510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K100728

B. Purpose for Submission: New device

C. Measurand: IgG/IgM antibodies to VlsE-1/pepC10 proteins of *Borrelia burgdorferi*

D. Type of Test: Multiplexed Microbead Immunoassay

E. Applicant: Zeus Scientific Inc.

F. Proprietary and Established Names: AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus Test System

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3830, Treponema pallidum treponemal test reagents
2. Product code: LSR; Reagent, Borrelia Serological Reagent
3. Classification: Class: II
4. Panel: 83 Microbiology

H. 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 Lyme 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.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s): For prescription use only

4. Special instrument requirements: Luminex® 200 IS and the AtheNA Multi-Lyte

data management package

I. Device Description:

The Zeus Scientific, Inc. AtheNA Multi-Lyte Borrelia VlsE-1/pepC10 Plus Test System is designed to detect IgG class antibodies in human sera to VlsE-1 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 VlsE-1 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 microtiter 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 VlsE-1 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 microtiter 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*™, internal calibration bead sets are used to convert raw fluorescence into outcome (AtheNA Score).

Detection of antigen specific IgG and IgM antibodies: After the bead set mix is allowed to react with human serum as described in steps 1 through 4, human antibodies if present, will bind to the bead sets and then will be analyzed. The amount of reporter is a direct measurement of IgM, IgG or both IgM and IgG if a patient has both IgG and IgM to the same antigen. The amount of IgG in such an instance can be determined by a mathematical formula of like-coated bead set results using a sequence of measurements patented by Zeus Scientific, Inc**.**

J. Substantial Equivalence Information:

1. Predicate device name(s): Zeus Scientific *Borrelia burgdorferi* ELISA Test System

2. Predicate 510(k) number(s): K885317

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended use	See above for the complete Intended Use statement (for the detection of IgG and IgM antibodies to *B. burgdorferi* as a 1-step assay. All positives should be tested by Western blot)	For the 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.
Specimen type	Serum	Serum
Method	Qualitative	Qualitative
Differences		
Item	**Device**	**Predicate**
Type of assay	Multiplex Microbead Immunoassay (MMIA) based on Luminex XMAP technology.	ELISA
Detection Method	Fluorescent	Colorimetric
Antigens	VlsE-1 and pepC10 proteins of *Borrelia burgdorferi*	Whole cell extract of *Borrelia burgdorferi* antigens

K. Standard/Guidance Document Referenced (if applicable): Not applicable

L. Test Principle: Multiplexed flow immunoassay

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

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.

Table .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

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 .Summary of Reproducibility

Panel Member	Sample N	Mean AU/mL	Within-Run		Within -Day		Between-Run/day		Between-Site		Total	
			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

b. *Linearity/assay reportable range:* Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Not applicable

d. *Detection limit:* Not applicable

e. *Analytical specificity:* 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. The cross reactivity data has been summarized in table 12. In total, 90 samples were tested for possible cross reactivity with 9 analytes. None of the ninety samples showed cross-reactivity with any of the nine analytes tested.

Table: Cross Reactivity Summary

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

Specimens known to contain potentially cross reactive antibodies to *B.burgdorferi* with infections to tick-borne relapsing fever, rickettsial diseases, ehrlichiosis, babesiosis, and leptospirosis have not been tested with this device, therefore the performance of this device is unknown if there is any cross-reactivity with these antibodies. This is indicated in the limitation section of the package insert.

Interfering Substances: The effect of potential interfering substances on sample results generated using the AtheNA Multi-Lyte VlsE-1/pepC10 Plus test system was evaluated with the following possible interfering substances: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids.

The quantity of analyte in each interfering substance is as follows:

Bilirubin: 1mg/dL (low), 15 mg/dL (high)
Albumin: 3.5 g/dL (low), 5 g/dL (high)
Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
Triglycerides: 150 mg/dL (low), 500 mg/dL (high)
Hemoglobin: 20 g/dL (low), 20 g/dL (high)
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 mean 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. 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.

f. Assay cut-off:

The cut off for this assay was established using 25 negative control specimens as well as 5 clinically characterized specimens for each antigen. The mean and standard deviation was established for the negative population; using a mathematical calculation involving this data, a theoretical cut-off is established and validated with the characterized specimens. Based upon the results of this testing, the manufacturer has established the following guidelines for interpretation of patient samples.

AtheNA Score	Result	Interpretation
< 1	**Negative**	An AtheNA Multi-Lyte® score of < 1indicates no antibodies to VlsE1 and pepC10 detected. If exposure to *B. burgdorferi* is suspected, a second sample should be collected and tested two to four weeks later.
≥1	**Positive**	An AtheNA Multi-Lyte® score of ≥ 1 indicates that antibodies specific to *B.burgdoferi* were detected. This indicates presumptive evidence of probable exposure.

2. Comparison studies:

   a. *Method comparison with predicate device:*

Clinical Studies and Method Comparison with a Commercially Marketed ELISA Predicate Device: The clinical studies 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 (IgG/IgM) reference assay. The following populations were tested at a total of four clinical sites: Sites 1 and 2 were hospital laboratories located in the northeast. The third clinical site was a state Department of Health Laboratory located in the northeast and site 4 was the manufacturer's research facility.

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

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. Characterized Samples: Summary of Comparative Testing Results

Clinical Diagnosis	AtheNA Multi-Lyte				Predicate ELISA(IgG/IgM)				Western Blot(IgG and/or IgM)			
	Pos	Neg	Total	% agreement with clinical diagnosis & 95%CI	Pos	Neg or Eqv	Total	% agreement with clinical diagnosis	Pos	Neg	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%

*one sample invalid

*blot results unavailable for all 79 samples

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. Prospective Samples: Summary of Comparative Testing Results

		Predicate ELISA (IgG/IgM)					
		Positive	**Equivocal**	**Negative**	**Site Total**	**PPA NPA**	**95% CI**
Multi-Lyte VIse-1/pepC10	**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. Retrospective 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	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.

CDC Characterized Lyme Panel: 40 samples of various reactivity were acquired from the CDC and evaluated internally at the manufacturer' 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. CDC Characterized Lyme Panel: Summary of Comparative Testing Results

	AtheNA Multi-Lyte Borrelia Vlse-1/pepC10				Predicate ELISA(IgG/IgM)				Western Blot (IgG and/or IgM)			
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: 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. Analytical Specificity

Sample Type	Number	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%.

b. Matrix comparison: Not applicable

3. Clinical studies:

   a. Clinical Sensitivity: Not applicable

   b. Clinical specificity: Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):
   See comparison study

4. Clinical cut-off**:** Not applicable

5. Expected values/Reference range:

   Demographics and Age Distribution in a Prospective Population: 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. The available patient demographics and age distribution for 410 of the 756 samples are summarized below followed by expected results in the prospective population.

   Summary of Patient Demographics (Prospective Samples)

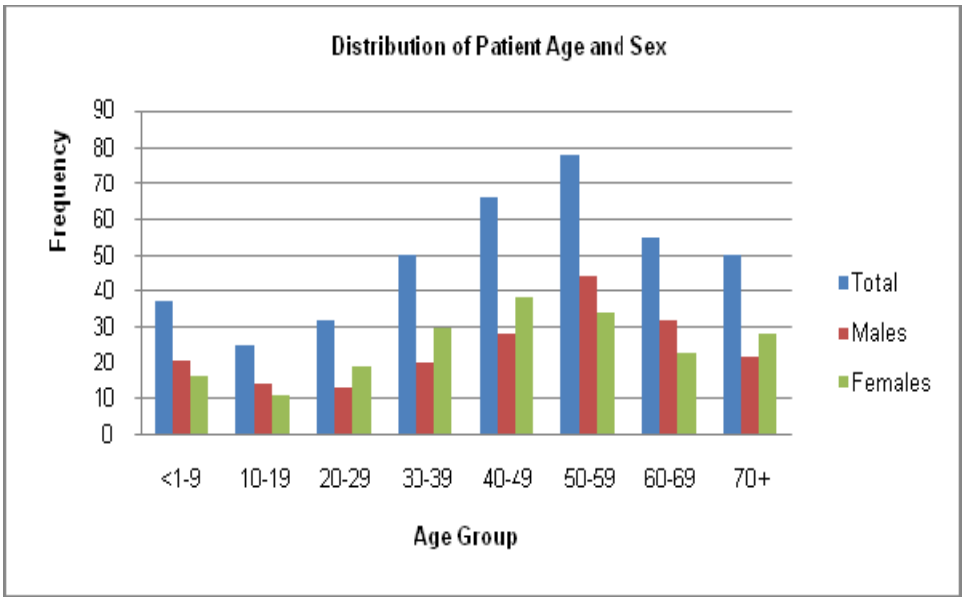

Table. Results from the Prospective Study

Age	Specimen Group	Positive	Negative
Total:	Males	26	168
	Females	5	194
	Female age Unknown		1
	Age / Sex Unknown	144	215
	Total	175	581
Grand Total			756

Other Studies: 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 below.

Table. 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

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.