510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K230863

B Applicant

ZEUS Scientific

C Proprietary and Established Names

ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System; ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LSR, QCH	Class II	21 CFR 866.3830 - Treponema Pallidum Treponemal Test Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System and the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit.

B Measurand:

IgM and/or IgG antibodies to *Borrelia burgdorferi*

C Type of Test:

Chemiluminescence immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System

The ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG/IgM antibodies to *Borrelia burgdorferi* in human serum. This assay is intended for use on samples from patients with signs and symptoms consistent with or patients suspected of having Lyme disease to assess the presence of IgG/IgM antibodies.

Positive results with the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System should be supplemented with additional testing with a Standard two-tier test (STTT) methodology using an IgG and/or IgM *Borrelia burgdorferi* immunoblot assay following current guidelines.

Positive supplemental results are supportive evidence of the presence of antibodies and exposure to *Borrelia burgdorferi* and may be used along with patient history, symptoms and other laboratory data to support a clinical diagnosis of Lyme disease.

Negative results by the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System should not be used to exclude Lyme disease. The test must be performed on the ZEUS Solinas α instrument.

ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit

The ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit is intended for use as assayed quality control samples to monitor the performance of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System. The performance characteristics of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit have not been established for any other assays or instrument platforms different from the ZEUS Solinas α instrument.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

ZEUS Solinas α instrument

IV Device/System Characteristics:

A Device Description:
The ZEUS Solinas VlsE1/pepC10 IgG/IgM Test System uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG/IgM antibodies to *Borrelia burgdorferi* in human serum. This assay is intended for use on samples from patients with signs and symptoms

consistent with or patients suspected of having Lyme disease to assess the presence of IgG/IgM antibodies. The test is performed in the Solinas α instrument.

The ZEUS Solinas α instrument is an automated immunoassay analyzer. Analyte detection relies on chemiluminescent technology using acridinium ester-labeled immunoglobulins. The system completely automates mixing of the test reagents and samples in a cuvette and measurement of the resulting luminescent reaction with a luminescent reader.

B Principle of Operation:

The ZEUS Solinas VlsE1/pepC10 IgG/IgM Test on the ZEUS Solinas α instrument uses chemiluminescent reactions for the detection of IgG and IgM antibodies against *Borrelia burgdorferi.* Specifically, serum samples are added to reaction cuvettes, which are loaded into the instrument. First, IgG and IgM antibodies against *Borrelia burgdorferi* in serum samples are bound to antigen-coated magnetic beads (solid phase). After binding, goat-anti-human acridinium-ester labeled conjugate antibodies are added to the reaction to bind to the target antibodies fixed on the solid phase. After several washing steps, trigger solutions are added to the reaction in the signal detection region of the instrument. Then, the reaction cuvettes are moved to the detection chamber where light emitted from the reaction between the trigger solutions and the acridinium ester-labeled conjugate is measured via a photomultiplier and reported by the software as relative light units (RLU). RLU values from each sample are converted to CLIA (Chemiluminescence immune assay) Units via the assay-specific two-point calibration curve. Because the data reduction portion of the Stratec software is unable to use calibrator Unit values containing decimals when calculating the standard curve, the software multiplies the arbitrary Unit values assigned to each calibrator by a factor of 100, which results in the POS/NEG cut-off of 100 Units.

The ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit is intended for use as assayed quality control samples to monitor the performance of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System. The positive control is optimized to yield an assay signal of approximately 200 to 300 Units (2-3 x the cut off), and the negative control is selected to yield an assay signal of ≤ 50 Units. The label on each control tube contains a 2D barcode, with an identification number used by the analyzer's software to ensure that each control is matched to the appropriate assay. This matching occurs automatically by the analyzer using the information stored within the control definition module. The controls are designed to assess performance across assay reagent lots, and thus are not assay reagent lot-specific. The performance characteristics of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit have not been established for any other assays or instrument platforms different from the ZEUS Solinas α instrument.

C Instrument Description Information:

1. Instrument Name:
   Solinas α

2. Specimen Identification:
   A camera reader located in the sample loading bay of the instrument can scan barcodes on test tubes for sample identification.

3. Specimen Sampling and Handling:
   The assay is intended for its use with human serum samples collected by aseptic venipuncture procedures. Fresh and properly stored samples should be used for this test. If testing is not performed within 8 hours from collection, sera may be stored at 2-8 °C for no longer than 3 days. 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. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory. The minimum serum volume required for testing is 100 µL per specimen (10 µL specimen + 90 µL dead volume). For testing in the Solinas α instrument, the sample loading bay is used to load test tubes via a special rack. The sample loading bay has a camera reader to read the information on the 1D or 2D barcode from the test tubes (containing samples such as patient-related specimen, or calibrators, or controls).

4. Calibration:
   Two assay-specific and lot-specific calibrators are utilized by the Solinas α analyzer. Each calibrator has a barcode containing information such as assay name, lot number, assigned unit value, and expiration date. The Solinas α analyzer automatically tests each calibrator in triplicate and the analyzer's software creates a two-point standard curve using the mean Unit value derived from triplicate analysis of each calibrator.

   A new calibration is necessary when a new lot of Reagent Cartridge is used, the existing calibration was performed more than eight weeks prior, the Solinas α analyzer underwent servicing, or the values of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Controls fall outside of the expected ranges.

5. Quality Control:
   Quality control testing using the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Controls should be performed once per day of use or in accordance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control procedures. It is recommended that the user refers to CLSI document, C24-A3, and 42 CFR 493.1256(c) for guidance on appropriate quality control practices.

   ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Positive and Negative Controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated. The performance of external controls (other than ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Positive and Negative Controls) should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all external quality control material used by the user.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Zeus ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System

B Predicate 510(k) Number(s):
K113397

C Comparison with Predicate(s):

Device & Predicate Device(s):	K230863	K113397
Device Trade Name	ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System	ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System
General Device Characteristic Similarities		
Intended Use/Indications For Use	The ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG/IgM antibodies to *Borrelia burgdorferi* in human serum. This assay is intended for use on samples from patients with signs and symptoms consistent with or patients suspected of having Lyme disease to assess the presence of IgG/IgM antibodies. Positive results with the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System should be supplemented with additional testing with a Standard two-tier test (STTT) methodology using an IgG and/or IgM *Borrelia burgdorferi* immunoblot assay following current guidelines.	The ZEUS ELISA *Borrelia* VIsE1/PepCl0 IgG/IgM Test System is intended for the qualitative detection of IgG and IgM class antibodies to VIsEl and pepC10 antigens from *Borrelia burgdorferi* in human serum. The assay is intended for testing serum samples from symptomatic patients or those with a history of Lyme Borreliosis. All positive and equivocal 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. This kit is for *in vitro* diagnostic use.

	Positive supplemental results are supportive evidence of the presence of antibodies and exposure to *Borrelia burgdorferi* and may be used along with patient history, symptoms and other laboratory data to support a clinical diagnosis of Lyme disease. Negative results by the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System should not be used to exclude Lyme disease. The test must be performed on the ZEUS Solinas α instrument. ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit The ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit is intended for use as assayed quality control samples to monitor the performance of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System. The performance characteristics of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Control Kit have not been established for any other assays or instrument platforms different from the	

	ZEUS Solinas α instrument. This kit is for *in vitro* diagnostic use.	
Sample	Human serum	Same
Measurand	IgG/IgM antibodies	Same
Antigens	VIsEl and/or pepC10 antigens from *Borrelia burgdorferi*	Same
General Device Characteristic Differences		
Solid Phase	Polystyrene microscopic magnetic beads	Polystyrene 96 well ELISA plate
Conjugate	Goat anti-human IgG/IgM labeled with acridinium ester	Goat anti-human IgG/IgM labeled with horse radish peroxidase
Detection	Chemiluminescence	Colorimetric
Automation	Fully automated on the Solinasα instrument	Manually performed
Interpretation criteria	<100 U = negative ≥ 100 U = positive	≤ 0.9 = negative 0.91 – 1.09 = equivocal ≥ 1.10 positive

VI Standards/Guidance Documents Referenced:

Guidance for the Content of Premarket Submission for Software Contained in Medical Devices, Guidance for Industry and Staff, May 2005

CLSI-EP7-A3

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision
For the precision study, a total of 48 data points per sample were collected using six serum samples with various levels of antibody concentrations, two calibrators (CAL-1 and CAL-2), and two controls (positive and negative) See Table 1.

Table 1: Precision Sample Panel

Sample #	Level	Approximate Antibody Concentration
1	High Positive	≥ 600 units
2	Medium Positive	300 units
3	Low Positive	100 to 150 units
4	Borderline	85 to 100 units
5	High Negative	60 to 85 units
6	Low Negative	0 to 50 units
ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Negative Control and Positive Control		
ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System assay Calibrator 1 (CAL-1) and Calibrator 2 (CAL-2).		

The samples, calibrators, and controls were tested in duplicate, twice per day during twelve separate days using a single Solinas α instrument. Samples, calibrators, and controls were blinded and randomized each day of testing. Results from the precision study are summarized in Table 2 below.

Table 2: Precision Study Results

Sample ID	N	Mean	Within Run		Between Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	48	682.8	29.1	4.3	20.5	3.0	17.0	2.5	39.5	5.8
2	48	334.5	8.4	2.5	11.9	3.6	2.5	0.7	14.8	4.4
3	48	136.2	3.5	2.5	3.2	2.3	8.5	6.3	9.7	7.1
4	48	98.9	4.7	4.8	0.0	0.0	5.1	5.1	6.9	7.0
5	48	78.1	4.3	5.6	1.9	2.5	4.8	6.2	6.8	8.7
6	48	20.4	2.8	13.9	1.9	6.4	2.8	13.7	4.4	21.7
*Neg Control	48	15659	1355.0	8.7	1826.0	11.7	2016.0	12.9	3039.0	19.4
Pos Control	48	228.5	8.0	3.5	7.7	3.4	0.0	0.0	11.1	4.9
CAL- 1	48	124.1	4.7	3.8	1.9	1.5	4.6	3.7	6.8	5.5
CAL- 2	48	69.4	3.3	4.7	1.5	2.2	3.5	5.0	5.0	7.2

*Raw RLU data points were utilized since some runs yielded unit values below zero.

Reproducibility

A reproducibility study was conducted over five days across three study sites using the same panel tested for the within laboratory precision study, including lot-specific calibrators and controls. Each sample was tested in triplicate and run twice per day, collecting 30 data points per site for a total of 90 data points per panel member. Each site used a different operator and instrument. Results are summarized in Table 3 below.

Table 3: Reproducibility Study Results

Sample ID	N	Mean (Units)	Within Run		Between Run		Between Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	90	658.2	83.4	12.7	0.0	0.0	10.8	1.6	90.8	13.8	84.1	12.8
2	90	351.3	40.0	11.4	0.0	0.0	0.0	0.0	40.7	11.6	40.0	11.4
3	90	113.9	7.4	6.5	2.4	2.1	1.4	1.2	4.3	3.8	7.9	6.9
4	90	91.1	8.2	9.0	3.6	3.9	0.0	0.0	1.9	2.1	8.9	9.8
5	90	76.5	10.4	13.6	0.0	0.0	3.2	4.2	9.6	12.6	10.9	14.2
6	90	22.1	9.4	42.6	0.0	0.0	0.6	2.6	10.6	48.0	9.4	42.7
Neg Control	90	40.3	6.5	16.2	0.0	0.0	0.8	2.0	7.2	17.9	6.6	16.3
Pos Control	90	298.9	41.3	13.8	0.0	0.0	3.6	1.2	44.8	15.0	41.4	13.9
CAL-1	90	123.8	11.9	9.6	0.0	0.0	2.1	1.7	12.3	10.0	12.1	9.8
CAL-2	90	84.4	5.6	6.6	0.9	1.1	1.5	1.8	4.6	5.4	5.8	6.9

*Raw RLU data points were utilized since some runs yielded unit values below zero.

2. Linearity:
   N/A

3. Interference/Cross-Reactivity:

Interference
Endogenous substances identified below (Table 4) were assessed for potential interference by testing a serum sample (90-100 units) in the presence of each of the substances. The samples spiked with interferents were tested in triplicate. The percent recovery for each interferent was calculated using the equation: Percent Recovery = (Mean unit value from three replicates of each sample spiked with an interferent/Mean unit value from the three replicates of each corresponding control sample) x100.

Table 4: Interferents Testing Results

Interferent	Testing concentration	Solvent/Control	Percentage Recovery
Hemoglobin	10 mg/mL	N/A	83%
Triglycerides	15 mg/mL	Methanol	106%
Bilirubin	0.4 mg/mL	PBS	106%
Albumin	120 mg/mL	PBS	93%
Cholesterol	5 mg/mL	Methanol	111%
Rheumatoid Factor (Rf)	100 IU/mL	PBS	98%

An interference effect was initially observed for hemoglobin at 10 mg/mL which resulted in a lower percent recovery. Additional testing was performed to confirm the effect and identify the hemoglobin concentration where this effect would disappear. However, lower than expected recovery was not observed at 10 mg/mL hemoglobin during repeat testing. Results are shown in Table 5 below.

Table 5: Repeat Evaluation of Hemoglobin Interference

Sample Concentration	CLIA Units	Percentage Recovery
	Mean of 3 replicates	
Positive Control	102.28	N/A
10mg/mL	96.28	94.13%
7.5 mg/mL	89.83	87.13%
5 mg/mL	94.25	92.15%
2.5 mg/mL	98.63	96.43%
1mg/mL	97.64	95.46%

Cross-Reactivity

A total of 246 samples containing antibodies against 23 potentially cross-reactive infections and conditions were tested. Results are summarized in Table 6 below.

Table 6: Cross-reactivity Study Results

Category	Tested Samples (n)	Positive
Tick-borne		
Babesiosis[a]	20	4
Autoimmune/Rheumatoid		
Anti-Nuclear Antibodies (ANA) Serological	10	0
Systemic Lupus Erythematosus (SLE)	10	0
Sjogrens Syndrome	10	0
Rheumatoid Arthritis[b]	30	1
Rheumatoid Factor[c]	15	0
Viral		
Cytomegalovirus (CMV)[d]	10	0
Epstein-Barr Virus (EBV) EA	10	0
Epstein-Barr Virus (EBV) EBNA IgG	10	0
Epstein-Barr Virus (EBV) VCA IgM[b]	9	2
Epstein-Barr Virus (EBV) VCA IgG	10	0
Human Immunodeficiency Virus (HIV)	5	0
Influenza Virus (Flu A)	10	0
Influenza Virus (Flu B)	10	0
Parvovirus IgM	7	0
Parvovirus IgG	10	0
Bacterial		
H. influenza b	10	0
H. pylori	10	0
B. pertussis	10	0
Legionella	10	0
T. pallidum[b]	20	3
Miscellaneous		
Human anti-mouse antibodies	5	0
Cancer	10	0

[a]All 4 samples positive by the VlsE1/pepC10 Solinas CLIA test were also positive by the predicate ELISA and *Borrelia* IgG and IgM immunoblots
[b]Samples positive on the VlsE1/pepC10 CLIA assay were negative by *Borrelia* IgG and IgM immunoblots
[c]15 of the 30 Rheumatoid Arthritis samples were positive for Rheumatoid Factor (RF) IgM. 0 of the 15 RF positives were positive on the VlsE1/pepC10 Solinas CLIA test
[d]The 10 CMV samples consisted of 8 IgG and 2 IgM positive samples

4. Assay Reportable Range:
   N/A

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Specimen stability

Stability of specimens stored at both room temperature and in refrigeration (2-8°C) were tested by using high positive, low positive and negative serum samples. One group of samples was aliquoted and tested in triplicate after storage at room temperature and a second group was tested in triplicate after storage in refrigeration. Subsequent measurements for both conditions were conducted at 48, 96, and 168 hours. Samples stored in refrigeration (2-8°C) demonstrate stability for up to 3 days. Results are summarized in Table 7 below.

Table 7: Sample Stability Study Results

Storage Time	Storage Condition	Result	Sample ID		
			Mean High Positive	Mean Low Positive	Mean Negative
T=0	Baseline	Units	1049	136	39
1 Day	Room Temperature	Units	985	131	37
		% of T = 0	94	96	95
	2-8°C	Units	1049	140	38
		% of T = 0	100	103	98
3 Days	Room Temperature	Units	951	136	39
		% of T = 0	91	100	100
	2-8°C	Units	1064	148	38
		% of T = 0	101	109	98
7 Days	Room Temperature	Units	1036	146	39
		% of T = 0	99	107	102
	2-8°C	Units	1098	151	40
		% of F/T 1	105	111	104
14 Days	Room Temperature	Units	1035	151	41
		% of T = 0	99	111	105
	2-8°C	Units	1064	159	41
		% of T = 0	101	117	106

Calibrators

Two lot-specific calibrators are provided with each assay kit. The calibrators are used to construct a two-point calibration curve with two signals: the first calibrator optimized to yield a signal of approximately 120 CLIA Units and the second calibrator optimized to yield an assay signal of approximately 80 CLIA Units. These unit values are approximately 20% above and 20% below the assay cut-off of 100 Units.

During the calibration process, the instrument assays each calibrator in triplicate, and the software creates a two-point calibration curve using the mean RLU value and mean Unit value of each calibrator. The calibration curve is stored within the software and is used to calculate results for patients and controls.

Calibrator frequency was determined by running eight samples and two controls in singlicate, once per week, over the course of nine weeks after the calibration event. Acceptance was determined by a variability not greater than 10% for all samples, except High Pos, Medium/High Neg, Low Neg and Low Neg (NC) where variability is expected to be higher. A single calibration event every eight weeks was determined to be acceptable (Table 8).

Table 8: Calibration Frequency Study Results

Sample	Category	Day1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
		Units	% Day 1 Units								
1	High Pos	1002	113	112	113	114	110	109	108	103	101
2	Medium/High Pos	659	98	98	103	106	96	96	100	93	89
3	Medium/High Pos	605	100	96	97	100	96	94	96	96	95
4	Low/Med Pos	333	87	95	99	92	95	97	94	93	94
5	Low Pos	124	109	96	106	106	101	100	93	98	100
6	Borderline/High Neg	86	108	103	104	105	97	95	94	107	98
7	Med/ High Neg	69	118	100	104	101	112	99	90	103	88
8	Low Neg	21	103	59	52	77	88	67	61	136	74
NC	Low Neg	4	82	52	130	195	91	80	38	116	112
PC	Low/Med Pos	203	101	97	106	103	99	105	103	106	93
CAL1	Low Pos	114	114	100	109	97	102	102	97	102	96
CAL2	High Neg	65	110	103	109	108	115	96	98	101	96

Controls

An assay-specific control kit is provided separately from the assay reagent kit. The control kit contains a positive control vial (1 mL) and a negative control vial (1 mL). During the manufacturing process, the positive control is optimized to yield an assay signal of approximately 200 to 300 Units (2-3X the cut off), and the negative control is selected to yield an assay signal of ≤ 50 Units. Each control tube label contains information such as the control expiration date, lot number, and recommended storage conditions. The label also has a 2D barcode, which contains an identification number used by the analyzer's software to match the control to the appropriate assay. This matching occurs automatically by the analyzer using the information stored within the control definition module. The controls are designed to assess performance across assay reagent lots, and thus are not assay reagent lot-specific.

The information from the certificate of analysis for each new lot of controls must be entered into the control definition module within the analyzer's software. The information entered into the control definition module include the assay name that each control is assigned to, the control lot number, the control expiration date, the number of replicates to be assayed during each control run, the acceptable control lot-specific Unit value ranges, and the maximum allowable time period between control runs. For the ZEUS Solinas VlsE1/pepC10 IgG/IgM Test System, a control run will be required every 24 hours.

Control process

During the sample loading process and the 2D barcode of each control is scanned, and a sample rack position is assigned for each control within the analyzer's software. The analyzer automatically assays each control in singlicate, and the software calculates a Unit value for each control. Then, the software determines if each control is valid, based upon the lot-specific ranges present within the corresponding control definition. If the controls yield valid results, then patient results can be reported until the next control run is requested by the analyzer's software (24 hours). However, if the controls yield invalid results, then the patient results cannot be reported until a valid control run has been achieved.

6. Detection Limit: N/A

7. Assay Cut-Off:
   A preliminary assay cut-off was established by testing 200 apparently healthy donor samples from endemic and non-endemic regions and calculating the mean and Standard Deviation of the RLU values. The preliminary assay cut-off was further adjusted after evaluation of the CDC 92-sample Lyme Research Panel II.

8. Accuracy (Instrument):
   Not applicable.

9. Carry-Over:
   Carry over contamination in the Solinas α instrument was evaluated using 36 high positive (≥ 600 units) and 36 negative samples (≤50 units). Additionally, lot specific calibrators and the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM controls were included in the study. Briefly, positive and negative samples were alternated and sequentially assayed using the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System. Acceptance criteria was determined by a within run CV of 15% or less for positive samples, and a CV of 50% or less for the negative samples. A summary of the study results is presented in Table 9 below.

Table 9: Carry-Over Study Results

Test Level	Number of Positive Panels Percentage	%CV Units
High Positive	36/36 100%	3.8
Negative	36/36 100%	16.7

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Method comparison using CDC Serum Panel
   The 280 samples of the CDC Pre-Marketing Borrelia panel were tested using the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System and the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System. This panel consisted of 60 cases of early Lyme disease (LD), 3 cases of cardiac LD, 7 cases of neurologic LD, 20 cases of late LD, 100 healthy controls and 90 non LD, diseased controls. The samples were tested, and their results were compared. Results for first and second tier testing comparisons can be seen in Tables 10 and 11 below, respectively.

Table 10: CDC Pre-market panel – First Tier Testing Results

Sample Category (N)	ZEUS VlsE1/pepC10 CLIA (Test)				ZEUS VlsE1/pepC10 ELISA (Predicate)			
	Pos	Neg	Sens/Spec	95% CI	Pos or Equiv	Neg	Sens/Spec	95% CI
Early Lyme (N=60)	48	12	80.0%	(68.22 – 88.17)	50	10	83.33%	(71.97 – 90.69)
Cardiac Lyme (N=3)	2	1	66.67%	(20.77 – 92.85)	3	0	100%	(43.85 – 100)
Neurologic Lyme (N=7)	7	0	100%	(64.57 – 100)	7	0	100%	(64.57 – 100)
Late Lyme (N=20)	20	0	100%	(83.89 – 100)	20	0	100%	(83.89 – 100)
Healthy Controls (N=100)	2	98	98.0%	(93 – 99.45)	4	96	96.0%	(90.16 – 98.43)
Disease Controls (N=90)	11	79	87.78%	(79.43 – 93.04)	7	83	92.22%	(84.88 – 96.18)

Additionally, samples were tested using the Gold Standard IgM and IgG immunoblots as the second-tier test for both first-tier methods, following a standard two-tier method. Results are shown in Table 11, below.

Table 11: CDC Pre-market panel – Standard Two-Tier Testing Results

Sample Category (N)	Test Assay + Line Blots IgG or IgM				Predicate Assay + Line Blots IgG or IgM			
	Pos	Neg	Sens/Spec	95% CI	Pos or Equiv	Neg	Sens/Spec	95% CI
Early Lyme (N=60)	43	17	71.67%	(59.23 – 81.49)	41	19	68.33%	(57.77 – 78.69)
Cardiac Lyme (N=3)	2	1	66.67%	(20.77 – 92.85)	2	1	66.67%	(20.77 – 92.85)
Neurologic Lyme (N=7)	7	0	100%	(64.57 – 100)	7	0	100%	(64.57 – 100)
Late Lyme (N=20)	20	0	100%	(83.89 – 100)	20	0	100%	(83.89 – 100)
Healthy Controls (N=100)	0	100	100%	(96.3 – 100)	0	100	100%	(96.3 – 100)
Disease Controls (N=90)	0	90	100%	(95.91 – 100)	0	90	100%	(95.91 – 100)

Multi-site Prospective Study

A study was executed to compare the performance of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System to the predicate ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System using prospectively collected patient samples. This study was conducted at three different Lyme disease endemic locations in the U.S. with three different Solinas α instruments and technicians. Sites collected routine, left-over, de-identified serum samples submitted to their respective facilities for routine Lyme disease serology testing.

Each site collected 600 samples, adding to a total of 1800 samples. Samples collected in each site were divided into three groups of 200 and redistributed among three testing sites, each testing site receiving a total of 600 samples representing all three collection sites. All samples were first tested using a first-tier method using both the ZEUS Solinas CLIA assay and the predicate. Any sample that generated a positive result by ZEUS Solinas VlsE1/pepC10 IgG/IgM Test System or a positive/equivocal result by ZEUS ELISA VlsE1/pepC10 IgG/IgM Test System was then tested in a Standard two-tier Testing method using the *B. burgdorferi* IgG and IgM Gold Standard immunoblots at ZEUS Scientific. One specimen was excluded from any data analyses as it was determined to be invalid according to the Gold Standard Diagnostics labeling due to excessively high background staining on the IgM immunoblot. Negative samples in the first-tier testing were not further tested. Results from

the first-tier testing are described in Table 12 below and results of the Standard Two-Tiered Testing method are described in Table 13 below.

Table 12: Prospective Study - First Tier Test Results Compared to Predicate

		ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System (Predicate Assay)		
		Positive	Equivocal	Negative
ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System	Positive	186	10	112
	Negative	29	10	1452
	Total	215	20	1799

PPA: 86.51% (196/235) 95% CI: 81.3 – 90.44%
NPA: 92.84% (1452/1564) 95% CI: 90.23 – 92.80%

Table 13: Prospective Study – Standard Two-Tier Test Results Compared to Predicate

		ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System (Predicate Assay) with Immunoblot (STTT)	
		Positive	Negative
ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM Test System with Immunoblot (STTT)	Positive	160	25
	Negative	7	1614
	Total	167	1799

PPA: 95.81% (160/167) 95% CI: 91.6 – 97.95%
NPA: 98.47% (1607/1632) 95% CI: 97.75 – 98.96%

Comparing the two methods in a STTT method using immunoblot resulted in PPA and NPA point estimates of 95.8% and 98.5%, respectively. Cumulatively these results demonstrate substantial equivalence to the predicate device.

2. Matrix Comparison: N/A

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

N/A

2. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

N/A

D Clinical Cut-Off:

N/A

E Expected Values/Reference Range:

The total number of samples tested and the number of positive samples for each population in the studies conducted to establish the performance characteristics of the ZEUS Solinas Borrelia VlsE1/pepC10 IgG/IgM are summarized in Table 14 below.

Table 14: Summary of Prospective Specimen Cohort

Populations		Number tested	Positive/Tested
Prospective Studies		1799	186/1799
Characterized Sample Study	Lyme disease	90	77/90
	Negative	190	13/190
Endemic Controls		100	1/100
Non-Endemic Controls		100	1/100

F Other Supportive Instrument Performance Characteristics Data:

N/A

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