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

I Background Information:

A 510(k) Number

K193051

B Applicant

DiaSorin Inc.

C Proprietary and Established Names

LIAISON Lyme Total Antibody Plus, LIAISON Lyme Total Antibody Plus Control Set

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LSR	Class II	21 CFR 866.3830 - Treponema Pallidum Treponemal Test Reagents	MI - Microbiology
QCH	Class II	21 CFR 866.3920 - Assayed quality control material for clinical microbiology assays	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination and FDA clearance for a new device.

B Measurand:

Anti-*Borrelia burgdorferi* total antibodies

C Type of Test:

Chemiluminescent immunoassay (CLIA)

III Intended Use/Indications for Use:

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

B Indication(s) for Use:
LIAISON Lyme Total Antibody Plus

The LIAISON Lyme Total Antibody Plus assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of IgG and IgM antibodies to *Borrelia burgdorferi* in human serum and plasma (K_2-EDTA, Li-heparin) samples. This assay is intended for use on samples from patients with signs and symptoms that are consistent with Lyme disease. Positive or equivocal results should be supplemented by testing with a standardized Western blot procedure. Positive supplemental results provide evidence of exposure to *B. burgdorferi* and can be used to support a clinical diagnosis of Lyme disease. Negative results by LIAISON Lyme Total Antibody Plus assay should not be used to exclude Lyme disease. The test has to be performed on the LIAISON XL Analyzer.

The LIAISON Lyme Total Antibody Plus Control Set

The LIAISON Lyme Total Antibody Plus Control Set is intended for use as assayed quality control samples to monitor the performance of the LIAISON Lyme Total Antibody Plus assay. The performance characteristics of LIAISON Lyme Total Antibody Plus Control Set have not been established for any other assays or instrument platforms different from the LIAISON XL.

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

D Special Instrument Requirements:
LIAISON XL Analyzer

IV Device/System Characteristics:

A Device Description:

The method for qualitative determination of IgG and IgM antibodies to *B. burgdorferi* is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the Analyzer. The principal components of the test are magnetic particles (solid phase) coated with recombinant Borrelia

antigens and a conjugate reagent containing two mouse monoclonal antibodies (anti-human IgG and anti- human IgM) linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, anti-*Borrelia burgdorferi* antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugates react with anti-*Borrelia burgdorferi* IgG and IgM antibodies that have bound to the solid phase. After each incubation, unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the presence of *B. burgdorferi* antibodies present in calibrators, samples or controls.

B Principle of Operation:

Chemiluminescence immunoassay (CLIA)

C Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☐	☒
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

1. Instrument Name:
   LIAISON XL Analyzer

2. Specimen Identification:
   Specimens are identified by barcode and each test parameter is identified via information encoded in the Reagent Integral Radio Frequency Identification transponder (RFID Tag).

   Control identification is detected by the bar code label or may be manually programmed into the instrument. Follow the analyzer operator's manual to start the run. Return controls to the refrigerator immediately after each use.

3. Specimen Sampling and Handling:
   Either human serum, SST serum, K_2-EDTA plasma and Lithium Heparin plasma may be used in this assay. Blood should be collected aseptically by venipuncture. Serum samples should be allowed to clot. Centrifuge samples and separate serum or plasma from the clot as soon as possible. Samples having particulate matter, turbidity, lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. Samples are stable at room temperature for up to 8 hours. If the assay is performed within 7 days of sample collection, the samples should be kept at 2-8°C; otherwise they

should be stored frozen (-20°C or below). If samples are stored frozen, mix thawed samples well before testing. Samples may be frozen-thawed 5 times. Self-defrosting freezers are not recommended for sample storage.

The minimum specimen volume required for a single determination is 155µL.

4. Calibration:
Individual LIAISON Lyme Total Antibody Plus Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows (5) calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:
    - With each new lot of reagents (Reagent Integral or Starter Reagents).
    - The previous calibration was performed more than 8 weeks prior.
    - Quality Control results are out of the acceptable range.
    - The Analyzer has been serviced.

    Refer to the analyzer operator's manual for calibration instructions.

    Measuring range: The LIAISON Lyme Total Antibody Plus assay measures between 0.01 and 10 Index units.

    The lowest reportable value is 0.01 Index unit. Values below 0.01 Index units should be reported as < 0.01 Index units. The highest reportable value without dilution is 10 Index units.

5. Quality Control:
Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3 and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

    LIAISON Lyme Total Antibody Plus controls are intended to monitor for substantial reagent failure. Single replicates of LIAISON controls should be run 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 other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

    The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Zeus ELISA Borrelia Vlse-1/pepc10 IgG/IgM Test System

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

C Comparison with Predicate(s):

Device & Predicate Device(s):	K193051	K113397
Device Trade Name	LIAISON Lyme Total Antibody Plus	Zeus ELISA Borrelia Vlse-1/pepc10 IgG/IgM Test System
General Device Characteristic Similarities		
Intended Use/ Indications For Use	LIAISON Lyme Total Antibody Plus: The LIAISON Lyme Total Antibody Plus assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of IgG and IgM antibodies of *Borrelia burgdorferi* in human serum and plasma (EDTA, Li-heparin) samples. This assay is intended for use on samples from patients with signs and symptoms that are consistent with Lyme disease. Positive or equivocal results should be supplemented by testing with a standardized Western blot procedure. Positive supplemental results provide evidence of exposure to *B. burgdorferi* and can be used to support a clinical diagnosis of Lyme disease. Negative results by LIAISON Lyme Total Antibody Plus assay should not be used to exclude Lyme disease. The test has to be performed on the LIAISON XL Analyzer.	Qualitative detection of IgG and IgM class antibodies to VIsE-l 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.

Results	Qualitative	Same
Measurand	IgG and IgM antibodies to *B. burgdorferi*	Same
Intended Population	Patients with signs and symptoms consistent with *Borrelia* infection (Lyme disease)	Same
Assay Principle	Uses Borrelia antigens coated on a solid phase to capture specific patient antibodies.	Uses *B. burgdorferi* antigen coated on a solid phase to capture specific patient antibodies.
Sample Type	Human serum, serum separator tubes, K_2-EDTA, lithium heparin plasma	Human serum
Conjugate antibody specificities	Anti-human IgG and anti-human IgM	Anti-human IgG/IgM
Assay Output	Index	Same

General Device Characteristic Differences	K193051	K113397
Test Format	CLIA (chemiluminescent assay)	ELISA
Reporter Molecule	Isoluminol derivative conjugated to anti-human IgG and IgM	TMB (as a substrate for Horseradish peroxidase conjugated to anti-human IgG/IgM).
Antigen	Recombinant VlsE antigen from *B. burgdorferi* strain B31 and from *B. garinii* strain Pbi, and OspC antigen from *B. afzelii*.	VlsE1 and pepC10 antigens of *B. burgdorferi*
Assay Procedure	Automated (on the LIAISON XL Analyzer)	Manual
Calibration	Two-point verification (in triplicate) of stored 10-point master curve	Single Cut-off Calibrator assayed in triplicate
Output Signal	Flash chemiluminescent response is integrated over a 3 second reading period to generate a relative light unit (RLU) value.	Microtiter well O.D. (450 nm) is measured after the enzyme reaction is halted by sulfuric acid.
Measurement System	Photomultiplier (flash chemiluminescence reader)	Spectrophotometer (EIA Microtiter plate reader)

VI Standards/Guidance Documents Referenced:

N/A

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision: A 12-day precision/repeatability study was conducted at DiaSorin on the LIAISON Lyme Total Antibody Plus assay. Six serum samples and one lot of LIAISON Lyme Total Antibody Plus Controls were tested for 12 days, 2 runs/day, and 2 replicates per run by multiple technologists for a total of 48 replicates. These test days span 2 calibration cycles. CLSI document EP05-A3 was consulted in the preparation of the testing protocol.

Table 1: Precision Study Results

Sample ID	N	Mean (Index)	Within Run		Between Run		Between Day		TOTAL	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg. Control	48	0.09	0.01	9.4	0.01	8.0	0.00	0.0	0.01	11.3
Pos. Control	48	1.96	0.13	6.7	0.03	1.6	0.15	7.7	0.20	10.4
Sample 1	48	0.09	0.00	5.6	0.01	6.0	0.00	2.2	0.01	8.5
Sample 2	48	0.84	0.04	4.5	0.02	2.8	0.05	5.6	0.07	7.7
Sample 3	48	1.59	0.12	7.7	0.00	0.0	0.10	6.0	0.15	9.4
Sample 4	48	4.51	0.21	4.7	0.24	5.4	0.25	5.5	0.41	9.0
Sample 5	48	0.82	0.06	7.3	0.07	8.4	0.06	7.0	0.11	13.2
Sample 6	48	1.39	0.08	6.0	0.12	8.7	0.07	5.2	0.16	11.8

Reproducibility: A five-day precision/reproducibility study was performed internally at DiaSorin Inc. and at two external U.S. laboratories with one lot of LIAISON Lyme Total Antibody Plus assay. The study was performed for 5 days, 2 runs/day, and 3 replicates/run. Each day, two operators, at each testing site performed the testing for a total of 30 replicates at each site. CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

Table 2: Reproducibility Study Results

Sample ID	n	mean	Within Run		Between Day		Between Run		Between Site		TOTAL	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg. Control	90	0.0509	0.002	4.7	0.002	3.4	0.002	3.4	0.002	4.7	0.004	8.2
Pos. Control	90	1.8	0.118	6.5	0.04	2.2	0.039	2.2	0.000	0.0	0.13	7.2
Sample 1	90	0.0463	0.002	4.6	0.002	4.3	0.001	3.1	0.000	0.0	0.003	7.0
Sample 2	90	0.654	0.028	4.2	0.036	5.5	0.026	3.9	0.024	3.7	0.057	8.8
Sample 3	90	1.55	0.07	4.5	0.055	3.6	0.046	3.0	0.077	5.0	0.126	8.2
Sample 4	90	4.66	0.207	4.4	0.161	3.5	0.107	2.3	0.000	0.0	0.283	6.1
Sample 5	90	0.86	0.052	6.1	0.04	4.6	0.000	0.0	0.035	4.1	0.074	8.7
Sample 6	90	1.42	0.065	4.6	0.075	5.3	0.041	2.9	0.064	4.5	0.125	8.8

2. Linearity: N/A

3. Analytical Specificity/Interference:

Cross-Reactivity Study: The cross-reactivity study evaluated 238 specimens from twenty-four disease states either known to contain potentially cross-reactive antibodies to *B. burgdorferi* or from patients with diagnoses that can exhibit signs and symptoms similar to late manifestations of Lyme disease and cause false positive results.

Table 3: Lyme Total Antibody Plus Cross-Reactivity

Organism Infected or Disease State	Samples Tested (n)	Pos. or Eqv.
Tick Borne Diseases		
Babesiosis	10	4
Tick Borne Relapsing Fever (TBRF)	8	2
Autoimmune Disorders		
Anti-Nuclear Antibodies (ANA)	10	0
Multiple Sclerosis	10	0
Sjogrens Syndrome	10	1
Viral Diseases		
Cytomegalovirus (CMV) IgM	10	0
Cytomegalovirus (CMV) IgG	10	0
Epstein-Barr Virus (EBV) VCA, and/or heterophile Ab IgM	10	0
Epstein-Barr Virus (EBV) VCA, NA-1 and/or EA-D IgG	10	0
Epstein-Barr Virus (EBV) EBNA IgG	10	1
Epstein-Barr Virus (EBV) VCA IgM	10	2
Epstein-Barr Virus (EBV) VCA IgG	10	0
Human Immunodeficiency Virus (HIV)	10	0
Influenza Virus	10	0
Parvovirus	10	3
Bacterial Diseases		
E. coli	10	0
H. pylori	10	0
Syphilis	10	0
Rheumatic Diseases		
Fibromyalgia	10	0
Rheumatoid Arthritis	10	0
Rheumatoid Factor	10	0
Systemic Lupus Erythematosus (SLE)	10	0
Additional Markers		
Chronic Fatigue Syndrome	10	0
Human Anti-mouse Antibodies (HAMA)	10	0
Total	238	13

Interfering Substances: Controlled studies of potentially interfering substances from endogenous interferents spiked into equivocal *B. burgdorferi* serum specimens showed that assay performance was not affected at the concentration for each substance listed below. The testing was based on CLSI-EP7-A3.

Table 4: Endogenous Interferents Study

Substance	Concentration
Hemoglobin	1000 mg/dL
Triglycerides	1500 mg/dL
Bilirubin	40 mg/dL
Total protein	12 g/dL
Cholesterol	500 mg/dL
Biotin	3600 ng/mL

4. Assay Reportable Range: N/A

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

6. Detection Limit: N/A

7. Assay Cut-Off:

   The cut-off for the LIAISON Lyme Total Antibody Plus assay was determined by evaluating U.S. sourced characterized samples from Lyme disease patients and routine clinical samples sent to the laboratory for Lyme disease serology testing. Based on available clinical and laboratory data and by comparison with other cleared serology assays, the samples were classified as expected positive or negative for *B. burgdorferi* antibodies and evaluated with the LIAISON Lyme Total Antibody Plus assay. An Index of 1.0 was determined to provide the best balance of sensitivity and specificity for the tested samples. An equivocal zone of 0.90 -1.10 was applied to the assay to account for normal measurement imprecision.

8. Accuracy (Instrument): N/A

9. Carry-Over: N/A

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Prospective Study: 1,550 samples collected from subjects sent for Lyme disease testing, were de-identified and tested. The collection consisted of subjects from five geographical regions of the U.S. The samples were tested with the LIAISON Lyme Total Antibody Plus assay on the LIASON XL and performed in three laboratories (two external and one internal at DiaSorin). Results were evaluated for first-tier testing.

Table 5: First-Tier Percent Agreement with Predicate Device

	Predicate Assay (IgG/IgM)			
LIAISON Lyme Total Antibody Plus	**Positive**	**Equivocal**	**Negative**	**Total**
Positive	62	1	9	72
Equivocal	2	0	10	12
Negative	42	8	1416	1466
Total	106	9	1435	1550

Positive % Agreement*: 56.5% (65/115) 95% CI: 47.4% - 65.2%
Negative % Agreement: 98.7% (1416/1435) 95% CI: 97.9% - 99.2%

*Includes Positive and Equivocal combined

Western blot testing was performed on the samples positive or equivocal by the test device and the predicate. The following results were obtained:

Table 6: Second-Tier Testing

Test System	Tier 1 $^{+}$ or Eqv.	Western Blot IgG/IgM +	Western Blot IgG/IgM -
LIAISON Lyme Total Antibody Plus	84	48	36
Predicate Assay	115	48	67
Predicate and LIAISON Lyme Total Antibody Plus	65	47	18

Agreement results:

2nd-Tier Positive % Agreement: 97.9% (47/48); 95% CI: 89.1%-99.6%

Analytical Specificity: The LIAISON Lyme Total Antibody Plus assay was used to test 300 samples from apparently healthy individuals in the U.S. This population was 55.3% female, 44.7% male with a mean age of 59 years. Fifty percent of the samples were collected in a Lyme disease endemic region and 50% were collected from a Lyme disease non-endemic region.

Table 7: Analytical Specificity Study

Population	N	% Positivity
Endemic	150	2
Non-Endemic	150	19.3*
All Specimens	300	10.7

*27/29 were confirmed positive by Western blot testing and 28/29 with the predicate first-tier assay.

Characterized Lyme Panel Testing: Two hundred eighty samples were acquired from the CDC and evaluated internally at the manufacturer's site. The results of the testing are presented here as a means of conveying further information on the performance of the

LIAISON Lyme Total Antibody Plus assay with a characterized serum panel. This does not imply an endorsement of the assay by the CDC.

Table 8: Testing of CDC Lyme Reference Sera

Sample Category (CDC Reference Classification)		Candidate			Predicate			N	LIAISON Lyme Total Antibody Plus % Agreement/ 95% Wilson CI	Predicate % Agreement/ 95% Wilson CI
		Pos.	Neg.	Eqv.	Pos.	Neg.	Eqv.			
Stage I	Acute	27	9	3	30	9	0	39	76.9% (30/39) 61.7% - 87.4%	76.9% (30/39) 61.7% - 87.4%
Stage II	Convalescent	29	2	0	29	2	0	31	93.5% (29/31) 79.3% - 98.2%	93.5% (29/31) 79.3% - 98.2%
Stage III	Late	20	0	0	20	0	0	20	100.0% (20/20) 83.9% - 100.0%	100.0% (20/20) 83.9% - 100.0%
Look-alike Diseases		2	86	2	4	85	1	90	95.6% (86/90) 89.1% - 98.3%	94.4% (85/90) 87.6% - 97.6%
Healthy Controls		2	98	0	3	95	2	100	98.0% (98/100) 93.0% - 99.4%	95.0% (95/100) 88.8% - 97.8%

2. Matrix Comparison:

Matrix Equivalence Study: Thirty-two matched patient sets of serum, SST serum, K_2-EDTA plasma and lithium heparin plasma samples were tested to determine if these sample types provide equivalent results. Sample regression analysis was done by Passing and Bablok method. All sample types met acceptance criteria for use in the LIAISON Lyme Total Antibody Plus assay. A summary of the results is shown in the following table.

Table 9: Sample Equivalence Results

Comparison to Serum	Bias	CI: 95%	
SST Serum Constant	0.00	0.01	0.01
SST Serum Proportional	0.99	0.97	1.01
K_2-EDTA Constant	-0.01	0.03	0.01
K_2-EDTA Proportional	0.99	0.95	1.01
Lithium Heparin Constant	0.01	0.02	0.01
Lithium Heparin Proportional	0.95	0.92	0.97

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:

Internal and external investigators assessed the device's performance with 1550 masked specimens prospectively collected from patients between the ages of 4 and 103 that were submitted for *Borrelia* antibody testing. Specimens were acquired from five distinct geographical regions within the U.S. The specimens were randomly distributed among three testing sites, one of which was the manufacturer's research facility for testing. The device's performance was also assessed at the manufacturer's research facility with specimens from an asymptomatic population obtained from both endemic and non-endemic regions. Available subject demographics, quantity of specimens tested and number of specimens which tested positive or equivocal for each population are summarized in Table 10.

Table 10: LIAISON XL Lyme Total Antibody Plus Testing Results

Populations	Number Tested	Gender		Age Range	Positive/Tested
		Male	Female		
Prospective	1550	641	909	4 - 103	84/1550
Endemic	150	49	101	18 - 87	3/150
Non-Endemic	150	85	65	16 - 99	29/150*

*27/29 were confirmed positive by Western blot testing and 28/29 with the predicate first-tier assay.

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.