Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K190907

B Applicant

ZEUS Scientific Inc.

C Proprietary and Established Names

Device 1: ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System
Device 2: ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System

D Regulatory Information (both devices):

Product Code(s)	Classification	Regulation Section	Panel
LSR	Class II	21 CFR 866.3830 -	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination and FDA clearance for modified intended uses of 2 previously cleared medical devices. This regulatory filing follows the FDA guidance document titled "Bundling Multiple Devices or Multiple Indications in a Single Submission"[1]. For these devices, bundling is appropriate since the device review presented scientific and regulatory issues that were most efficiently addressed during a single review. In determining whether a bundled submission was appropriate FDA considered that: (i) the supporting data are similar; (ii) primarily one review division/group will be involved; and (iii) the devices or indications for use are similar.

B Measurand:

Anti-*Borrelia burgdorferi* (IgM and/or IgG) antibodies

[1] https://www.fda.gov/media/73500/download

C Type of Test:

Enzyme-linked immunosorbent assay (ELISA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System

The ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System is intended for the qualitative detection of IgG and IgM class antibodies to VlsE1 and pepC10 antigens from *Borrelia burgdorferi* in human serum. The assay is intended for testing serum samples from symptomatic patients or those suspected of having Lyme disease.

Positive and equivocal test results with the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System for the presence of *Borrelia burgdorferi* antibodies must be confirmed through additional testing by one of the following methods:

(1) Standard two-tier test methodology (STTT) using IgG or IgM Western blot testing following current interpretation guidelines;

or

(2) Modified two-tier test methodology (MTTT) using one of the following three ELISA-based assays:
- ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System
- ZEUS ELISA *Borrelia burgdorferi* IgM Test System
- ZEUS ELISA *Borrelia burgdorferi* IgG Test System

Positive test results by either the STTT or MTTT methodology are supportive evidence for the presence of antibodies and exposure to *Borrelia burgdorferi*, the cause of Lyme disease. A diagnosis of Lyme disease should be made based on the presence of *B. burgdorferi* antibodies, history, symptoms, and other laboratory data.

Zeus ELISA *Borrelia burgdorferi* IgG/IgM Test System

The ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG and IgM class antibodies to Borrelia burgdorferi in human serum. The assay is intended for testing serum samples from symptomatic patients or those suspected of Lyme Disease.

Positive and equivocal test results with the ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System for the presence of *Borrelia burgdorferi* antibodies must be confirmed through additional testing by one of the following methods:

(1) Standard two-tier test methodology (STTT) using IgG or IgM Western blot testing following current guidelines;

or

(2) Modified two-tier test methodology (MTTT) using the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System.

Positive test results by either the STTT or MTTT methodology are supportive evidence for the presence of antibodies and exposure to *Borrelia burgdorferi*, the cause of Lyme disease. A diagnosis of Lyme disease should be made based on the presence of *Borrelia burgdorferi* antibodies, history, symptoms, and other laboratory data.

C Special Conditions for Use Statement(s):

For Prescription Use Only

D Special Instrument Requirements:

N/A

IV Device/System Characteristics:

A Device Description:

The ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System is designed to detect IgG and IgM class antibodies in human sera to VlsE1 and pepC10 antigens. The test procedure involves three incubation steps:

1. Diluted test sera are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgG and IgM is added to the wells and the plate is incubated. The conjugate will react with IgG and/or IgM antibodies immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microwells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution is proportional to the antibody concentration in the original test sample.

The ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System is designed to detect IgM and IgG class antibodies to *B. burgdorferi* in human sera. The wells in the plastic strips were sensitized by passive adsorption with *B. burgdorferi* whole cell antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgM/IgG is added to the wells and the plate is incubated. The conjugate will react with IgM and/or IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microwells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution proportional to the antibody concentration in the original test sample.

B Principle of Operation:

Enzyme Immunoassay

V Substantial Equivalence Information:

A Predicate Device Name(s):

ZEUS ELISA Borrelia VlsE-1/pepc10 IgG/IgM Test System and ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System

B Predicate 510(k) Number(s):

K113397 and K885317

C Comparison with Predicate(s):

Device & Predicate Device(s):	K190907	K113397	K885317
Device Trade Names	(Device 1): ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System (Device 2): ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System	ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System	ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System

General Device Characteristic Similarities			
Summary of the Intended Use/ Indications for Use	These are two ELISA assays for the qualitative detection of *B. burgdorferi* antibodies in human serum. For a complete description of the Intended Use please see Item III B. above.	The ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System is intended for the qualitative detection of IgG and IgM class antibodies to VlsE1 and pepC10 antigens from *Borrelia burgdorferi* in human serum. The assay is intended for testing serum samples from symptomatic patients or those suspected of having Lyme disease. Positive and equivocal test results with the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System for the presence of *Borrelia burgdorferi* antibodies must be confirmed by a Western blot.	The ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG/IgM class antibodies to *Borrelia burgdorferi* in human serum. The assay is intended for testing serum samples from symptomatic patients or those suspected of Lyme disease. Positive and equivocal test results with the ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System for the presence of *Borrelia burgdorferi* antibodies must be confirmed by Western blot
Sample Type	Serum	Same	Same
Assay Type	Qualitative	Same	Same
Instrumentation	None, manual assay	Same	Same
General Device Characteristic Differences			
Assay Technology	ELISA	Same	Same
Antigens	VlsE, pepC10, and whole cell extract of *B. burgdorferi*	VlsE, pepC10 antigens of *B. burgdorferi*	whole cell extract of *B. burgdorferi*
Signal Detection	Colorimetric	Same	Same

VI Standards/Guidance Documents Referenced:

N/A

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Note: *This clearance is for a modified use for two previously cleared IVDs, the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System (K113397) and the ZEUS ELISA Borrelia burgdorferi IgG/IgM Test System (K885317). Representative analytical study data are presented below.*

1. Precision/Reproducibility:
   a) Reproducibility: a five-day reproducibility study at three external sites was conducted with assay samples representing Negative, High Negative, Low Positive, and Moderate Positive reactivity with ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System. Results are presented in Table 1 below.

Table 1: Reproducibility Study Results

Panel Member		Sample Size (n)	Mean IV	Within-Run		Within -Day		Between-Run		Between-Site		Total	
				SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Sample 3	Moderate +	180	2.56	0.14	5.3	0.17	6.4	0.11	4.3	0.19	7.3	0.19	7.4
Sample 6	Low +	180	1.36	0.09	6.6	0.11	8.2	0.08	6.1	0.12	8.7	0.12	8.9
Sample 7	High -	180	0.74	0.05	6.6	0.06	7.8	0.04	4.9	0.06	8.1	0.06	8.1
Sample 10	Negative	180	0.25	0.03	11.5	0.04	14.6	0.03	10.1	0.04	15.2	0.04	15.8
Control	Negative	180	0.16	0.01	7.9	0.02	10.7	0.01	8.7	0.02	11.5	0.02	11.7
Calibrator	Positive	180	2.34	0.12	5.3	0.13	5.5	0.03	1.4	0.13	5.6	0.13	5.6
Control	Positive	180	4.23	0.26	6.0	0.28	6.6	0.12	2.8	0.34	8.0	0.34	8.1

   b) Repeatability: A 20-day repeatability study was conducted at the manufacturer's site with assay samples representing Negative, High Negative, Low Positive, and Moderate Positive reactivity with ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System. Results of the repeatability study are presented in Table 2 below.

Table 2: Repeatability Study Results

Panel Member		Sample Size (n)	Mean IV	Within-Run		Within -Day		Between-Run		Total	
				SD	% CV	SD	% CV	SD	% CV	SD	% CV
Sample 3	Moderate +	80	2.28	0.09	4.1	0.17	7.4	0.16	7.0	0.22	9.5
Sample 6	Low +	80	1.32	0.08	5.8	0.11	8.1	0.08	6.1	0.12	9.3
Sample 7	High +	80	0.72	0.04	5.2	0.05	7.2	0.04	5.7	0.08	10.9
Sample 12	Negative	80	0.32	0.03	7.9	0.04	11.5	0.03	8.7	0.06	20.0
Control	Negative	80	0.18	0.02	11.6	0.03	14.9	0.02	10.0	0.03	18.0
Calibrator	Positive	80	2.34	0.19	7.9	0.20	8.5	0.09	4.0	0.23	9.8
Control	Positive	80	4.41	0.15	3.4	0.31	6.9	0.31	6.9	0.34	7.7

2. Analytical Specificity/Interference:

Cross Reactivity: A study was conducted to assess cross reactivity with the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System using patient sera that was seropositive to different microorganisms and samples from patients diagnosed with certain disease conditions. The results are shown in Table 3.

Table 3: Cross Reactivity Results

Cross Reactivity Study	
Possible Cross-Reactant	**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
Rheumatoid Arthritis	0/10
Parvovirus	1/10
Fibromyalgia	0/10
Multiple Sclerosis	0/10
Infection with *H. pylori*	2/10

Interfering Substances: The following potentially interfering substances were evaluated in serum to establish their effect on the investigational device: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. The concentration of analyte of each potential interfering substance was as follows:

Bilirubin: 1mg/dL (low), 15mg/dL (high)
Albumin: 3.5g/dL (low), 5g/dL (high)
Cholesterol: 150mg/dL (low), 250mg/dL (high)
Triglycerides: 150mg/dL (low), 500mg/dL (high)
Hemoglobin: 10g/dL (low), 20g/dL (high)
Intralipid: 300mg/dL (low), 750mg/dL (high)

Three clinical samples exhibiting differing reactivities with the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System were tested for interference with each substance listed in Table 3: a positive sample, a negative sample, and a borderline sample. All positive samples exhibited a change of signal less than 15% when tested with each potential interferant. Borderline samples showed a change of signal of less than 15% with all the interferants with the exception of testing with low and high concentrations of hemoglobin, where a reduction of signal greater than 15% (16.5% and 17.3% respectively) was observed. All negative samples showed a change of signal less than 15% except for the sample spiked with a low concentration of hemoglobin which exhibited a 22.2% reduction in signal.

3. Assay Reportable Range: N/A

4. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods): N/A

5. Detection Limit: N/A

6. Assay Cut-Off: N/A

B Comparison Studies: Method comparison approaches were used to establish test performance as published literature from multiple independent sources provided scientific support that these modifications are expected to yield clinically relevant information.

1. Method Comparison Studies

Clinical performance was established by two identical method comparison studies using different cohorts, where the modified two-tier test (MTTT) was compared to the standard two-tiered testing (STTT). All samples were tested with the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System; samples testing positive or equivocal by the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System were subsequently tested with either the EIA *Borrelia burgdorferi* IgG/IgM Test System (MTTT) or a *B. burgdorferi* IgM and/or IgG Western blot (STTT). A positive *B. burgdorferi* IgM Western blot result was defined by at least 2 out of 3 positive antibody bands and IgG Western blot result was defined by at least 5 out of 10 positive antibody bands.

The STTT and MTTT methodologies used in the method comparison studies are diagrammed below in Figures 1 and 2:

Fig. 1: STTT IgM Western Blot Algorithm

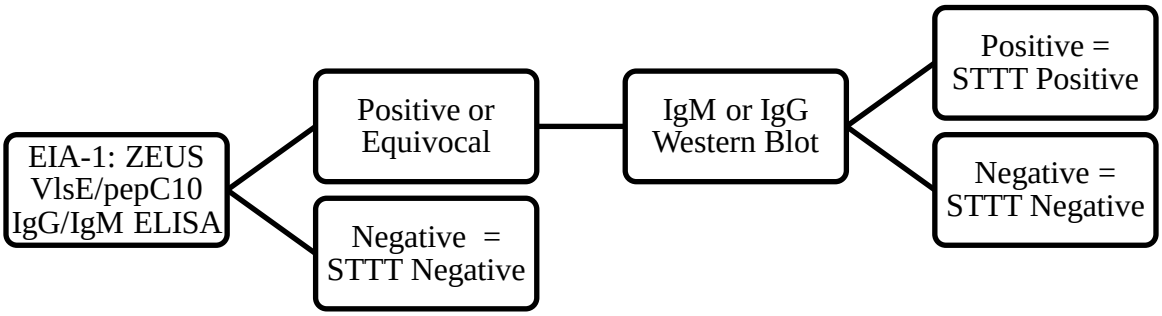
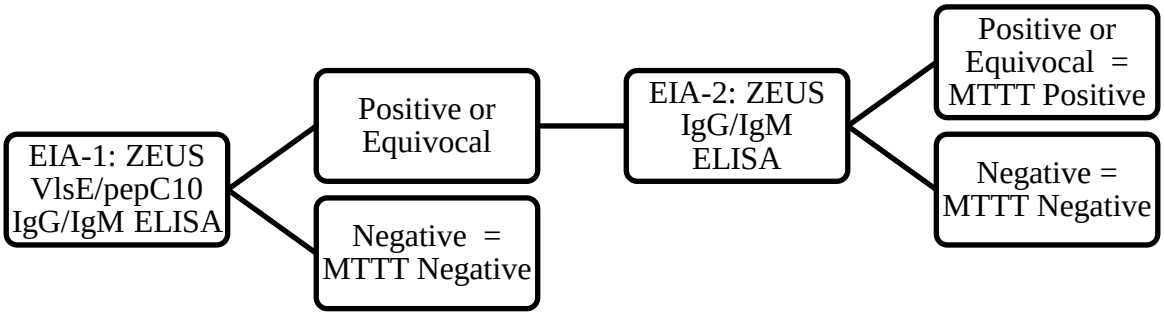


Fig. 2: MTTT IgM EIA Algorithm



Studies were performed with a retrospective cohort and a prospective cohort.

Retrospective Cohort Testing: A 356-sample retrospective cohort consisting of a 280 member samples obtained from CDC supplemented by an additional 46 Stage 2 Lyme Disease (LD) specimens and an additional 30 Stage 3 LD specimens. Overall, the retrospective panel consisted of specimens from 166 patients known to have LD (60 Stage 1, 56 Stage 2 and 50 Stage 3), 90 specimens from patients with diseases other than LD, and 100 specimens from healthy controls in endemic and non-endemic answer (50 specimens from each group).

The 356 retrospective samples were first tested with the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System yielding 160 positive samples and 6 samples with equivocal results. The 166 positive and equivocal samples were then tested by the IgM and/or IgG Western blot (STTT) or the ZEUS IgM ELISA (MTTT). Table 4 shows the outcome of the MTTT and WB-STTT test results.

Table 4: Comparison of EIA-MTTT and WB-STTT Results for Retrospective Cohort

	Stage I (n=60)		Stage II (n=56)		Stage III (n=50)		Healthy Controls (n=100)		Disease Controls (n=90)	
	STTT-IgG/IgM	**MTTT-IgG/IgM**	**STTT-IgG/IgM**	**MTTT-IgG/IgM**	**STTT-IgG/IgM**	**MTTT-IgG/IgM**	**STTT-IgG/IgM**	**MTTT-IgG/IgM**	**STTT-IgG/IgM**	**MTTT-IgG/IgM**
Positive	38	47	34	37	50	50	0	0	0	2
Negative	22	13	22	19	0	0	100	100	90	88
Sensitivity	**63.3%**	**78.3%**	**60.7%**	**66.1%**	**100%**	**100%**	N/A	N/A	N/A	N/A
Specificity	N/A	N/A	N/A	N/A	N/A	N/A	**100%**	**100%**	**100%**	**97.8%**

Prospective Cohort Testing: A prospectively collected cohort of 2932 patient serum samples was elected from samples sent for routine LD testing from three different areas in the US considered endemic for LD. Specimens were both collected and tested at two of the three sites (Massachusetts and Minnesota); at the third site (Wisconsin), specimens were collected and then sent to the manufacturer for the testing. The distribution of specimens from the three collection sites is summarized in Table 5 below:

Table 5: Summary of the Prospective Specimen Cohort.

Geographic Location:	**n**
Massachusetts	900
Wisconsin	990
Minnesota	1042
Total	**2932**

Testing of the 2,932 samples by the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System resulted in 363 positive and 58 equivocal results. The 421 positive or equivocal samples were then tested with a *B. burgdorferi* IgM and/or IgG Western blot (STTT) and second EIA, the ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System (MTTT); for the MTTT, specimens which returned an equivocal or positive result from the ZEUS ELISA *Borrelia burgdorferi* IgG/IgM Test System were considered MTTT positive. A summary of the outcome of STTT versus the MTTT appears in Table 6 below:

Table 6: EIA-MTTT Method compared to WB-STTT Method for the Prospective Cohort

		WB-STTT(IgG and/or IgM)		
		Positive	Negative	Total
EIA-MTTT (IgG/IgM)	Positive	167	63**	230
	Negative	12*	2690	2702
Total		179	2753	2932
Positive agreement: 93.3% (167/179)			95% CI: 88.6% – 96.12%	
Negative agreement: 97.7% (2690/2753)			95% CI: 97.1% – 98.2%	

*Of the 12 samples that were STTT positive/MTTT negative, one of the 12 was confirmed to be a case of Stage 1 Lyme Disease. One sample had no clinical information available and the remaining ten did not have clinical information consistent with Lyme disease.

**Of the 63 samples that were MTTT positive/STTT negative, four samples were from confirmed cases of Lyme disease (three Stage 1 and one late disease). Thirty two samples had no clinical information available and the remaining twenty seven specimens did not have clinical information consistent with Lyme disease.

2. Matrix Comparison: N/A

C Clinical Studies:

1. Clinical Sensitivity: N/A

2. Clinical Specificity: N/A

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable): N/A

D Clinical Cut-Off: N/A

E Expected Values/Reference Range:

Available patient demographics, total number of samples tested and the number of positive samples for each population in the clinical studies conducted to establish the performance characteristics of ZEUS ELISA *Borrelia* VlsE1/pepC10 IgG/IgM are summarized in Table 7.

Table 7: ZEUS ELISA *Borrelia* VlsE1/pepC10 IgG/IgM Study Demographics

Populations	Number Tested	Gender		Age Range	Positive/Tested
		Male	Female		
Prospective Sample Study	775	297	474	3 - 105	63/775*
Characterized Sample Study	100	58	42	3 - 91	92/100
Endemic Controls	200	78	122	5 - 99	10/198
Non-Endemic Controls	200	100	100	18 - 88	3/200

* Demographic information for sex was missing for four subjects.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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