AIV Plus Test System

REF A96101

IVD

CE

Rx Only

INTENDED USE

The ZEUS **AtheNA Multi-Lyte®** Autoimmune Vasculitis (AIV) Plus Test System is intended for the qualitative and semi-quantitative detection of IgG class antibody to three separate antigens; Glomerular Basement Membrane (GBM), Myeloperoxidase (MPO) and Proteinase 3(PR-3) in human serum. The test system is intended to be used as an aid in the diagnosis of various autoimmune vasculitic disorders characterized by elevated levels of selected autoantibodies. MPO and/or PR-3 may be associated with autoimmune disorders such as Wegener's Granulomatosis, ICGN, MPA and PRS. Anti-GBM antibodies aid in the diagnosis of Goodpasture's syndrome. This test is for *In Vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Anti-neutrophil cytoplasmic antibody (ANCA) was initially described by Davies, *et al* in 1982 (1). Since this initial discovery, ANCA has been found to be associated with a number of Systemic Vasculitides (SV). ANCA is now recognized to include two primary specificities: C-ANCA directed against PR-3, and P-ANCA directed against MPO. Testing for both P-ANCA and C-ANCA is highly recommended in the laboratory workup of patients who present with clinical features suggestive of SV. The clinical syndromes most frequently associated with ANCA are as follows; Wegener's granulomatosis (2), Polyarteritis (3), "Overlap" Vasculitis (4), Idiopathic Crescentic Glomerulonephritis (ICGN) (5), Kawasaki Disease (6), and Autoimmune renal disorders such as Goodpasture's Syndrome (7).

Although the initial identification of C-ANCA and P-ANCA was based on the indirect immunofluorescence procedures, further identification and purification of PR-3 and MPO has resulted in the development of enzyme immunoassays (ELISA) and microparticle based immunoassays for both PR-3 and MPO. Goodpasture syndrome is characterized by lung hemorrhage, renal failure and the presence of anti-GBM antibodies (8). In Goodpasture's syndrome a part of the globular domain of the collagen IV chains are antigenic and are responsible for development of anti-GBM antibodies in progressive glomerulonephritis (9, 10, and 11).

PRINCIPLE OF THE ASSAY

The ZEUS **AtheNA Multi-Lyte** AIV Plus Test System is designed to detect IgG class antibodies in human sera to MPO, PR-3 and GBM. 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 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: Myeloperoxidase (MPO), Proteinase 3 (PR3) and glomerular basement membrane (GBM). 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	-

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

DIL	SPE

6. 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

7. 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

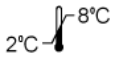
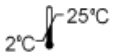
1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of 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 (20).
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 (13, 14). 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 (15).

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 two Positive Controls with each run. The Negative Control should be tested in well A1, Positive Control 1 in well B1 and Positive Control 2 in well C1. 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	Patient 1	
E	Patient 2	
F	Patient 3	
G	Patient 4	
H	Patient 5	

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 AIV 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 two Positive Controls (in wells B1 through C1).
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 two 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. 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 **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 CLSI 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** AIV 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** AIV 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:** Specimen unit values for GBM, MPO and PR-3 are interpreted as follows:

Negative Specimens	< 100 AU/mL
Positive Specimens	> 120 AU/mL
Equivocal Specimens	100 – 120 AU/mL

LIMITATIONS OF THE ASSAY

1. The ZEUS **AtheNA Multi-Lyte** AIV 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

1. **MPO/PR-3**

The clinical investigation included 122 specimens that were sent to a lab for routine ANCA testing, 173 specimens from clinically diagnosed patients, and 150 specimens from normal blood donors. The results of the MPO and PR-3 ELISA were used to demonstrate the expected outcome for such groups. The results of each population are depicted in the Table 1 and Table 2 below:

Table 1: Expected Outcome of MPO and PR-3 Analyte Results using Different Specimen Populations

				AtheNA Multi-Lyte Result			
Group	**N**	**Analyte**		**Invalid**	**Equivocal**	**Positive**	**Negative**
Routine	122	MPO	Quantity	2	2	21	97
			%	1.6	1.6	17.2	79.5
		PR-3	Quantity	2	2	50	68
			%	1.6	1.6	41.0	55.7
Clinical	173	MPO	Quantity	1	2	104	66
			%	0.6	1.2	60.1	38.2
		PR-3	Quantity	1	6	104	62
			%	0.6	3.5	60.1	35.8
Normal	150	MPO	Quantity	0	1	9	140
			%	0.0	0.7	6.0	93.3
		PR-3	Quantity	0	6	27	117
			%	0.0	4.0	18.0	78.0

Table 2: Expected Outcome of MPO and PR-3 Analyte Results using Different Specimen Populations

		AtheNA Multi-Lyte Results			
Group	**Analyte**	**Mean Result**	**Median Result**	**Range**	
				Low Value	**High Result**
Routine	MPO	211.6	35.5	0	1887
	PR-3	396.3	73	5	3405
Clinical	MPO	272	50	14	2451
	PR-3	267.2	62	18	3041
Normal	MPO	53	35	0	684
	PR-3	128	57	0	1636

2. **GBM**

The clinical investigation included 115 specimens that were sent to a lab for ANCA (Systemic Vasculitidis) testing and 115 specimens that were submitted for GBM (Goodpasture's Syndrome) testing. The resulting data was used to demonstrate the expected outcome for such groups.

Table 3: Expected Outcome of ANCA and GBM Analytes using Different Specimen Populations

			AtheNA Multi-Lyte Result			
N	**Analyte**		**Invalid**	**Equivocal**	**Positive**	**Negative**
115	ANCA	Quantity	0	0	20	95
		%	1.6	1.6	17.4	82.6
	GBM	Quantity	0	0	13	102
		%	0.00	0.00	11.3	88.7

Table 4: Expected Outcome of ANCA and GBM Analytes using Different Specimen Populations

	AtheNA Multi-Lyte Results			
Analyte	**Mean Result**	**Median Result**	**Range**	
			Low Value	**High Result**
ANCA	105	26	2	1180
GBM	85	39	13	1336

PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

An in-house comparative study was performed to demonstrate the equivalence of the ZEUS **AtheNA Multi-Lyte** AIV Plus Test System to commercially available ELISA Test Systems. Performance was evaluated using 445 specimens; 150 normal donor sera, 122 specimens previously sent to a lab for routine ANCA autoantibody testing, and 173 disease-state specimens from clinically diagnosed patients with SV disorders. The results of the investigation have been summarized in Tables 5 and 6 below. Comparative data for GBM, performed using 230 samples (including 115 sera from patients suspected of having Goodpasture's syndrome) are shown in Table 7.

Table 5: Performance of ZEUS AtheNA Multi-Lyte AIV Plus Test System (MPO Anaylyte) Relative to the ZEUS ELISA MPO IgG Test Sytem

		ELISA Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte Results	Positive	55	39	2	96
	Negative	2	338	1	341
	Equivocal*	1	4	0	5
	Total	59	383	3	445
Relative Sensitivity = 55/57 = 96.5%		Relative Specificity = 338/377 = 89.6%		Relative Agreement = 393/434 = 90.6%	
* Equivocal samples were excluded from agreement calculations					

Table 6: Performance of ZEUS AtheNA Multi-Lyte AIV Plus Test System (PR-3 Anaylyte) Relative to the ZEUS ELISA PR-3 IgG Test Sytem

		ELISA Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte Results	Positive	85	53	1	139
	Negative	6	283	0	289
	Equivocal*	4	10	0	14
	Total	96	348	1	445
Relative Sensitivity = 85/91 = 93.4%		Relative Specificity = 283/336 = 84.2%		Relative Agreement = 368/427 = 86.2%	
* Equivocal samples were excluded from agreement calculations					

Table 7: Performance of ZEUS AtheNA Multi-Lyte AIV Plus Test System (GBM Anaylyte) Relative to the ZEUS ELISA ANCA Test Sytem

		ELISA Results			
		Positive	Negative	Equivocal*	Total
AtheNA Multi-Lyte Results	Positive	31	2	0	33
	Negative	1	195	1	197
	Equivocal*	0	0	0	0
	Total	32	197	1	230
Positive Percent Agreement = 31/33 = 93.9%		Negative Percent Agreement = 195/197 = 99.0%		Overall Percent Agreement = 226/230 = 98.3%	
* Equivocal samples were excluded from agreement calculations					

Table 8: Summary of Comparative Performance

Analyte	N	Relative Sensitivity	Relative Specificity	Overal Agreement
MPO	445	55/57 = 96.5%	338/377 = 89.6%	393/439 = 90.6%
PR-3	445	85/91 = 93.4%	283/336 = 84.2%	368/427 = 86.2%
GBM	230	31/33 = 93.9%	195/197 = 99.0%	226/230 = 98.3%

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 AU/mL values, standard deviations, and percent CV. Specimens were selected in such a way that resulted in Samples 5 and 6 being clearly negative, Samples 1 and 2 being clearly positive and Samples 3 and 4 being near the assay cutoff. The results of this study have been summarized in Tables 9, 10 and 11 below:

Table 9: MPO Precision Study

Sample	Day 1 Results		Day 2 Results		Day 3 Results		Intra-Assay Precision				Inter-Assay Precision	
	Dilution 1	Dilution 2	Dilution 1	Dilution 2	Dilution 1	Dilution 2		Day 1	Day 2	Day 3		
1	1350	1275	1905	1642	1478	1619	Mean	1349	1684	1683	Mean	1572
	1339	1453	1623	1623	1759	1655	StD	61.94	115.78	99.70	StD	184.98
	1298	1297	1817	1682	1778	1712	% CV	4.6	6.9	5.9	% CV	11.8
	1420	1360	1611	1571	1695	1766						
2	461	458	380	479	503	389	Mean	431	436	455	Mean	440
	422	397	412	454	482	417	StD	26.58	33.03	44.00	StD	35.33
	396	454	453	457	489	449	% CV	6.2	7.6	9.7	% CV	8.0
	441	416	405	444	496	412						
3	175	178	144	167	164	209	Mean	175	160	182	Mean	172
	169	173	156	175	195	187	StD	7.88	15.47	14.93	StD	15.58
	166	192	158	180	178	179	% CV	4.5	9.7	8.2	% CV	9.1
	171	175	134	167	169	171						
4	100	104	91	87	86	101	Mean	113	97	93	Mean	101
	110	99	114	98	94	84	StD	11.41	10.68	9.86	StD	13.32
	118	125	105	99	79	92	% CV	10.1	11.0	10.6	% CV	13.2
	130	114	101	80	108	101						
5	25	24	23	26	19	20	Mean	25	25	23	Mean	24
	27	27	29	19	23	23	StD	2.97	3.85	3.21	StD	3.39
	31	21	30	23	26	20	% CV	11.7	15.4	13.9	% CV	13.9
	24	24	28	22	28	25						
6	17	24	19	28	15	21	Mean	19	18	24	Mean	20
	19	19	17	21	17	23	StD	2.60	5.88	9.22	StD	6.76
	19	22	15	7	14	33	% CV	13.5	32.7	38.2	% CV	33.1
	18	16	18	19	31	39						

Table 9: PR-3 Precision Study

Sample	Day 1 Results		Day 2 Results		Day 3 Results		Intra-Assay Precision				Inter-Assay Precision	
	Dilution 1	Dilution 2	Dilution 1	Dilution 2	Dilution 1	Dilution 2		Day 1	Day 2	Day 3		
1	2535	2631	2723	2541	2447	2718	Mean	2583	2620	2609	Mean	2604
	2582	2674	2591	2713	2611	2596	StD	85.75	83.58	90.77	StD	84.36
	2501	2496	2649	2474	2597	2728/	% CV	3.3	3.2	3.5	% CV	3.2
	2726	2521	2632	2636	2542	2633						
2	1349	1536	1434	1501	1785	1414	Mean	1387	1427	1491	Mean	1435
	1318	1348	1408	1383	1489	1438	StD	96.71	68.84	144.02	StD	111.88
	1268	1445	1367	1517	1520	1452	% CV	7.0	4.8	9.7	% CV	7.8
	1323	1505	1325	1484	1547	1280						
3	113	118	109	119	107	116	Mean	120	113	112	Mean	115
	118	114	132	96	137	93	StD	7.76	11.92	14.42	StD	11.82
	137	118	108	124	98	110	% CV	6.4	10.6	12.8	% CV	10.3
	119	126	111	101	127	110						
4	79	79	73	85	81	79	Mean	78	80	79	Mean	79
	73	98	87	84	88	81	StD	9.67	7.06	5.80	StD	7.39
	70	76	76	73	73	72	% CV	12.4	8.8	7.3	% CV	9.4
	82	66	72	89	84	73						
5	15	22	28	15	14	16	Mean	22	20	22	Mean	21
	26	18	21	23	15	29	StD	3.74	6.75	6.57	StD	5.63
	21	23	7	27	22	19	% CV	17.3	33.5	30.6	% CV	26.7
	26	22	19	21	26	31						
6	53	53	54	48	52	56	Mean	56	52	56	Mean	55
	57	63	54	50	58	51	StD	4.43	3.56	6.52	StD	5.10
	52	61	56	50	66	46	% CV	7.6	6.8	11.6	% CV	9.3
	52	57	57	48	63	58						

Table 9: GBM Precision Study

Sample	Day 1 Results		Day 2 Results		Day 3 Results		Intra-Assay Precision				Inter-Assay Precision	
	Dilution 1	Dilution 2	Dilution 1	Dilution 2	Dilution 1	Dilution 2		Day 1	Day 2	Day 3		
1	773	611	798	672	901	782	Mean	733	740	812	Mean	762
	829	809	651	700	812	752	StD	91.15	75.47	48.50	StD	79.35
	706	638	883	705	796	803	% CV	12.4	10.2	60	% CV	10.4
	840	659	743	766	865	782						
2	796	728	651	646	882	794	Mean	742	714	842	Mean	766
	736	674	729	824	970	790	StD	54.40	61.63	62.90	StD	80.31
	715	706	676	700	844	821	% CV	7.3	8.6	7.5	% CV	10.5
	846	732	777	706	859	779						
3	115	124	90	93	119	128	Mean	116	87	112	Mean	105
	127	95	76	80	118	107	StD	11.93	12.40	10.69	StD	17.28
	115	118	101	64	95	101	% CV	10.3	14.3	9.6	% CV	16.5
	130	103	98	91	116	109						
4	163	130	107	83	94	92	Mean	112	98	98	Mean	103
	118	89	103	101	115	106	StD	27.21	7.86	8.67	StD	17.74
	122	86	103	96	95	102	% CV	24.2	8.1	8.8	% CV	17.3
	108	82	97	90	89	94						
5	41	15	29	36	14	2	Mean	22	21	25	Mean	23
	25	29	21	20	24	29	StD	9.68	9.74	11.63	StD	10.06
	13	17	20	26	30	31	% CV	44.0	45.6	46.5	% CV	44.1
	13	23	16	3	38	32						
6	41	26	16	50	39	24	Mean	28	33	21	Mean	27
	7	18	35	32	17	27	StD	10.91	14.10	9.05	StD	12.51
	33	31	55	33	19	11	% CV	39.1	45.9	43.6	% CV	46.0
	30	37	32	10	15	14						

3. **Cross Reactivity**

The ZEUS **AtheNA Multi-Lyte** AIV Plus Test System MPO and PR-3 analytes were evaluated for potential cross reactivity to other antibodies and interference from serum components. For this study, a total of 35 specimens were evaluated. Fifteen of the specimens were positive for various autoimmune and infectious disease antibodies. Of the 15 evaluated, one was reactive for the MPO analyte and the same sample was positive for the PR-3 analyte. The GBM analyte was evaluated for potential cross reactivity to other antibodies and interference from serum components using a total of 26 specimens. Twenty-six specimens were positive for various autoimmune and infectious disease antibodies. Of the 26 samples evaluated, all remained negative for GBM demonstrating that there is little likelihood of cross reactivity. Additionally, 10 samples with high levels of MPO and 10 samples with high levels of PR-3 were tested for cross reactivity with GBM. All 20 samples remained negative for GBM demonstrating there is little likelihood of cross reactivity between MPO and PR-3 with GBM.

4. **Interfering Substances**
   There were a total of 20 MPO/PR-3 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). Two of the specimens were positive for both the MPO and PR-3analytes. There were a total of six GBM specimens evaluated with potential interfering substances. These six specimens were spiked with abnormal levels of hemolysis, (n=2), bilirubin (n=2), above normal lipid levels (n=2), albumin (n=2), cholesterol (n=2) or triglycerides (n=2). The qualitative outcome for all six samples remained unchanged with the exception of one sample spiked with a high level of cholesterol and two samples spiked with high levels of triglycerides. Lipemic samples may interfere with the outcome of this assay. Use of these types of specimens should be avoided.

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