510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
  k071219
B. Purpose for Submission:
  New analyte added to a previously cleared device
C. Measurand:
  Glomerular basement membrane (GMB) autoantibodies
D. Type of Test:
  Qualitative and semi-quantitative flow cytometry-based, homogeneous, multiplexed, microparticle fluorescent immunoassay,
E. Applicant:
  Zeus Scientific, Inc.
F. Proprietary and Established Names:
  Zeus Scientific, Inc. AtheNA Multi-Lyte™ ANCA
G. Regulatory Information:
  1. <u>Regulation section:</u>
     21 CFR §866.5660 Multiple autoantibodies immunological test system
  2. <u>Classification:</u>
     Class II
  3. <u>Product code:</u>
     MVJ Devices, measure, antibodies to glomerular basement membrane (GBM)
  4. <u>Panel:</u>
     Immunology 82
H. Intended Use:
  1. <u>Intended use(s):</u>
     The Zeus Scientific, Inc. AtheNA Multi-Lyte ANCA Test System is intended for the qualitative and semi-quantitative detection of IgG class antibody to 3 separate ANCA 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 anti-neutrophil cytoplasmic antibodies (ANCA). 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.**
  2. <u>Indication(s) for use:</u>
     The Zeus Scientific, Inc. AtheNA Multi-Lyte® GBM Test System as part of the Zeus Scientific, Inc. AtheNA Multi-Lyte ANCA Test System is intended for the qualitative and semi-quantitative detection of IgG class antibodies to glomerular basement membrane in human serum. The test system is intended to be used as an aid in the diagnosis of Goodpasture's Syndrome.
  3. <u>Special conditions for use statement(s):</u>
     For prescription use only

4. Special instrument requirements:
   The reagents are for use on the Zeus Scientific AtheNA Multi-Lyte analyzer.

I. **Device Description:**

The assay consists of a multiplexed bead suspension containing separate distinguishable 5.6 micron polystyrene bead sets that are conjugated with the following antigens: myeloperoxidase (MPO), proteinase 3 (PR3), and **glomerular basement membrane (GBM)**, each bead set conjugated with a different antigen. 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; phycoerythrin conjugated goat anti-human IgG (γ-chain specific); human positive serum control; human negative serum control; and diluent containing phosphate-buffered-saline. The test system also includes the following non-reactive components: a 96-well polystyrene assay plate, a 96-well dilution plate and a 96-well filtration plate.

J. **Substantial Equivalence Information:**

1. Predicate device name(s):
   INOVA QUANTA Lite GBM
2. Predicate 510(k) number(s):
   k984336
3. Comparison with predicate:

Similarities		
Item	Device: AtheNA ANCA	Predicate: QUANTA Lite GBM
Intended Use	For the qualitative and semi-quantitative detection of IgG class antibody to 3 separate ANCA Antigens (**GBM**, MPO and PR3) in human serum. The test system is intended to be used as an aid in the diagnosis of autoimmune vasculitic disorders characterized by elevated levels of anti-neutrophil cytoplasmic antibodies (ANCA). 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**	QUANTA Lite GBM is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of glomerular basement membrane (GBM) IgG antibodies in human serum. The presence of GBM antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of autoimmune renal disorders such as Goodpasture's syndrome.

Similarities		
Item	Device: AtheNA ANCA	Predicate: QUANTA Lite GBM
	Syndrome.	
Analyte	**Anti-GMB** autoantibodies (along with anti-PR3 and anti-MPO autoantibodies)	Anti-GMB autoantibodies
Sample type	Serum	same
Conjugate	Goat anti-human IgG	Goat anti-human IgG

Differences		
Item	Device	Predicate
Analytes detected	Anti-MPO, anti-PR3 and **anti-GBM**	Anti-GBM
Assay method	Flow cytometry based, microparticle fluorescent immunoassay	ELISA
Instrument	AtheNA Multi-Lyte analyzer	ELISA plate reader
Solid phase	Differentially colored, carboxylated microspheres	Polystyrene microtiter plate
Conjugate label	Phycoerythrin	Horseradish peroxidase
Chromogenic substrate	Not applicable	TMB
Stop solution	Not applicable	0.344M sulfuric acid
Wash	Sheath fluid reagent in the instrument	HRP wash concentrate
Interpretation of results	<100 AU/mL = negative 100-129 = equivocal >120 = positive	0-20 Units = negative 21-30 = weak positive >30 = moderate to strong positive
Control materials	Positive and negative serum controls	High and low positive controls and a negative control

K. Standard/Guidance Document Referenced (if applicable):
   None referenced

L. Test Principle:
   Diluted test sera are incubated in a vessel containing the multiplexed mixture of the bead suspension. 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. Phycoerythrin-conjugated goat anti-human IgG is added to the vessel and the plate in incubated. The conjugate will react with IgG antibody immobilized on the solid phase beads. The bead suspension is then analyzed by the AtheNA instrument. The bead sets 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).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
      To demonstrate intra-assay and inter-assay reproducibility, a panel of six samples was prepared: two negative, two strong positive, and two near the assay cut-off. 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. The study was performed for three days.

Intra-assay

	Strong positive		Near cut-off		Negative	
	1	2	3	4	5	6
Mean AU/mL	761.5	765.9	104.7	102.7	22.8	27.2
%CV range	6.0-12.4%	7.3-8.6%	9.6-14.3%	8.1-24.3%	[44-46.5%]	[39.1-45.9%]

Inter-assay

	Strong positive		Near cut-off		Negative	
	1	2	3	4	5	6
Mean AU/mL	761.5	765.9	104.7	102.7	22.8	27.2
%CV	10.4%	10.5%	16.5%	17.3%	[44.1%]	[46.%]

   b. Linearity/assay reportable range:
      The measuring range for the assay is 0-1100 AU/mL. It should be noted that the amount of fluorescence is related to the quantity of autoantibody present on the bead but in a non-linear fashion. The antibody concentrations correspond to fluorescent signal changes but are not directly proportional.
   c. Traceability, Stability, Expected values (controls, calibrators, or methods):
      There is no reference standard or method available for GMB antibodies. The multiplex bead suspension is stable for 12 months at 2-8° C; the phycoerythrin conjugate, controls and calibrators are stable for 18 months at 2-8° C; and the sample diluent is stable for 36 months at 2-8° C.
   d. Detection limit:
      Not relevant for this assay
   e. Analytical specificity:
      The 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 that were positive for various autoimmune and infectious disease antibodies. Of the 26 samples, all were negative for GBM antibodies. Additionally, 10 samples with high levels of MPO antibodies and 10 samples with high levels of PR3 antibodies were tested for cross-reactivity with the GBM beads. All 20 samples tested negative for GBM antibodies.

Interference from serum components was evaluated by testing 6 GBM antibody positive specimens. These 6 specimens were spiked with abnormal levels of hemoglobin, bilirubin, lipids, albumin, cholesterol, and triglycerides. One sample spiked with cholesterol and 2 with triglycerides showed interference. The user is instructed to avoid using lipemic samples.

f. Assay cut-off:

The clinical investigation included 115 specimens that were sent to a lab for ANCA (systemic vasculitides) testing and 115 specimens that were submitted for GBM (Goodpasture's syndrome) testing. The resulting data were used to demonstrate the expected outcome for such groups. Samples from a normal population consisting of healthy donors were used to establish and validate the cut-off for the assay. The cut-off were set at <100 AU/mL is negative; >120 AU/mL is positive; and 100 to 120 is considered equivocal.

2. Comparison studies:
   a. Method comparison with predicate device:
      Comparative studies were performed with 230 samples that include 115 samples from patients suspected of having Goodpasture's syndrome.

		ELISA			
		Positive	Negative	Equivocal*	Total
AtheNA	Positive	31	2	0	33
	Negative	1	195	1	197
	Equivocal*	0	0	0	0
	Total	32	197	1	230

* Equivocal result was omitted from the calculations

Positive percent agreement (PPA):   31/32 = 90.9%
Negative percent agreement (NPA):   195/197 = 99.0%
Overall agreement: 226/229 = 98.7%

   b. Matrix comparison:
      Both assays use serum as the only matrix.

3. Clinical studies:
   a. Clinical Sensitivity:
      Not determined
   b. Clinical specificity:
      Not determined
   c. Other clinical supportive data (when a. and b. are not applicable):
      Not applicable.
4. Clinical cut-off:
   See assay cut-off
5. Expected values/Reference range
   The expected value in the normal population is negative.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.