K100728

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Zeus Scientific Inc. 510(k) AtheNA Multi-Lyte Borrelia VlsE-1/ pepC10 Plus Test System Summary of Safety and Effectiveness

Section I: Administrative Information

1. Submission Purpose: Intent to market referenced device for the qualitative detection of VlsE-1 and pepC10 antibodies.
2. Measurand: VlsE-1 IgG and pepC10 IgM antibodies.
3. Type of Test: Sandwich Immunoassay.
4. Applicant: Zeus Scientific, Inc. , PO Box 38, Raritan, NJ 08869 (908)526-3744
5. Establishment Registration Number: 2242426
6. Contact: Ewa Nadolczak, Manager of Clinical Affairs (347)731-0402 enadolczak@zeusscientific.com
7. Proprietary Name: AtheNA Multi-Lyte® Borrelia VlsE-1/ pepC10 Plus Test System.
8. Established name: Borrelia serological reagents.

Section 2: Regulatory Information

1. Device Classification: Borrelia Serological Reagent
2. Class: Class 2
3. Product Code: LSR
4. Panel Microbiology
5. Form 3454: Appendix A
6. Device Hazard Analysis: Appendix B

Section 3: Intended Use

The Zeus Scientific, Inc AtheNA Multi-Lyte Borrelia VlsE-1/ pepC10 Plus Test System is a multiplexed sandwich immunoassay for the qualitative detection of IgG class antibody to recombinant VlsE-1 and the IgM class of antibody to synthetic pepC10 in human serum. The AtheNA Multi-lyte Borrelia VlsE-1/pepC10 Plus Test System is intended for use with the Luminex® 200 IS and the AtheNA Multi-Lyte data management package in testing serum samples from symptomatic patients or those with a history of Lyme Borreliosis. All positive specimens should be tested with a second-tier test such as Western Blot which if positive, is supportive evidence of infection with *B.burgdorferi*. Diagnosis of Borreliosis should be made based on the presence of *B.burgdorferi* antibodies, history, symptoms and other laboratory data. Negative first or second tier results should not be used to exclude Borreliosis.

Section 3A: Special Conditions for Use

The AtheNA Multi-Lyte Borrelia VlsE-1/ pepC10 Plus Test System is for *in vitro* diagnostic use only.

The AtheNA Multi-Lyte Borrelia VlsE-1/ pepC10 Plus Test System is for prescription use only.

Section 4: Device Description

The AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus Test System is a micro particle immunoassay intended for the qualitative detection of distinct IgG class antibody to VlsE-1 and distinct IgM antibody to pepC10. The assay is a multiplexed immunoassay designed to simultaneously detect, distinguish and identify IgG reactivity to recombinant VlsE-1 antigen and IgM reactivity to synthetic pepC10 antigen. The test system is comprised of the AtheNA Multi-Lyte test kit and the Luminex Corp instrument model number Luminex™ 200 IS and software version 3.

The AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus test system provides the following Components:

Reactive Reagents:

1. All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).
2. Multiplexed bead suspension 1. Ready to use, 5.5 mL bottle. The suspension contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with recombinant VlsE1 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.
3. Multiplexed bead suspension 2. Ready to use, 5.5 mL bottle. The suspension contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with the following antigens: recombinant VlsE-1 and synthetic pepC10.
4. Conjugate 1: Phycoerythrin conjugated goat anti-human IgG (γ chain specific). Ready to use, 15 mL amber bottle.
5. Conjugate 2: Phycoerythrin conjugated goat anti-human IgM (μ chain specific). Ready to use, 15 mL amber bottle.
6. Human positive serum controls. Two, 0.2 mL vials.
7. Human negative serum control. One, 0.2 mL vial.
8. SAVe Diluent®. One 50 mL bottle containing phosphate-buffered-saline. Ready to use. NOTE: the sample diluent will change color in the presence of serum.
9. Wash Buffer Concentrate: dilute 1 part concentrate + 9 parts deionized or distilled water. One bottle containing 10 X concentrate of phosphate buffered saline.

Non-Reactive Reagents:

1. One, 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

Instrument and Software

This assay is platform dependant and used in conjunction with the Luminex 100 IS and the AtheNA Multi-Lyte® Test System Data Analysis Package Version 3.0.

Materials required but not provided:

1. **AtheNA Multi-Lyte®** System (Luminex® instrument)
2. Pipettes capable of accurately delivering 10 to 200 µL
3. Multichannel pipette capable of accurately delivering (10 to 200 µL)
4. Reagent reservoirs for multichannel pipettes
5. Disposable pipette tips
6. Laboratory timer to monitor incubation steps
7. Small bath sonicator
8. Plate shaker capable of shaking at 800 RPM (optional for mixing)
9. Vacuum aspirator and vacuum manifold for washing the microspheres

Section 5: Substantial Equivalence

Examination of enclosed data indicates that the Zeus Scientific, Inc AtheNA Multi-Lyte Borrelia VlsE-1/pepC1- Plus Test System for the detection of IgG class antibody to VlsE-1 and IgM antibody to pepC10 is substantially equivalent to a commercially marketed test system which has been previously cleared by the FDA for *in vitro diagnostic use*.

1. Name of Predicate Device: Zeus Scientific *Borrelia burgdorferi* ELISA Test System
2. Manufacturer of Predicate Device: Zeus Scientific, Inc.
3. Methodology of Predicate: enzyme-linked immunosorbent assay

Section 5A: Interpretation of Results

Investigational Device: AtheNA Score: raw fluorescence is converted into intermediate AtheNA Unit values (iAU/mL). These values correspond to the amount of human IgG and/or human IgM antibody bound to the VlsE-1 and/or pepC10 antigen coated beads respectively. iAU/mL values are converted to a single outcome (AtheNA Score).

Investigational Device: AtheNA Scores
Negative: <1.0
Positive ≥1.0

Predicate Device: Index Value
Negative: ≤ 0.90
Equivocal: 0.91 to 1.09
Positive: ≥ 1.10

Section 5B: Comparison of Investigational Device to Predicate Device

The comparison of the AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus to the predicate device follows, including intended use and various aspects of the procedure.

Comparison of AtheNA Multi-Lyte VlsE-1 PepC10 Plus Test System versus Predicate ELISA

Characteristic	AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus		Predicate ELISA
Use	For in vitro diagnostic use only		For in vitro diagnostic use only
Intended Use	The Zeus Scientific, Inc AtheNA Multi-Lyte Borrelia VlsE-1/ pepC10 Plus Test System is a multiplexed sandwich immunoassay for the qualitative detection of IgG class antibody to recombinant VlsE-1 and the IgM class of antibody to synthetic pepC10 in human serum. The AtheNA Multi-lyte Borrelia VlsE-1/pepC10 Plus Test System is intended for use in testing serum samples from symptomatic patients or those with a history of Borreliosis.		qualitative detection of human (IgG and IgM) antibodies to individual proteins of *Borrelia burgdorferi* in human serum. This test system should only be used with patients with signs and symptoms that are consistent with Lyme disease. Equivocal or positive results must be supplemented by testing with a standardized Western Blot procedure. Positive supplemental results are supportive evidence of exposure to *B. burgdorferi* and can be used to support a clinical diagnosis of Lyme disease.
Assay	Immunoassay		Immunoassay
Detection Method	Fluorescent		Colorimetric
Solid Phase	Polystyrene micro particle		Polystyrene micro wells
Antigen Used	Recombinant VlsE-1 antigen and synthetic pepC10 antigen		Inactive Borrelia burgdorferi (B31 Strain)
Specimen Tested	Human Serum		Human Serum
Controls	Two PC and one NC		One PC and one NC
Calibration	Includes Intra-Well Calibration® that provides a separate calibration curve for every sample		One calibrator
Analyte Measured	Human IgG and IgM		Human IgG and IgM
Sample Dilution	1:21 in SAVe Diluent		1:21 in SAVe Diluent
Sample Incubation	30 +/- 10 minutes at room temperature		25 +/- 5 minutes at room temperature
Post Sample Wash	3x wash (vacuum filtration)		5X wash (manual or automated)
Conjugate	Goat anti-human IgG; *γ* chain specific Goat anti-human IgM; *μ* chain specific		Goat anti-human IgM/IgG
Conjugate Label	Phycoerythrin		horseradish peroxidase
Conjugate Incubation	30 +/- 10 minutes at room temperature		25 +/- 5 minutes at room temperature
Post Conjugate Wash	N/A		5X wash (manual or automated)
Substrate	N/A		TMB
Reading	Read the fluorescence on the beads		Read the optical density against the blank
Data Points	Read a minimum of 50 beads (events) for each bead in the bead mix.		Read one OD value for each control and sample
Math	Multi-point curve, regression analysis		Single point regression
Scale	Intra-Well Calibration determines a unit value for each sample from the regression curve. Unit values for each analyte are converted to a single AtheNA Score.		Calculate the index value of unknown samples by comparing their OD to the cut-off OD
Interpretation Criteria	Negative is < 1.0 AtheNA Score, Positive is ≥ 1.0 AtheNA Score		Negative is ≤ 0.90, equivocal is 0.91 to 1.09 and positive is ≥1.1

Section 6: Test Principle

The Zeus Scientific, Inc. **AtheNA Multi-Lyte** Borrelia VlsE1/pepC10 Plus Test System is designed to detect IgG class antibodies in human sera to VlsE1 antigen and IgM class antibodies to pepC10 antigen. The test procedure involves four incubation steps:

1. Test sera (properly diluted) are incubated in a filter plate well containing a multiplexed mixture of Bead Suspension-1. The multiplexed Bead Suspension-1 contains a mixture of distinguishable sets of polystyrene microspheres; one of these bead sets is conjugated with the VlsE1 antigen. The bead mix also contains one bead set designed to detect non-specific binding and four separate bead sets used for assay calibration. 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. Conjugate-1 is added to the micro titer well and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The microspheres are rinsed to remove unbound conjugate.
3. Bead Suspension-2 is added to the wells. The bead set contains beads conjugated with pepC10 and VlsE1 antigens. A second aliquot of test sera at the same dilution as in step 1 is added to the well and mixed. The bead and specimen suspension is incubated. Following incubation, the microspheres are rinsed to remove the non-reacting serum proteins.
4. Conjugate 2 is added to the micro titer well and the plate is incubated. The conjugate will react with IgM antibody immobilized on the solid phase in step 1 and step 3.
5. The entire 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*™ (see following page for an explanation of this technology), internal calibration bead sets are used to convert raw fluorescence into intermediate AtheNA Unit values (iAU/mL). These values correspond to the amount of human IgG and/or human IgM antibody bound to the VlsE-1 and/or pepC10 antigen coated beads respectively. iAU/mL values are converted to a single outcome (AtheNA Score).

Section 7: Analytical Performance

7A: Linearity

A strong positive sample was determined using a FDA cleared test system to determine the sample reactivity. A fresh, clean, dead negative sample was used as the diluent to prepare serial dilutions of the positive sample. Each dilution was tested in duplicate, the mean calculated and the result plotted. The linearity is acceptable if the R-squared value obtained through regression analysis is ≥0.90. The results of this study demonstrated that the dilutions recovered were within the acceptance criteria.

Section 7B: Analytical Specificity-Interfering Substances

The effect of potential interfering substances on sample results generated using the AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus test system was evaluated with the following possible interfering substances at two different concentrations: bilirubin, albumin, cholesterol, triglycerides, hemoglobin and intralipid. The quantity of analyte in each interfering substance is as follows:

1. Bilirubin: 1mg/dL (low), 15 mg/dL (high)
2. Albumin: 3.5 g/dL (low), 5 g/dL (high)
3. Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
4. Triglycerides: 150 mg/dL (low), 500 mg/dL (high)
5. Hemoglobin: 20 g/dL (low), 20 g/dL (high)
6. Intralipid: 300 mg/dL (low), 750 mg/dL (high)

Three samples each for VlsE-1 and pepC10 were chosen based on their performance on the AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus test system: (strongly reactive, weakly reactive and negative). The samples were exposed to the possible interfering substance, tested in duplicate and the mean iAU/mL was determined.

All samples showed less than a 20% change in signal in the Vlse-1 study with the exception of the borderline VlsE-1 sample which exhibited an increase in signal of 32% with the high spike of bilirubin and an increase in signal of 27% with the high spike of cholesterol. The negative VlsE-1 sample showed a reduction in signal of 36% with the high spike of hemoglobin, a change in signal of 23% with the low spike of bilirubin and 27% with the high spike of bilirubin, a change in signal of 25% with the low spike of cholesterol and 45% with the high spike of cholesterol and a change in signal of 45% with both the low and high spikes of triglycerides. The change of signal in these negative samples did not change the qualitative outcome, the results remained negative.

All samples showed less than a 20% change in signal in the pepC10 study with the exception of the borderline pepC10 sample which exhibited a reduction in signal of 24% with the high spike of hemoglobin and an increase in signal of 28% with the low spike of triglyceride.

Section 7C: Cross Reactivity

A study was conducted at Zeus Scientific to assess cross reactivity with the Athena Multi-Lyte Borrelia VlsE-1/pepC10 Plus test system using sera that were sero-positive to EBV VCA IgG, RF, ANA, Syphilis, CMV IgG, CMV IgM, Rubella, VZV IgM and Toxoplasma. ELISA, IFA and micro-particle immunoassay test systems manufactured by various companies for commercial distribution were used to determine the

sero-positivity of the samples. Ten samples for each possible cross-reactant were tested. None of the ninety samples showed cross-reactivity with any of the nine analytes tested.

AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus Cross Reactivity Study	
Possible Cross-Reactants	Positive Results/ Number Tested
EBV VCA IgG	0 / 10
ANA	0 / 10
Syphilis	0 / 10
CMV IgG	0 / 10
CMV IgM	0 / 10
Rubella IgG	0 / 10
Toxo IgG	0 / 10
VZV IgM	0 / 10
RF	0 / 10

Section 8: Comparative Data

A total of 410 prospective samples were tested at four sites for the presence of VlsE-1 and pepC10 using the AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus test system and predicate ELISA test system commercially marketed for the detection of Borrelia burgdorferi IgG and IgM antibodies namely the Zeus Scientific *Borrelia burgdorferi* ELISA Test System. These samples were submitted for *B. burgdorferi* antibody testing, sequentially numbered and archived. All positive samples were furthered tested using western blot methodology to confirm reactivity. 342 retrospective samples, acquired from various sources, were tested at three sites. Additional testing consisted of 100 low prevalence population samples, 300 endemic and 300 non-endemic control samples, 246 random samples submitted for Borrelia burgdorferi antibodies, 229 characterized serum samples and a panel of 40 characterized samples acquired from the CDC.

Summary of Specimens Included in Study:

Site of Testing	Number of Samples	Sample Status	
1	124	retrospective	previously screened positive by laboratory
1	107	prospective	submitted for B.burgdorferi antibody testing
2	118	retrospective	previously screened positive by laboratory
2	103	prospective	submitted for B.burgdorferi antibody testing
3	446	prospective	submitted for B.burgdorferi antibody testing in 2006
4	100	prospective	submitted for B.burgdorferi antibody testing
4	100	non-endemic controls	samples collected in non-endemic area for testing non-infectious in nature
3	150	endemic controls	samples from blood donors collected in endemic area (New England)
3	150	non-endemic controls	samples from blood donors collected in non-endemic area (New Mexico)
4	150	endemic controls	samples from blood donors collected in endemic area (New England)
4	150	non-endemic controls	samples from blood donors collected in non-endemic area (New Mexico)
3	21	characterized samples	samples from patients with clinical history of Borreliosis (acute)
3	50	characterized samples	samples from patients with clinical history of Borreliosis (convalescent)
3	158	characterized samples	78 paired samples from patients with clinical history of Borreliosis (acute & convalescent)

4	40	characterized samples	sample panel acquired from the CDC
Total	1967		

Section 8A: Expected results

Internal and external investigators assessed the device's performance with 756 masked samples prospectively collected from patients between the ages of 1 and 94 which were submitted for Lyme antibody testing. Site 1, a hospital laboratory located in the northeast tested 107 samples. Site 2, a hospital laboratory in the northeast tested 103 samples. The third clinical site was a state Department of Health located in the northeast. This facility tested 446 samples collected in the northeast. Demographics for 346 of the 756 samples were unavailable. Site 4, the manufacturer's research facility tested 100 samples collected in Connecticut.

AtheNA Multi-Lyte Vlse-1/pepC10 Results From the Prospective Study

Age	Specimen Group	Positive	Negative
1-9	Males	7	14
	Females	1	15
10-19	Males	1	13
	Females	1	10
20-29	Males		13
	Females		19
30-39	Males	1	19
	Females		30
40-49	Males	3	25
	Females	1	37
50-59	Males	3	41
	Females		34
60-69	Males	4	28
	Females	1	22
70+	Males	7	15
	Females	1	27
Age / Sex Unknown		1	15
Total:	Prospective Males	26	168
	Prospective Females	5	194
	Female age Unknown		1
	Age / Sex Unknown	144	215
	Total	175	581

Grand Total	756

Internal and external investigators assessed the device's performance with varying populations. The available patient demographics, volume of samples tested and the number of samples which tested positive for each population are summarized in the following table.

AtheNA Multi-Lyte Vlse-1/pepC10 Results from Other Populations

Populations	Number Tested	Gender		Age Range	Positive/ Tested
		Male	Female		
Characterized	229	*NA	NA	NA	171/229
Retrospective	242	128	113	4-85	191/242
Endemic Controls	300	NA	NA	NA	38/300
Non-Endemic Controls	400	NA	NA	NA	39/400

*Not available

Section 9: Clinical Studies

Clinical Data Generated for Submission: Method Comparison with Predicate Device
Clinical studies consisted of 1,967 serum samples evaluated at a total of four US sites. The following populations were tested.

1. Characterized Samples
2. Prospective Population
3. Retrospective Samples
4. CDC Lyme Panel
5. Endemic and Non-Endemic Control Samples
6. Precision and Reproducibility

PERFORMANCE CHARACTERISTICS
The clinical study consisted of 1,967 serum samples evaluated at four sites located in the United States. All serum samples evaluated for concordance were tested with the ELISA reference assay. Samples that were positive by ELISA were reference assay positive. Samples that were negative by ELISA were reference assay negative.

Study 1. Characterized Samples: 229 characterized serum samples were acquired and tested at a northeastern state Department of Health Laboratory. 21 samples were acute patients with a history of Borreliosis. 50 samples were from convalescent patients with a history of Borreliosis. 14 of these patients present with neurological, 2 with cardiac and 34 with arthritic symptoms, 79 samples were paired acute (culture proven, early acute Lyme disease) and early convalescent sera from these same patients.

Table 1. Characterized Samples. Summary of Comparative Testing Results.

	AtheNA Multi-Lyte				Predicate ELISA				Western Blot			
Clinical Diagnosis	Pos	Neg or Eqv	Total	% agreement with clinical diagnosis & 95%CI	Pos	Neg or Eqv	Total	% agreement with clinical diagnosis	Pos	Neg or Eqv	Total	% agreement with clinical diagnosis
Acute	21	0	21	100% (21/21) 86.7%-100%	21	0	21	100% (21/21) 86.7%-100%	20	1	21	95.2% (20/21) 76.2%-99.9%
Convalescent	47	3	50	94% (47/50) 83.5%-98.8%	50	0	50	100% (50/50) 94.2%-100%	43	7	50	86% (43/50) 73.3%-9$.2%
Culture (+) early acute	41	38	79	51.9% (41/79) 40.4%-63.3%	37	42	78*	47.4 (37/78) 36.0%-59.1%	31	22	53*	58.5% (31/53) 44.1%-71.9%
Early Convalescent	62	17	79	78.5% (62/79) 67.8%-86.9%	73	5	78*	93.6% (73/78) 85.7%-97.9%	62	13	75*	82.7% (62/75) 72.2%-90.4%
Total	171	58	229	74.7 (171/229) 68.5%-80.2%	181	47	227	79.7% (181/227) 73.9%-84.8%	156	43	199	78.4% (156/199) 72.0%-83.9%

*invalid sample

*blot results unavailable

Study 2. Prospective Population: A total of 756 unselected samples from patients with an order for a Lyme antibody test were included in the study. The samples submitted for Lyme antibody testing were sequentially numbered, de-identified and archived. After the collection, 103 samples were tested at a hospital laboratory located in the Mid-Atlantic, 100 samples were tested at a hospital laboratory in upper Connecticut, 107 samples were tested at a hospital laboratory in lower Connecticut and 446 samples were tested at a state Department of Health Lab also located in the northeast.

Table 2. Prospective Samples. Summary of Comparative Testing Results.

		Predicate ELISA (IgG/IgM)					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
AtheNA Multi-Lyte Vlse-1/pepC10 Plus							
	Positive	162	3	45	210	81.4% (162/199)	75.3-86.6
	Equivocal	0	0	0	0		

	Negative	31	6	509	546	91.4% (509/557)	88.7-93.6
	Site Total	193	9	554	756		

If available, western blot testing was performed on the discrepant results. 12/31 samples were negative by blot and 9/31 samples were positive by blot for sera which tested negative on the AtheNA Multi-lyte test system and positive by ELISA (10/31) samples had no blot data provided). The 3 equivocal samples by the predicate would be considered for second step Western blot testing along with positives.7/45 samples tested positive and 5/45 samples tested negative by blot for the discrepant samples that were positive on the AtheNA Multi-Lyte test system and negative by ELISA (33 samples had no blot data available).

Study 3. Retrospective Samples: 242 samples believed to have screened positive for *Borrelia burgdorferi* antibodies were tested at two external sites. 124 samples were tested in a hospital facility in Connecticut and 118 samples were tested in a Pennsylvania hospital laboratory.

Table 3. Retrospective Samples. Summary of Comparative Testing Results.

		Predicate ELISA (IgG/IgM)					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
AtheNA Multi-Lyte VIse-1/pepC10 Plus							
	Positive	180	7	4	191	80% (180/225)	74.2-85.0
	Equivocal	0	0	0	0		
	Negative	39	6	6	51	35.3% (6/17*)	17.3-59.0
	Site Total	219	13	10	242		

Western blot testing was done on discrepant results. 2/37 samples were negative by blot and 37/39 samples were positive by blot for sera which tested negative on the AtheNA Multi-lyte test system and positive by ELISA . 4/4 samples tested positive by blot for the discrepant samples that were positive on the AtheNA Multi-Lyte test system and negative by ELISA.
*Statistical significance evaluation can not be made on limited number of samples.

Study 4. CDC Characterized Lyme Panel: 40 samples of various reactivity were acquired from the CDC and evaluated internally at the manufacturer's site. 5 samples were from normal blood donors. 35 samples were from patients diagnosed with Borreliosis. The results of the testing are presented here as a means of conveying further information on the performance of this assay with a characterized serum panel. This does not imply an endorsement of the assay by the CDC.

Table 4. CDC Characterized Lyme Panel. Summary of Comparative Testing Results

	AtheNA Multi-Lyte Borrelia VIse-1/pepC10				Predicate ELISA				Western Blot			
Time From Onset	Pos	Neg	Total	% agreement with clinical diagnosis	Pos	Neg or Eqv	Total	% agreement with clinical diagnosis	Pos	Neg	Total	% agreement with clinical diagnosis
normals	0	5	5	100% (5/5)	0	5	5	100% (5/5)	0	5	5	100% (5/5)

0-1 month	3	0	3	100% (3/3)	3	0	3	100% (3/3)	3	0	3	100% (3/3)
1-2 months	5	4	9	55.6% (5/9)	8	1	9	88.9% (8/9)	6	3	9	66.7% (6/9)
3-12 months	11	5	16	68.8% (11/16)	13	3	16	81.3% (13/16)	11	5	16	68.8% (11/16)
> 12 months	6	1	7	85.7% (6/7)	7	0	7	100% (7/7)	6	1	7	85.7 (6/7)
Total	25	15	40	62.5% (25/40)	31	9	40	77.5% (31/40)	26	14	40	65.0% (26/40)

Analytical Specificity

Study 5. Analytical Specificity: Testing of normal population was done on 300 samples acquired from blood donors in the New England endemic area and 400 samples acquired from blood donors and individuals undergoing routine testing not infectious in nature in the New Mexico non-endemic area.

Table 5. Analytical Specificity.

Sample Type	Volume	Negative	Positive	% Positivity
Endemic	300	262	38	12.7%
Non-endemic	400	361	39	9.8%

***% positivity with the predicate was found to be: endemic =14.3%; non-endemic-= 6.5%.**

Section 9A: Precision Studies

Reproducibility

Assay reproducibility was evaluated at three external clinical sites. The study was conducted as follows: Five samples were identified and/or prepared (by Zeus Scientific, Inc.) for use in the study based upon their activity on the **AtheNA Multi-Lyte** assay. Selected samples were negative, near cut-off, low positive, and moderate and high positive. To assess reproducibility, on each day of testing, each sample was diluted twice and then each dilution was run in triplicate. This was done twice per day by two different technicians, and was repeated for five days.

Table 6. Summary Of Reproducibility

Panel	Sample	Mean	Within-Run		Within -Day		Between-Run		Between-Site		Total	
Member	N	AU/mL	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
VlsE-1 Negative	180	31	4.5	14.7	5.5	17.9	3.7	12.1	5.9	18.8	6.3	19.7
VlsE-1 Near Cut-off	180	110.4	12.8	11.6	14	12.6	7.5	6.7	15.2	13.3	16.6	13.5
VlsE-1 Low Positive	180	136.7	13.8	20.2	15.6	11.4	9.1	6.7	16.8	11.5	16.8	12.4
VlsE-1 Moderate Positive	180	312.7	24.5	7.7	32.7	10.2	26.5	8.2	43.4	9.7	49.8	10.2
VlsE-1 High Positive	180	1869	103.1	5.5	105.6	5.6	37.5	2	107.6	5.4	112.8	5.3
pepC10 Negative	180	23.6	1.9	7.8	2.3	9.8	7.5	6.4	3.4	10.2	3.7	11.1
pepC10 Near Cut-off	180	108.9	10	9	11	10	5.4	4.9	13	10.3	13	10.4
pepC10 Low Positive	180	150.8	10.5	6.9	15.2	9.9	12.7	8.1	17.9	9.5	20.3	10

pepC10 Moderate Positive	180	192.3	12.8	6.8	15.6	8.1	10.7	5.5	23	8	124.3	8.1
pepC10 High Positive	180	1222.0	58.1	4.7	70.8	5.7	49	3.8	93.4	5.9	129.3	6.2

Precision

Assay repeatability was evaluated at the manufacturer site. The study was conducted as follows: six samples were identified and/or prepared (by Zeus Scientific, Inc.) for use in the study based upon their activity on the **AtheNA Multi-Lyte** assay. Selected samples were negative, high negative, near cut-off, low positive, and moderate and high positive. On each day of testing, the samples were diluted twice and tested. This was repeated in a second run on the same day by a different technologist for a total of twelve days. This study is summarized in iAU to assess detailed individual bead performance.

Table 7. Summary of Repeatability

Panel Member	Sample N	Mean AU/mL	Within Run		Within Day		Total	
			SD	%CV			SD	%CV
VlsE-1Negative 1	48	37.9	4.5	11.4	9.2	25.1	5.7	29.7
VlsE-1 high negative	48	91.7	6.4	7.1	7.1	7.7	10.5	11.4
near cut-off	48	119.6	8.1	6.6	9.9	8.3	13.1	11.0
low pos	48	129.8	8.2	6.4	11.6	9.0	14.4	11.1
mod pos	48	157.3	11.3	7	11.1	6.9	15.1	9.6
high pos	48	2031	111.1	5.4	119.8	5.9	139.7	6.9
pepC10 Negative 1	48	36.8	3.9	10.5	4.1	11.0	5.1	13.1
pepC10high neg	48	95.2	6.3	6.3	8.9	9.4	11.3	11.8
pepC10 near cut-off	48	119.4	7.4	6.1	7.9	6.6	11.4	9.5
pepC10 low pos	48	130.4	9.7	7.4	10.5	8.0	12.2	9.4
pepC10 mod pos	48	295.4	28.4	9.5	34.0	11.5	41.1	13.9
pepC10 high pos	48	1207.2	38.4	3.2	52.8	4.4	65	5.4

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
10903 New Hampshire Avenue
Document Mail Center – WO66-0609
Silver Spring, MD 20993-0002

Zeus Scientific, Inc.
C/O Ewa K. Nadolczak
Manager, Clinical Affairs
P.O. Box 38
Raritan, NJ 08869

JUL 0 6 2010

Re: k100728
Trade/Device Name: AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus Test System
Regulation Number: 21 CFR 866.3830
Regulation Name: Treponema pallidum treponemal test reagents
Regulatory Class: Class II
Product Code: LSR
Dated: March 10, 2010
Received: April 9, 2010

Dear Ms. Nadolczak:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): k 100728

Device Name: AtheNA Multi-Lyte® Borrelia VLSe-1/pepC10 Plus Test System

Indications for Use:

The Zeus Scientific, Inc AtheNA Multi-Lyte Borrelia VlsE-1/ pepC10 Plus Test System is a multiplexed sandwich immunoassay for the qualitative detection of IgG class antibody to recombinant VlsE-1 and the IgM class of antibody to synthetic pepC10 in human serum. The AtheNA Multi-lyte Borrelia VlsE-1/pepC10 Plus Test System is intended for use with the Luminex® 200 IS and the AtheNA Multi-Lyte data management package in testing serum samples from symptomatic patients or those with a history of Lyme Borreliosis. All positive specimens should be tested with a second-tier test such as Western Blot which if positive, is supportive evidence of infection with *B.burgdorferi.* Diagnosis of Borreliosis should be made based on the presence of *B.burgdorferi* antibodies, history, symptoms and other laboratory data. Negative first or second tier results should not be used to exclude Borreliosis.
This kit is for *in vitro* diagnostic use only.

Prescription Use X (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) k 100728