.
4. Human controls: Store at 2-8°C.
5. Sample Diluent: Store at 2-8°C.
6. Wash Buffer Concentrate (10X). Store between 2-25°C. Diluted Wash Buffer (1X) is stable at room temperature (20-25°C) for up to 7 days or for 30 days at 2-8°C.

SPECIMEN COLLECTION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (6,7). No anticoagulants or preservatives should be added. Avoid using hemolyzed, icteric, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-8°C for no longer than 48 hours. If 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

ASSAY PROCEDURE

Materials required but not provided:

1. Pipettes capable of accurately delivering 10 to 200µL
2. Multichannel pipette capable of accurately delivering (10-200µL)
3. Reagent reservoirs for multichannel pipettes
4. Serological pipettes
5. Disposable pipette tips
6. Laboratory timer to monitor incubation steps

Set-up of the Assay:

Remove the individual components from storage and allow them to warm to room temperature (20-25°C). Determine the total number of controls and samples to be tested. It is necessary to include a the Negative Control and the Positive Control with each run. The Negative Control should be tested in well A1. The Positive Control should be tested in well B1. Each control and sample requires one microwell for processing.

Note 1. To optimize read times the bead suspension must be thoroughly mixed just prior to use. The most effective means to resuspend the beads is to first vortex the bead suspension for approximately 30 seconds and then sonicate for approximately 30 seconds in a small bath sonicator

Note 2. 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 RPM 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.

Serum Incubation

1. Prepare a 1:21 dilution of the Negative Control, the Positive Control and each of patient sera. (***Example****: Combine 10µL of serum with 200µL of Sample Diluent*). The sample 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. Mix according to **Note 2** above.
2. After determining the total number of wells to process, use a multichannel or a repeating pipette to dispense 50 µL of the bead suspension into each of the wells of the filtration plate.
3. 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. Mix according to **Note 2** above
4. Incubate the plate at room temperature (20-25°C) for 30 +/- 10 minutes.
5. After the incubation, rinse the beads by vacuum filtration:
    a. Place the filtration plate on the vacuum manifold and remove the solution, leaving the beads behind.
    b. Turn off the vacuum and add 200 µL of Diluted Wash Buffer (1X).
    c. Apply the vacuum and remove the solution.
    d. Repeat steps 5.b and 5.c for a total of three rinses with the Diluted Wash Buffer (1X).
6. Following the final wash gently blot the bottom of the filter plate and allow plate to air dry for 3-5 minutes before proceeding to the next step.

Conjugate Incubation

1. Add 150µL of the Conjugate solution to each well at the same rate and in the same order as the specimens were added. For proper performance, it is important that the conjugate solution and bead suspension are thoroughly mixed. Suitable means of mixing include shaking the plate for approximately 15 seconds, or repeatedly withdraw and expel the suspension several times to ensure that the suspension is properly mixed. Use a different pipette tip for each well. As an option, while mixing the conjugate and the beads, one may transfer the bead/conjugate mixture to empty wells of a polystyrene reaction plate.
2. Incubate the plate at room temperature (20-25°C) 30 +/- 10 minutes.

Specimen Analysis

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.

1. Set the **AtheNA Multi-Lyte** instrument to analyze the reactions by selecting the anti-Thyroid template. Refer to the operators manual for details regarding the operation of the **AtheNA Multi-Lyte** instrument.
2. 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.

ABBREVIATED ASSAY PROTOCOL:

Step	Procedure
1	Dilute specimens 1:21 in Sample Diluent. Mix well.
2	Combine 50 µL of bead suspension and 10 µL of diluted specimen in an empty well. Mix well.
3	Incubate at room temperature for 30 +/- 10 minutes
4	Rinse the microspheres 3X with Diluted Wash Buffer (1X).
5	Gently blot the bottom of the plate and air dry for 3-5 minutes.
6	Add 150 µL of conjugate to each well. Mix well and transfer to a reaction plate (optional).
7	Incubate at room temperature for 30 +/- 10 minutes.
8	Shake plate (optional).
9	Read results within 60 minutes.

CONVERSION OF FLUORESCENCE TO UNIT VALUES

A. Calculations:

1. Assay Calibration

The **AtheNA Multi-Lyte** 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 Scientific, Inc. These values are lot specific and are encoded within the lot specific Calibration CD included in the kit box.

2. Analyte Cut Off Values

Each analyte of the **AtheNA Multi-Lyte** Plus Test System has an assigned cut off value. Cut off values are determined by Zeus Scientific, Inc. for each kit lot, and are encoded within the lot specific Calibration CD included in the kit box.

3. Calculations

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.**

B. Interpretations:

1. **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 120IU/mL

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 Control and the Positive Control 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 multiplexed bead suspension.
    c. The Positive Control must be positive for both Tg and TPO 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.
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 the AtheNA® 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.

LIMITATIONS

1. The **AtheNA Multi-Lyte** 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

Comparative Study:

An in-house comparative study was performed to demonstrate the equivalence of the Zeus Scientific, Inc., 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 AtheNA TPO IgG 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 AtheNA Multi-Lyte TPO test system.

Clinical sensitivity of the AtheNA Multi-Lyte TPO IgG 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 AtheNA Multi-Lyte TPO test system.

Clinical specificity of the AtheNA Multi-Lyte TPO IgG 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 AtheNA Multi-Lyte TPO 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 AtheNA Tg IgG Relative to the Tg IgG ELISA

AtheNA Results	ELISA Results: Positive	Negative	Equivocal**	Total:
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 AtheNA Multi-Lyte Tg test system.
Clinical sensitivity of the AtheNA Multi-Lyte Tg IgG 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 AtheNA Multi-Lyte Tg IgG test system was therefore determined to be 300/300 or 100%.

Clinical Specificity of the AtheNA Multi-Lyte Tg test system.
Clinical specificity of the AtheNA Multi-Lyte TPO IgG 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 AtheNA Multi-Lyte RF IgM 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%

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: Summary of TPO Precision Testing.

TPO Precision Study

Sample:	Characteristics:	Day One Results:		Day Two Results:		Day Three Results:	
		Dilution 1	Dilution 2	Dilution 1	Dilution 2	Dilution 1	Dilution 2
Sample 1	Strong Positive	1229	1196	1202	1254	1224	1252
Sample 1	Strong Positive	1085	1223	1098	1164	1191	1118
Sample 1	Strong Positive	1170	1218	1189	1083	1191	1206
Sample 1	Strong Positive	1201	1174	1314	1136	1343	1174
Sample 2	Strong Positive	1185	1134	1103	1122	1138	1117
Sample 2	Strong Positive	1126	1160	1140	1150	1175	1122
Sample 2	Strong Positive	1083	1079	1050	1087	1185	1164
Sample 2	Strong Positive	1149	1181	1055	1008	1060	1067
Sample 3	Positive	259	267	351	328	312	346
Sample 3	Positive	249	266	261	295	362	360
Sample 3	Positive	295	259	309	207	327	297
Sample 3	Positive	297	289	313	296	314	310
Sample 4	Positive	266	296	310	235	317	346
Sample 4	Positive	293	276	263	235	309	311
Sample 4	Positive	270	269	283	253	295	322
Sample 4	Positive	318	276	247	260	242	303
Sample 5	Negative	4	5	6	7	7	6
Sample 5	Negative	4	5	6	6	5	6
Sample 5	Negative	4	5	8	6	5	5
Sample 5	Negative	3	4	6	7	6	7
Sample 6	Negative	6	5	6	7	8	8
Sample 6	Negative	5	4	6	7	5	7
Sample 6	Negative	5	5	6	4	6	6
Sample 6	Negative	6	5	7	6	8	7

		Day 1 Intra-assay Precision:		Day 2 Intra-assay Precision:		Day 3 Intra-assay Precision:		Inter-assay Precision	
	Sample 1	mean	1187	mean	1180	mean	1212	mean	1193
		stdev	46.53724	stdev	77.75603	stdev	65.57643	stdev	63.32340
		%CV	3.9%	%CV	6.6%	%CV	5.4%	%CV	5.3%
	Sample 2	mean	1137	mean	1089	mean	1129	mean	1118
		stdev	40.21882	stdev	49.05955	stdev	46.83405	stdev	48.40290
		%CV	3.5%	%CV	4.5%	%CV	4.2%	%CV	4.3%
	Sample 3	mean	273	mean	295	mean	329	mean	299
		stdev	18.39206	stdev	44.15233	stdev	24.61126	stdev	37.82509
		%CV	6.7%	%CV	15.0%	%CV	7.5%	%CV	12.7%
	Sample 4	mean	283	mean	261	mean	306	mean	283
		stdev	17.91249	stdev	25.35885	stdev	29.84693	stdev	30.24439
		%CV	6.3%	%CV	9.7%	%CV	9.8%	%CV	10.7%
	Sample 5	mean	4	mean	7	mean	6	mean	6
		stdev	0.707107	stdev	0.755929	stdev	0.834523	stdev	1.21509
		%CV	16.6%	%CV	11.6%	%CV	14.2%	%CV	21.9%
	Sample 6	mean	5	mean	6	mean	7	mean	6
		stdev	0.64087	stdev	0.991031	stdev	1.125992	stdev	1.16018
		%CV	12.5%	%CV	16.2%	%CV	16.4%	%CV	19.2%

Table 4. Summary of Tg Precision Testing.

Tg Precision Study

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

		Day 1 Intra-assay Precision:		Day 2 Intra-assay Precision:		Day 3 Intra-assay Precision:		Inter-assay Precision	
	Sample 1	mean	2816	mean	2771	mean	2875	mean	2821
		stdev	139.5575	stdev	119.7411	stdev	223.3076	stdev	165.43184
		%CV	5.0%	%CV	4.3%	%CV	7.8%	%CV	5.9%
	Sample 2	mean	2857	mean	2780	mean	2848	mean	2828
		stdev	105.3768	stdev	132.3545	stdev	93.18453	stdev	112.31470
		%CV	3.7%	%CV	4.8%	%CV	3.3%	%CV	4.0%
	Sample 3	mean	305	mean	322	mean	217	mean	282
		stdev	17.09428	stdev	33.09186	stdev	15.15633	stdev	52.08605
		%CV	5.6%	%CV	10.3%	%CV	7.0%	%CV	18.5%
	Sample 4	mean	273	mean	275	mean	323	mean	290
		stdev	16.53568	stdev	42.67736	stdev	17.46783	stdev	36.04323
		%CV	6.1%	%CV	15.5%	%CV	5.4%	%CV	12.4%
	Sample 5	mean	20	mean	17	mean	21	mean	19
		stdev	1.832251	stdev	1.846812	stdev	2.815772	stdev	2.66995
		%CV	9.3%	%CV	10.6%	%CV	13.3%	%CV	13.7%
	Sample 6	mean	18	mean	17	mean	20	mean	18
		stdev	2.03101	stdev	2.748376	stdev	3.251373	stdev	2.88424
		%CV	11.4%	%CV	16.0%	%CV	16.3%	%CV	15.7%

Cross Reactivity and Interfering Substances:

The 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 AtheNA Multi-Lyte Tg 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 AtheNA Multi-Lyte TPO test system. Four of those five were also positive on the TPO ELISA. Four of the specimens were positive using the AtheNA Multi-Lyte Tg test system. Two of the four were also positive on the Tg ELISA.

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