Autoimmune Vasculitis Plus Test System

A Multiplexed, Microparticle-Based Immunoassay for Antibodies to glomerular basement membrane, MPO and PR3 Antigens

Product Number: A96101

INTENDED USE

The Zeus Scientific, Inc. **AtheNA Multi-Lyte®** Autoimmune Vasculitis Plus Test System is intended for the qualitative and semi-quantitative detection of IgG class antibody to 3 separate Antigens (Glomerular Basement Membrane, Myeloperoxidase and Proteinase 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 PR3 may be associated with autoimmune disorders such as Wegener's Granulomatosis, ICGN, MPA and PRS. Anti-Glomerular Basement Membrane (GBM) antibodies aid in the diagnosis of Goodpasture's syndrome.
These tests are 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 Proteinase 3 (PR3), and P-ANCA directed against Myeloperoxidase (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)
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 PR3 and MPO has resulted in the development of enzyme immunoassays (ELISA) and microparticle based immunoassays for both PR3 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, 11).

PRINCIPLE OF THE AtheNA Multi-Lyte Autoimmune Vasculitis Plus TEST SYSTEM

The Zeus Scientific, Inc. **AtheNA Multi-Lyte** Autoimmune Vasculitis Plus Test System is designed to detect IgG class antibodies in human sera to MPO, PR3 and GBM. 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: Myeloperoxidase (MPO), Proteinase 3 (PR3) and glomerular basement membrane. 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, 30 mL amber bottle.
3. Human positive serum control. Two, 0.2mL vial.
4. Human negative serum control. One, 0.2mL vial.
5. SAVe Diluent®. One 50 mL bottle containing phosphate-buffered-saline. Ready to use. NOTE: The diluent will change color in the presence of serum.
6. Wash Buffer Concentrate: Dilute 1 part concentrate + 9 parts deionized or distilled water. One 50mL bottle containing 10X concentrate of phosphate buffered saline.

Non-reactive Components:

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

WARNINGS

1. **CAUTION! POTENTIAL BIOHAZARD:** The controls contain human source material. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by FDA-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 (12); and OSHA's Standard for Bloodborne Pathogens (13). The **AtheNA Multi-Lyte** Plus Test System conjugated microspheres do not contain viable organisms. However, the reagent should be considered a **POTENTIAL BIOHAZARD** and handled accordingly.
2. Caution: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides if disposal into a drain is in compliance with Federal, State and local requirements.
3. Normal precautions exercised in handling laboratory reagents should be followed when performing the **AtheNA Multi-Lyte** Plus assay. 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. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes. Do not breathe vapor. Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all Federal, State and local requirements.

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 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. SAVe Diluent: Store at 2-8°C.
6. Wash Buffer Concentrate (10X): Stre 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/CLSI document M29: Protection of Laboratory Workers from Infectious Disease (14). 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 stored sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Do not use 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 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. Specimen containers should be tightly sealed before storage. For freezing, the use of self-sealing, air tight tubes is recommended. Whenever possible, avoid the use of self-defrosting freezers because of the danger of desiccation of specimens.

ASSAY PROCEDURE

Materials required but not provided:

1. **AtheNA Multi-Lyte** system (Luminex® instrument)
2. Pipettes capable of accurately delivering 10 to 200µL
3. Multichannel pipette capable of accurately delivering (10-200µL)
4. Reagent reservoirs for multichannel pipettes
5. Serological pipettes
6. Disposable pipette tips
7. Laboratory timer to monitor incubation steps
8. Distilled or deionized water

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 the Negative Control and the Positive Controls with each run. The Negative Control should be tested in well A1. The Positive Control-1 should be tested in well B1, and Positive Control-2 should be tested in well C1. 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 Controls 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 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 200 µL of the 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 the 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. Mix according to **Note 2** above. As an option, while mixing the conjugate 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 AI Vasculitis 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.
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 1X Wash Buffer.
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.
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.

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:

Autoimmune Vasculititis Plus Test System Result Interpretation:

Specimen unit values for GBM, MPO and PR3 are interpreted as follows:

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

QUALITY CONTROL

1. Each time the assay is run it is necessary to include the Negative Control (in well A1), the Positive Control-1 in well B1, and Positive Control-2 in well 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 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 Controls must be positive for a predetermined group of analytes included in the multiplexed 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 the **AtheNA Multi-Lyte** Plus 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.

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 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 PR3 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 AtheNA MPO and PR-3 assays using different specimen populations.

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

Table 2. Expected outcome of AtheNA MPO and PR-3 assays using different specimen populations.

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

EXPECTED RESULTS (GBM)
The clinical investigation included 115 specimens that were sent 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 AtheNA Multi-Lyte® GBM assay using different specimen populations.

			AtheNA Multi-Lyte Results			
Samples	**n**		**Invalid**	**Equivocal**	**Positive**	**Negative**
Submitted for:						
ANCA (Vasculitidis)	115	Quantity	0	0	20	95
		%	1.6%	1.6%	17.4%	82.6%
GBM (Goodpastures)	115	Quantity	0	0	13	102
		%	0.00%	0.00%	11.3%	88.7%

Table 4. Expected outcome of AtheNA Multi-Lyte® GBM using different specimen populations.

		AtheNA Multi-Lyte Results			
Samples				Range	
Submitted for:	**n**	**Mean Result**	**Median Result**	**Low Value**	**High Result**
ANCA (Vasculitidis)	115	105	26	2	1180
GBM (Goodpastures)	115	85	39	13	1336

PERFORMANCE CHARACTERISTICS
Comparative Study:

An in-house comparative study was performed to demonstrate the equivalence of the Zeus Scientific, Inc., **AtheNA Multi-Lyte** Plus MPO/PR3 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 AtheNA Multi-Lyte MPO IgG Relative to the MPO IgG ELISA

		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 AtheNA Multi-Lyte PR3 IgG Relative to the PR3 IgG ELISA

AtheNA Multi-Lyte Results	ELISA Results: Positive	Negative	Equivocal*	Total:
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 AtheNA Multi-Lyte GBM IgG Relative to the GBM IgG ELISA

AtheNA Multi-Lyte Results	ELISA Results: Positive	Negative	Equivocal*	Total:
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

	N	Relative Sensitivity		Relative Specificity		Overall Agreement	
MPO	***445***	96.5%	55/57	89.6%	338/377	90.6%	393/439
PR3	***445***	93.4%	85/91	84.2%	283/336	86.2%	368/427
GBM	***230***	93.9%	31/33	99.0%	195/197	98.3	226/230

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 two of them being clearly negative, two being clearly positive and two were selected that were near the assay cut off.

The results of this study have been summarized in Table 9, 10 and 11 below:

Table 9. MPO 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	1350	1275	1905	1642	1478	1619
Sample 1	Strong Positive	1339	1453	1623	1623	1759	1655
Sample 1	Strong Positive	1298	1297	1817	1682	1778	1712
Sample 1	Strong Positive	1420	1360	1611	1571	1695	1766
Sample 2	Strong Positive	461	458	380	479	503	389
Sample 2	Strong Positive	422	397	412	454	482	417
Sample 2	Strong Positive	396	454	453	457	489	449
Sample 2	Strong Positive	441	416	405	444	496	412
Sample 3	Near Cut Off	175	178	144	167	164	209
Sample 3	Near Cut Off	169	173	156	175	195	187
Sample 3	Near Cut Off	166	192	158	180	178	179
Sample 3	Near Cut Off	171	175	134	167	169	171
Sample 4	Near Cut Off	100	104	91	87	86	101
Sample 4	Near Cut Off	110	99	114	98	94	84
Sample 4	Near Cut Off	118	125	105	99	79	92
Sample 4	Near Cut Off	130	114	101	80	108	101
Sample 5	Negative	25	24	23	26	19	20
Sample 5	Negative	27	27	29	19	23	23
Sample 5	Negative	31	21	30	23	26	20
Sample 5	Negative	24	24	28	22	28	25
Sample 6	Negative	17	24	19	28	15	21
Sample 6	Negative	19	19	17	21	17	23
Sample 6	Negative	19	22	15	7	14	33
Sample 6	Negative	18	16	18	19	31	39

		Day 1 Intra-assay Precision:		Day 2 Intra-assay Precision:		Day 3 Intra-assay Precision:		Inter-assay Precision	
	Sample 1	mean	1349	mean	1684	mean	1683	mean	1572
		stdev	61.94467578	stdev	115.781506	stdev	99.69919043	stdev	184.98343
		%CV	4.6%	%CV	6.9%	%CV	5.9%	%CV	11.8%
	Sample 2	mean	431	mean	436	mean	455	mean	440
		stdev	26.5757005	stdev	33.03245158	stdev	44.00304373	stdev	35.33104
		%CV	6.2%	%CV	7.6%	%CV	9.7%	%CV	8.0%
	Sample 3	mean	175	mean	160	mean	182	mean	172
		stdev	7.881941385	stdev	15.46828553	stdev	14.92840055	stdev	15.58334
		%CV	4.5%	%CV	9.7%	%CV	8.2%	%CV	9.1%
	Sample 4	mean	113	mean	97	mean	93	mean	101
		stdev	11.41427677	stdev	10.68293031	stdev	9.862446813	stdev	13.32101
		%CV	10.1%	%CV	11.0%	%CV	10.6%	%CV	13.2%
	Sample 5	mean	25	mean	25	mean	23	mean	24
		stdev	2.973093627	stdev	3.854496447	stdev	3.207134903	stdev	3.38769
		%CV	11.7%	%CV	15.4%	%CV	13.9%	%CV	13.9%
	Sample 6	mean	19	mean	18	mean	24	mean	20
		stdev	2.604940361	stdev	5.879747322	stdev	9.218575967	stdev	6.76294
		%CV	13.5%	%CV	32.7%	%CV	38.2%	%CV	33.1%

Table 10. PR-3 Precision

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	2535	2631	2723	2541	2447	2718
Sample 1	Strong Positive	2582	2674	2591	2713	2611	2596
Sample 1	Strong Positive	2501	2496	2649	2474	2597	2728
Sample 1	Strong Positive	2726	2521	2632	2636	2542	2633
Sample 2	Strong Positive	1349	1536	1434	1501	1785	1414
Sample 2	Strong Positive	1318	1348	1408	1383	1489	1438
Sample 2	Strong Positive	1268	1445	1367	1517	1520	1452
Sample 2	Strong Positive	1323	1505	1325	1484	1547	1280
Sample 3	Near Cut Off	113	118	109	119	107	116
Sample 3	Near Cut Off	118	114	132	96	137	93
Sample 3	Near Cut Off	137	118	108	124	98	110
Sample 3	Near Cut Off	119	126	111	101	127	110
Sample 4	Near Cut Off	79	79	73	85	81	79
Sample 4	Near Cut Off	73	98	87	84	88	81
Sample 4	Near Cut Off	70	76	76	73	73	72
Sample 4	Near Cut Off	82	66	72	89	84	73
Sample 5	Negative	15	22	28	15	14	16
Sample 5	Negative	26	18	21	23	15	29
Sample 5	Negative	21	23	7	27	22	19
Sample 5	Negative	26	22	19	21	26	31
Sample 6	Negative	53	53	54	48	52	56
Sample 6	Negative	57	63	54	50	58	51
Sample 6	Negative	52	61	56	50	66	46
Sample 6	Negative	52	57	57	48	63	58

		Day 1 Intra-assay Precision:		Day 2 Intra-assay Precision:		Day 3 Intra-assay Precision:		Inter-assay Precision	
	Sample 1	mean	2583	mean	2620	mean	2609	mean	2604
		stdev	85.75338394	stdev	83.57791489	stdev	90.7650027	stdev	84.36488
		%CV	3.3%	%CV	3.2%	%CV	3.5%	%CV	3.2%
	Sample 2	mean	1387	mean	1427	mean	1491	mean	1435
		stdev	96.71017083	stdev	68.84130301	stdev	144.0217617	stdev	111.87636
		%CV	7.0%	%CV	4.8%	%CV	9.7%	%CV	7.8%
	Sample 3	mean	120	mean	113	mean	112	mean	115
		stdev	7.763237543	stdev	11.91637529	stdev	14.41972855	stdev	11.81921
		%CV	6.4%	%CV	10.6%	%CV	12.8%	%CV	10.3%
	Sample 4	mean	78	mean	80	mean	79	mean	79
		stdev	9.672309519	stdev	7.059694449	stdev	5.792544469	stdev	7.38572
		%CV	12.4%	%CV	8.8%	%CV	7.3%	%CV	9.4%
	Sample 5	mean	22	mean	20	mean	22	mean	21
		stdev	3.739270364	stdev	6.749338592	stdev	6.568322247	stdev	5.63311
		%CV	17.3%	%CV	33.5%	%CV	30.6%	%CV	26.7%
	Sample 6	mean	56	mean	52	mean	56	mean	55
		stdev	4.242640687	stdev	3.563204817	stdev	6.519202405	stdev	5.09884
		%CV	7.6%	%CV	6.8%	%CV	11.6%	%CV	9.3%

Table 11. GBM Precision

Sample:	Level:	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	773	611	798	672	901	782
Sample 1	Strong Positive	829	809	651	700	812	752
Sample 1	Strong Positive	706	638	883	705	796	803
Sample 1	Strong Positive	840	659	743	766	865	782
Sample 2	Strong Positive	796	728	651	646	882	794
Sample 2	Strong Positive	736	674	729	824	970	790
Sample 2	Strong Positive	715	706	676	700	844	821
Sample 2	Strong Positive	846	732	777	706	859	779
Sample 3	Near Cut Off	115	124	90	93	119	128
Sample 3	Near Cut Off	127	95	76	80	118	107
Sample 3	Near Cut Off	115	118	101	64	95	101
Sample 3	Near Cut Off	130	103	98	91	116	109
Sample 4	Near Cut Off	163	130	107	83	94	92
Sample 4	Near Cut Off	118	89	103	101	115	106
Sample 4	Near Cut Off	122	86	103	96	95	102
Sample 4	Near Cut Off	108	82	97	90	89	94
Sample 5	Negative	41	15	29	36	14	2
Sample 5	Negative	25	29	21	20	24	29
Sample 5	Negative	13	17	20	26	30	31
Sample 5	Negative	13	23	16	3	38	32
Sample 6	Negative	41	26	16	50	39	24
Sample 6	Negative	7	18	35	32	17	27
Sample 6	Negative	33	31	55	33	19	11
Sample 6	Negative	30	37	32	10	15	14

		Day 1 Intra-assay Precision		Day 2 Intra-assay Precision		Day 3 Intra-assay Precision		Inter-assay Precision	
	Sample 1	mean	733.125	mean	739.75	mean	811.625	mean	761.5
		stdev	91.14734931	stdev	75.46759002	stdev	48.50018409	stdev	79.34898153
		%CV	12.4%	%CV	10.2%	%CV	6.0%	%CV	10.4%
	Sample 2	mean	741.625	mean	713.625	mean	842.375	mean	765.875
		stdev	54.39521381	stdev	61.63240683	stdev	62.89887008	stdev	80.31422664
		%CV	7.3%	%CV	8.6%	%CV	7.5%	%CV	10.5%
	Sample 3	mean	115.875	mean	86.625	mean	111.625	mean	104.7083333
		stdev	11.93359603	stdev	12.39743637	stdev	10.68961445	stdev	17.28150347
		%CV	10.3%	%CV	14.3%	%CV	9.6%	%CV	16.5%
	Sample 4	mean	112.25	mean	97.5	mean	98.375	mean	102.7083333
		stdev	27.20687938	stdev	7.855844048	stdev	8.667467912	stdev	17.73593544
		%CV	24.2%	%CV	8.1%	%CV	8.8%	%CV	17.3%
	Sample 5	mean	22	mean	21.375	mean	25	mean	22.79166667
		stdev	9.680613912	stdev	9.738546386	stdev	11.62509601	stdev	10.05627283
		%CV	44.0%	%CV	45.6%	%CV	46.5%	%CV	44.1%
	Sample 6	mean	27.875	mean	32.875	mean	20.75	mean	27.16666667
		stdev	10.9078936	stdev	15.10380747	stdev	9.051440296	stdev	12.50623033
		%CV	39.1%	%CV	45.9%	%CV	43.6%	%CV	46.0%

Cross Reactivity and Interfering Substances:

The **AtheNA Multi-Lyte** MPO/PR3 assays 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 fifteen evaluated, one was reactive on the **AtheNA Multi-Lyte** MPO assay and the same sample was positive on the **AtheNA Multi-Lyte** PR3 assay. The **AtheNA Multi-Lyte** GBM assay was evaluated for potential cross reactivity to other antibodies and interference from serum components. For this study, a total of 26 specimens were evaluated. 26 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 PR3 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 PR3 with GBM.

There were a total of 20 MPO/PR3 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 on both the **AtheNA Multi-Lyte** MPO assay and the PR3 assay. There were a total of 6 GBM specimens evaluated with potential interfering substances. These 6 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 6 samples remained unchanged with the exception of 1 sample spiked with a high level of cholesterol and 2 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.*

REFERENCES

1. Davies D, Moran ME, Niall JF, Ryan GB: Segmental glomerulonephritis with antineutrophil antibody: Possible arbovirus aetiology. Br. J. Med. 285: 606, 1982
2. van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, van Es LA: Autoantibodies against neutrophils and monocytes: Tools for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1: 425-429, 1985.
3. Feehally J, Wheeler DC, Walls J, *et al*: A case of microscopic polyarteritis associated with antineutrophilia cytoplasmic antibodies. Clin. Nephrol. 27: 214-215, 1987
4. Falk RJ, Becker M, Terrell R, Jennette JC: Antigen specificity of P-ANCA and C-ANCA (Abstract). The 3rd International Workshop on ANCA, Washington, DC 1990: 2-3.
5. Jennette JC, Falk RJ: Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. N.Engl.J. Med. 318: 1651-1657, 1988.
6. Savage COS, Tizard J, Jayne D, *et al*: Anti-neutrophil cytoplasmic antibodies in Kawasaki disease. Arch. Dis. Child. 64: 360-363, 1989.
7. Saxena R, Bygren P, Arvastson B, Wieslander J: Circulating autoantibodies as serological markers in the differential diagnosis of pulmonary renal syndrome. J. Intern. Med. 238: 143-152, 1995.
8. Hellmark T, Johansson C, Wieslander J. Characterisation of anti-GBM antibodies involved in Goodpasture syndrome: Kidney Int. 46: 823-829, 1994.
9. Wieslander J, Barr J, Butkowski R, Edwards S, Bygren P, Heinegard D, Hudson B: Goodpasture antigen of the glomerular basement membrane: Localization to noncollagenous regions of type IV collagen. Proc. Natl. Acad. Sci. USA 81:3838-42, 1984.
10. Butkowski R, Wieslander J, Wisdom B, Barr J, Noelken M, Hudson B: Properties of the globular domain of type IV collagen and its relationship to the Goodpasture antigen. J. Biol. Chem. 262: 7874-7877, 1987.
11. Butkowski R, Langveld J, Wieslander J, Hamilton J, Hudson B: Localization of Goodpasture epitope to novel chain of basement membrane collagen. J. Biol. Chem. 260: 3739-3747, 1985.
12. U.S. Department of Health and Human Services. Public Health Service. Centers for Disease Control and Prevention and National Institutes of Health. U.S. Government Printing Office, Washington D.C., 4th Ed., 1999.
13. U.S. Department of Labor, Occupational Safety and Health Administration; Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed.Register 56:64175-64182, 1991.
14. Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissues; Approved Guideline. NCCLS/CLSI Document M29, Vol.17(12), 1997.

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AtheNA Multi-Lyte® AIV Plus/Issue Date: 2008-04-28/K071219/R2720PE/FCD-0557