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

I Background Information:

A 510(k) Number

K202574

B Applicant

DiaSorin Inc.

C Proprietary and Established Names

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

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 and to obtain a substantial equivalence determination and FDA clearance for a modified intended use of a previously cleared medical device. 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.

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

B Measurand:

Anti-*Borrelia burgdorferi* 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 IgG

The LIAISON Lyme IgG assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative detection of IgG antibodies to Borrelia burgdorferi in human serum and plasma specimens (K2-EDTA, Li-heparin). 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 antibodies and exposure to Borrelia burgdorferi. In addition, the LIAISON Lyme IgG assay may be used as a confirmatory test in the modified two-tier test (MTTT) in combination with the DiaSorin LIAISON Lyme Total Antibody Plus assay.

If used as a first stage test, positive or equivocal results with the LIAISON Lyme IgG assay should be confirmed through additional testing with a Standard two-tier test (STTT) methodology using an IgG Borrelia burgdorferi Western blot 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 LIAISON Lyme IgG assay should not be used to exclude Lyme disease.

The test must be performed on the LIAISON XL Analyzer.

LIAISON Lyme IgG Control Set

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

LIAISON Lyme Total Antibody Plus

The LIAISON Lyme Total Antibody Plus assay uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of lgG and IgM antibodies of Borrelia burgdorferi in human serum and plasma (K2-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 with the LIAISON Lyme Total Antibody Plus should be confirmed with additional testing by one of the following methodologies:

a). Standard two-tier test (STTT) methodology using an IgG and/or IgM Western blot test for Borrelia burgdorferi.

or -

b). Modified two-tier test (MTTT) methodology using one or more of the following LIAISON assays: LIAISON Lyme IgM and/or LIAISON Lyme IgG

Positive supplemental results either by the STTT or MTTT are supportive evidence of the presence of antibodies and of 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.

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 LIAISON Lyme IgG assay is an indirect chemiluminescence immunoassay (CLIA) for the qualitative detection of IgG antibodies to *Borrelia burgdorferi* in human serum and plasma samples. 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 an anti-human IgG mouse monoclonal antibody 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. Unbound material is then removed with a wash cycle. During the second incubation, the antibody conjugate reacts with anti-*Borrelia burgdorferi* IgG antibodies that have bound to the solid phase. Excess antibody conjugate is then removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is 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 *Borrelia burgdorferi* IgG antibodies present in calibrators, samples or controls.

B Principle of Operation:

Chemiluminescence immunoassay (CLIA)

C Instrument Description Information:

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, K2-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 three days. If the assay is performed within 14 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.

4. Calibration:

   The DiaSorin LIAISON Lyme IgG assay generates a continuous response (relative light units, RLU) which is used in sample grading to provide a qualitative (positive, negative, or equivocal) reportable result. The sample grading is based on the use of a calibration curve referenced to an 'In-house' anti-Lyme IgG reference standard curve (Master Curve) and is controlled by the use of two calibrators (Calibrator 1 and Calibrator 2) provided in the Reagent Integral.

   Calibration is required every 8 weeks, or whenever one of the following conditions occurs:

   • If Quality Control results are out of the acceptable range
   • With each new lot of reagents (Reagent Integral or Starter Reagents)
   • After each servicing of the LIAISON XL Analyzer

The assay measuring range is 0.01 to 45.0 Index value. Samples that read lower than the measuring curve are reported as < 0.01 Index, and samples that read above the measuring curve are reported as > 45.0 Index.

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 IgG 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 burgdorferi IgG Test System; LIAISON Lyme Total Antibody Plus

B Predicate 510(k) Number(s):
K895292, K191398; K193051

C Comparison with Predicate(s):

Device & Predicate Device(s):	K202574	K895292 / K191398	K193051
Device Trade Name	Device 1: LIAISON Lyme IgG Device 2: LIAISON Lyme Total Antibody Plus	ZEUS ELISA *Borrelia burgdorferi* IgG Test System	LIAISON Lyme Total Antibody Plus

General Device Characteristic Similarities			
Intended Use/Indications For Use	These are two indirect chemiluminescence 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 burgdorferi* IgG Test System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG class antibody 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 Test System for the presence of *Borrelia burgdorferi* antibodies must be confirmed through additional testing by one of the following approaches: (1) Standard two-tier test methodology (STTT) using IgG 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	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.

		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.	
Measurand	Antibodies to *B. Burgdorferi*	Same	Same
Intended Population	Patients with signs and symptoms consistent with *Borrelia* infection (Lyme disease)	Same	Same
Assay Type	Qualitative	Same	Same
Assay Principle	*B. burgdorferi* antigens coated on a solid phase to capture specific patient antibodies	*B. burgdorferi* antigen coated on a solid phase to capture specific patient antibodies	*B. burgdorferi* antigens coated on a solid phase to capture specific patient antibodies
Assay Output	Index	Same	Same
General Device Characteristic Differences			
Assay Technology	CLIA (Indirect chemiluminescent assay)	ELISA	CLIA (Indirect chemiluminescent assay)
Sample Type	Serum, Serum Separator Tubes, K_2-EDTA, lithium heparin plasma	Serum	Serum, Serum Separator Tubes, K_2-EDTA, lithium heparin plasma
Instrumentation	LIAISON XL Analyzer	None	LIAISON XL Analyzer
Signal Detection	Chemiluminescence	Colorimetric	Chemiluminescence
Antigen	Recombinant antigens: VlsE (*B. burgdorferi* strain B31 and *B. garinii* strain Pbi)	Whole cell antigen from *B. burgdorferi* (strain B31)	Recombinant antigens: VlsE from *B. Burgdorferi* strain B31 and *B. garinii* strain Pbi

	Peptide for C6 region of VlsE OspC from *B. afzelii* strain pKo		OspC antigen from *B. afzelii*
Assay Procedure	Automated	Manual	Automated

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Methods; Approved Guideline – Third Edition
- CLSI EP15-A3, User Verification of Precision and Estimation of Bias; Approved Guideline
- CLSI EP07-A3, Interference Testing in Clinical Chemistry; Approved Guideline, Second Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Note: This clearance is to support use a new device, the LIAISON Lyme IgG assay, as both a first-tier test as part of a standard two-tiered test (STTT) algorithm and a second-tier test when used as part of a modified two-tiered test algorithm (MTTT) with the already cleared LIAISON Lyme Total Antibody Plus assay. The LIAISON Lyme Total Antibody Plus intended use has been modified to include this new indication.

1. Precision/Reproducibility:

A 12-day precision/repeatability study was conducted at DiaSorin Inc. on two lots of the LIAISON Lyme IgG assay. Six serum samples corresponding to one negative specimen, two high negative specimens, two low positive specimens, and one moderate positive specimen were evaluated with two lots of LIAISON Lyme IgG controls. Testing was performed by multiple technicians over 12 days with two runs per day and two replicates per run for a total of 48 replicates per lot spanning two calibration cycles.

Table 1. Precision Study Results – Combined

Sample ID	N	Mean	Between Lot		Total (Across Lots)	
			SD	% CV	SD	%CV
Sample 1	96	0.75	0.02	2.6%	0.04	4.7%
Sample 2	96	1.31	0.08	6.0%	0.06	4.2%
Sample 3	96	4.70	0.27	5.7%	0.16	3.4%
Sample 4	96	0.79	0.05	6.1%	0.06	7.5%
Sample 5	96	1.44	0.05	3.6%	0.06	4.3%
Sample 6	96	0.47	0.01	2.6%	0.02	3.4%
Negative Control Lot 1	96	0.42	0.06	13.3%	0.03	6.0%
Positive Control Lot 1	96	2.14	0.11	5.3%	0.11	5.2%
Negative Control Lot 2	96	0.08	0.01	7.1%	0.00	5.9%
Positive Control Lot 2	96	2.33	0.09	3.7%	0.13	5.6%

Table 2. Precision Study Results Single – Kit Lot 1

Sample ID	N	Mean	Within Run		Between Run		Between Day		Total	
			SD	% CV	SD	% CV	SD	% CV	SD	%CV
Sample 1	48	0.77	0.03	3.9%	0.04	5.4%	0.00	0.0%	0.05	6.1%
Sample 2	48	1.37	0.04	2.7%	0.05	3.8%	0.04	1.5%	0.07	5.4%
Sample 3	48	4.89	0.11	2.3%	0.11	2.3%	0.11	4.7%	0.19	3.9%
Sample 4	48	0.82	0.05	6.4%	0.04	5.2%	0.04	0.0%	0.08	9.8%
Sample 5	48	1.47	0.05	3.3%	0.06	4.2%	0.00	0.0%	0.08	5.2%
Sample 6	48	0.48	0.02	3.3%	0.01	1.7%	0.01	2.7%	0.02	4.4%
Negative Control Lot 1	48	0.46	0.02	4.2%	0.02	4.7%	0.00	2.2%	0.03	5.9%
Positive Control Lot 1	48	2.22	0.1	4.4%	0.11	4.8%	0.03	5.4%	0.15	6.7%
Negative Control Lot 2	48	0.09	0.00	4.1%	0.00	3.5%	0.00	0.0%	0.01	7.2%
Positive Control Lot 2	48	2.39	0.07	2.9%	0.21	8.6%	0.00	2.3%	0.17	7.2%

A five (5) day precision/reproducibility study was performed internally at DiaSorin Inc. and at two (2) external U.S. laboratories with one (1) lot of the LIAISON Lyme IgG 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. The combined results from the 3 sites are provided. CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

Table 3. Multi-Site Reproducibility Study Results

Sample ID	N	Mean	Within Run		Between Run		Between Day		Between Site		Total	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	%CV
Sample 1	90	0.820	0.018	2.2	0.016	0	0.032	4	0.043	5.3	0.060	7.3
Sample 2	90	1.40	0.039	2.5	0.041	2.9	0.035	2.5	0.039	2.8	0.077	5.5
Sample 3	90	5.05	0.113	2.2	0.068	1.3	0.142	2.8	0.203	4	0.280	5.5
Sample 4	90	0.735	0.021	2.	0.017	2.2	0.030	4.1	0.034	4.6	0.053	7.2
Sample 5	90	1.63	0.048	3	0.035	2.1	0.029	1.8	0.065	4	0.092	5.7
Sample 6	90	0.542	0.013	2.5	0.006	1.1	0.02	3.7	0.033	6.2	0.041	7.7
Negative Control	90	0.128	0.006	4.3	0.00	0	0.004	3.3	0.006	4.8	0.009	7.3
Positive Control	90	2.25	0.064	2.9	0.037	1.7	0.048	2.1	0.113	5	0.144	6.4

2. Linearity:

   Not Applicable

3. Analytical Specificity/Interference:

   Cross-reactivity study: The cross-reactivity study evaluated 222 specimens from 22 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 4. Cross-reactivity Study Results

Organism/Disease State	Samples Tested (N)	LIAISON Lyme IgG Positive or Equivocal Result
Tick Borne Diseases		
Babesiosis IgG	10	5[a]
Autoimmune Disorders		
Anti-Nuclear Antibodies (ANA)	10	0
Multiple Sclerosis	10	0
Viral Diseases		
Cytomegalovirus (CMV) IgG	10	0
Epstein-Barr Virus (EBV IgG)	10	0
Epstein-Barr Virus (EBV) EBNA IgG	10	1[b]
Epstein-Barr Virus (EBV) VCA IgG	10	0
Herpes Simplex Virus (HSV) IgG	10	0

Human Immunodeficiency Virus (HIV)	10	0
Influenza Virus	10	0
Parvovirus IgG	10	0
Varicella Zoster Virus (VZV) IgG	10	0
Bacterial Diseases		
H. pylori	10	1
Syphilis	10	0
Leptospirosis	2	1
Rheumatic Diseases		
Fibromyalgia	10	0
Rheumatoid Arthritis	10	0
Rheumatoid Factor	10	0
Systemic Lupus Erythematosus (SLE)	10	0
Sjogrens Syndrome	10	1
Additional Markers		
Chronic Fatigue Syndrome	10	1
Human Anti-mouse Antibodies (HAMA)	10	0
E. coli	10	0

[a] Four out of five samples were also positive by the predicate device
[b] Positive by the predicate device

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.

Table 5. Interference Testing Results

Substance	Concentration
Hemoglobin	1000 mg/dL
Triglycerides	1500 mg/dL
Cholesterol	400 mg/dL
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Total protein	15 g/dL
Biotin	3600 ng/mL

Class Specificity Study: Dithiothreitol **(**DTT) was used to specifically inactivate IgM antibodies without affecting IgG antibodies. Five *Borrelia burgdorferi* positive specimens containing various levels of IgG antibodies and known to be IgM positive by Western blot were used for this testing. Samples were divided into two aliquots: one aliquot was spiked with DTT to a final concentration of 10 mM and the second aliquot was spiked with the same volume of vehicle control. Results are summarized in the table below.

Table 6. Class Specificity Study Results

Sample ID	Vehicle Control IgM Assay	DTT Treated IgM Assay	Vehicle Control IgG Assay	DTT Treated IgG Assay	% Difference IgG
1	1.78	<0	41.1	40.0	2.7%
2	6.13	0.0264	28.6	28.5	0.3%
3	7.70	0.134	39.6	41.0	3.5%
4	5.31	<0	4.35	4.7	-9.7%
5	1.19	<0	5.68	5.54	2.5%

4. Assay Reportable Range:

Not applicable.

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

Specimen Stability

Freeze-thaw Study

A study was performed using six samples for each of the following sample types: serum (no preservative), Serum-Separating Tube (SST) serum, EDTA plasma and lithium heparin plasma. Each sample type includes neat or contrived serum samples spiked to the following levels: one negative, two high negative, two low positive, and one moderate positive.

Samples were aliquoted, frozen and thawed for up to six freeze/thaw cycles before being tested in duplicate in the same assay.

Table 7. Freeze-thaw (FT) Sample Stability Results (Mean Index Value)

Serum						
Sample	**0 FT**	**1 FT**	**3 FT**	**4 FT**	**5 FT**	**6 FT**
Negative	0.233	0.247	0.251	0.255	0.244	0.250
High Negative 1	0.804	0.736	0.714	0.692	0.700	0.699
High Negative 2	0.868	0.957	1.08	1.02	1.05	1.10
Low Positive 1	1.50	1.42	1.38	1.44	1.32	1.39
Low Positive 2	1.70	1.62	1.86	1.84	1.81	1.91
Moderate Positive	4.96	4.75	4.50	4.62	4.48	4.56
SST Serum						
Sample	**0 FT**	**1 FT**	**2 FT**	**4 FT**	**5 FT**	**6 FT**
Negative	0.230	0.238	0.250	0.248	0.251	0.254

High Negative 1	0.856	0.717	0.696	0.700	0.682	0.709
High Negative 2	0.888	0.957	1.12	1.06	1.08	1.17
Low Positive 1	1.52	1.46	1.36	1.39	1.30	1.36
Low Positive 2	1.65	1.557	1.87	1.83	1.87	2.01
Moderate Positive	4.99	4.77	4.60	4.47	4.51	4.66
K_2-EDTA Plasma						
Sample	**0 FT**	**1 FT**	**2 FT**	**4 FT**	**5 FT**	**6 FT**
Negative	0.225	0.247	0.246	0.242	0.243	0.244
High Negative 1	0.776	0.657	0.711	0.641	0.649	0.643
High Negative 2	0.975	0.958	1.12	1.03	1.07	1.10
Low Positive 1	1.37	1.30	1.18	1.26	1.15	1.22
Low Positive 2	1.51	1.46	1.80	1.83	1.82	1.97
Moderate Positive	4.75	4.55	4.26	4.47	4.37	4.31
Lithium Heparin Plasma						
Sample	**0 FT**	**1 FT**	**2 FT**	**4 FT**	**5 FT**	**6 FT**
Negative	0.251	0.290	0.272	0.289	0.282	0.295
High Negative 1	0.844	0.694	0.721	0.717	0.690	0.737
High Negative 2	1.00	0.943	1.07	1.09	1.01	1.11
Low Positive 1	1.49	1.34	1.26	1.32	1.28	1.26
Low Positive 2	1.49	1.41	1.71	1.70	1.72	1.67
Moderate Positive	4.98	4.85	4.58	4.68	4.60	4.62

Regression of the mean percent difference versus freeze thaw counts does not cross ±10% for any of the sample types tested and thus demonstrate the stability of all claimed specimen types over the sponsor's stability claim of five freeze-thaw cycles.

Room Temperature Stability

Another stability study was performed to demonstrate analyte stability when stored at room temperature. Six samples containing the same analyte levels as described above were aliquoted and stored at 22°C for the indicated times prior to testing. Samples were tested in duplicate at the following time points: 0, 4, 8, 9, 24, 25, 48, 49, 72, 73, 168 and169 hours. Data is summarized in the table below.

Table 8. Room Temperature Stability Results (Mean Index Values)

Serum												
Sample	**T0**	**4 h**	**8 h**	**9 h**	**24 h**	**25 h**	**48 h**	**49 h**	**72 h**	**73 h**	**168 h**	**169 h**
Negative	0.233	0.235	0.217	0.229	0.232	0.223	0.237	0.238	0.232	0.237	0.258	0.230
High Negative 1	0.804	0.705	0.679	0.707	0.714	0.712	0.729	0.743	0.749	0.741	0.722	0.725
High Negative 2	0.868	0.940	0.943	0.919	1.04	0.987	1.06	0.961	1.06	1.00	1.03	1.03
Low Positive 1	1.50	1.39	1.46	1.40	1.31	1.40	1.46	1.40	1.52	1.50	1.52	1.47
Low Positive 2	1.70	1.63	1.60	1.61	1.74	1.59	1.68	1.68	1.79	1.67	1.59	1.64
Moderate Positive	4.96	4.40	4.55	4.42	4.72	4.66	4.64	4.64	4.63	4.71	4.67	4.67
SST Serum												
Sample	**T0**	**4 h**	**8 h**	**9 h**	**24 h**	**25 h**	**48 h**	**49 h**	**72 h**	**73 h**	**168 h**	**169 h**
Negative	0.230	0.235	0.233	0.227	0.232	0.228	0.241	0.232	0.246	0.242	0.248	0.241
High Negative 1	0.856	0.702	0.689	0.729	0.704	0.753	0.712	0.751	0.750	0.740	0.720	0.715
High Negative 2	0.888	0.955	0.947	0.966	1.18	1.10	1.10	1.03	1.18	0.984	1.11	1.04
Low Positive 1	1.52	1.33	1.41	1.45	1.26	1.45	1.53	1.41	1.41	1.45	1.46	1.41
Low Positive 2	1.65	1.70	1.66	1.64	1.86	1.63	1.78	1.72	1.83	1.66	1.64	1.60
Moderate Positive	4.99	4.40	4.59	4.50	4.52	4.59	4.75	4.69	4.64	4.66	4.68	4.72
K_2-EDTA Plasma												
Sample	**T0**	**4 h**	**8 h**	**9 h**	**24 h**	**25 h**	**48 h**	**49 h**	**72 h**	**73 h**	**168 h**	**169 h**
Negative	0.225	0.233	0.226	0.221	0.226	0.231	0.234	0.238	0.230	0.236	0.238	0.239
High Negative 1	0.776	0.687	0.651	0.654	0.712	0.684	0.678	0.682	0.704	0.684	0.695	0.696
High Negative 2	0.975	0.988	0.914	0.955	1.05	1.04	1.03	1.02	1.10	0.986	1.04	1.01
Low Positive 1	1.37	1.24	1.24	1.22	1.27	1.35	1.27	1.30	1.28	1.33	1.27	1.27
Low Positive 2	1.51	1.59	1.58	1.53	1.85	1.61	1.70	1.75	1.82	1.63	1.65	1.70
Moderate Positive	4.75	4.34	4.29	4.32	6.51	4.44	4.49	4.45	4.46	4.54	4.38	4.50
Lithium Heparin Plasma												
Sample	**T0**	**4 h**	**8 h**	**9 h**	**24 h**	**25 h**	**48 h**	**49 h**	**72 h**	**73 h**	**168 h**	**169 h**
Negative	0.251	0.284	0.255	0.268	0.276	0.248	0.294	0.272	0.288	0.287	0.296	0.270
High Negative 1	0.844	0.696	0.724	0.735	0.724	0.761	0.678	0.749	0.784	0.756	0.751	0.754

High Negative 2	1.00	0.949	0.948	0.959	1.10	1.01	0.994	0.990	1.05	0.941	1.01	0.954
Low Positive 1	1.49	1.28	1.31	1.34	1.46	1.43	1.36	1.34	1.34	1.38	1.37	1.36
Low Positive 2	1.49	1.53	1.51	1.56	1.72	1.49	1.68	1.63	1.79	1.60	1.55	1.60
Moderate Positive	4.98	4.59	4.66	4.64	4.77	4.76	4.89	4.89	4.74	4.92	4.80	4.80

No significant trend over a period of 169 hours was observed and the mean percent difference versus time does not cross ±10% over the course of the study. These data are sufficient to support the sponsor's recommended storage claim of three days at room temperature.

An additional specimen stability study was performed to assess stability of samples when stored at 4°C. Six samples containing the same analyte levels as described above were aliquoted and stored at 22°C for the indicated times prior to testing. Samples were tested in duplicate at the following time points: 0, 3, 4, 7, 8, 14 and 15 days. Data is summarized in the table below.

Table 9. Storage at 4°C Stability Results (Mean Index Values)

Serum							
Sample	**0 d**	**3 d**	**4 d**	**7 d**	**8 d**	**14 d**	**15 d**
Negative	0.181	0.202	0.187	0.196	0.199	0.212	0.229
High Negative 1	0.725	0.691	0.663	0.724	0.712	0.747	0.659
High Negative 2	0.632	0.598	0.627	0.645	0.631	0.616	0.695
Low Positive 1	1.39	1.28	1.33	1.38	1.38	1.32	1.34
Low Positive 2	1.34	1.34	1.34	1.34	1.34	1.34	1.34
Moderate Positive	2.09	1.84	1.88	1.92	1.98	1.93	2.14
SST Serum							
Sample	**0 d**	**3 d**	**4 d**	**7 d**	**8 d**	**14 d**	**15 d**
Negative	0.180	0.199	0.185	0.200	0.191	0.211	0.206
High Negative 1	0.878	0.798	0.820	0.856	0.877	0.844	0.771
High Negative 2	0.659	0.659	0.663	0.682	0.662	0.641	0.725
Low Positive 1	1.51	1.41	1.36	1.40	1.46	1.39	1.45
Low Positive 2	1.28	1.23	1.27	1.24	1.21	1.28	1.12
Moderate Positive	2.14	1.94	1.99	1.96	1.98	1.97	2.13

K_2 EDTA Plasma							
Sample	**0 d**	**3 d**	**4 d**	**7 d**	**8 d**	**14 d**	**15 d**
Negative	0.205	0.212	0.207	0.209	0.210	0.212	0.209
High Negative 1	0.666	0.592	0.626	0.625	0.590	0.635	0.598
High Negative 2	0.638	0.627	0.638	0.651	0.635	0.644	0.742
Low Positive 1	1.40	1.37	1.32	1.21	1.26	1.17	1.30
Low Positive 2	1.20	1.12	1.18	1.15	1.12	1.14	1.07
Moderate Positive	2.06	1.84	1.89	1.86	1.84	1.79	2.08
Lithium Heparin Plasma							
Sample	**0 d**	**3 d**	**4 d**	**7 d**	**8 d**	**14 d**	**15 d**
Negative	0.200	0.217	0.206	0.212	0.213	0.206	0.202
High Negative 1	0.573	0.534	0.551	0.565	0.551	0.534	0.546
High Negative 2	0.614	0.594	0.610	0.620	0.591	0.604	0.722
Low Positive 1	1.36	1.21	1.25	1.22	1.29	1.22	1.38
Low Positive 2	1.17	1.04	1.13	1.09	1.04	1.10	1.02
Moderate Positive	2.16	1.99	2.03	2.09	2.01	1.95	2.28

The regression of mean percent difference versus time does not cross +10% for any of the sample types tested. These data support stability of all claimed specimen types over a period of 14 days when stored at 4°C.

Finally, sample stability was also assessed when stored at -20°C. Six samples containing the same analyte levels as described above were aliquoted and stored at 22°C for the indicated times prior to testing. Samples were tested in duplicate at designated intervals of 1, 2, 3, 6, 7, 9, 10, 12 and 13 months. Results for up to three months have been obtained, additional testing is currently ongoing. Available data is summarized in the table below.

Table 10. Frozen Sample Stability Results (Mean Index Values)

Serum				
Sample	**0 Mo.**	**1 Mo.**	**2 Mo.**	**3 Mo.**
Negative	0.181	0.190	0.198	0.213
High Negative 1	0.725	0.668	0.687	0.778
High Negative 2	0.632	0.700	0.616	0.75

Low Positive 1	1.39	1.25	1.26	1.37
Low Positive 2	1.34	1.08	0.977	1.14
Moderate Positive	2.09	1.96	1.84	2.03
SST Serum				
Sample	**0 Mo.**	**1 Mo.**	**2 Mo.**	**3 Mo.**
Negative	0.180	0.197	0.178	0.201
High Negative 1	0.878	0.811	0.759	0.916
High Negative 2	0.659	0.723	0.648	0.793
Low Positive 1	1.51	1.34	1.24	1.48
Low Positive 2	1.28	1.18	1.13	1.35
Moderate Positive	2.14	2.05	1.84	2.19
K_2 EDTA Plasma				
Sample	**0 Mo.**	**1 Mo.**	**2 Mo.**	**3 Mo.**
Negative	0.205	0.218	0.211	0.24
High Negative 1	0.666	0.614	0.628	0.682
High Negative 2	0.638	0.73	0.665	0.744
Low Positive 1	1.40	1.23	1.18	1.38
Low Positive 2	1.20	1.10	1.14	1.23
Moderate Positive	2.06	1.99	1.85	1.95
Lithium Heparin Plasma				
Sample	**0 Mo.**	**1 Mo.**	**2 Mo.**	**3 Mo.**
Negative	0.200	0.210	0.200	0.249
High Negative 1	0.573	0.580	0.537	0.618
High Negative 2	0.614	0.634	0.622	0.689
Low Positive 1	1.36	1.29	1.22	1.43
Low Positive 2	1.17	1.03	1.00	1.23
Moderate Positive	2.16	2.09	2.16	2.29

6. Detection Limit:

Not Applicable.

7. Assay Cut-Off:

The cut-off for the LIAISON Lyme IgG 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 IgG assay. A cut-off index of 1.0 was determined to provide the best balance of sensitivity and specificity. An equivocal zone of 0.90-1.10 was applied to the assay to account for normal measurement imprecision.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Prospective Cohort Testing: A total of 2,643 human serum specimens were obtained from four approved commercial vendors. The specimens were collected from non-selected subjects sent to the lab for Lyme disease testing in 14 states which represented five distinct geographical regions. Twenty-two (22) samples did not have enough volume to complete all testing. A total of 2,621 samples were evaluated of which 44.1% were male, 55.7% were female, and 0.2% did not have a gender reported.

Samples were tested with the LIAISON Lyme IgG assay on the LIAISON XL instrument at three laboratories (two external and one internal at DiaSorin). Results were evaluated for first-tier testing and compared to the predicate device.

Table 11. First-Tier Percent Agreement with the Predicate Device

	Predicate Assay (ZEUS ELISA B. Burgdorferi IgG Test System)			
LIAISON Lyme IgG	**Positive**	**Equivocal**	**Negative**	**Total**
Positive	156	4	86	246
Equivocal	6	0	24	30
Negative	119	16	2210	2345
Total	281	20	2320	2621

Positive % Agreement*: 55.1% (166/301) 95% CI: 49.5-60.7%
Negative % Agreement: 96.5% (2210/2320) 95% CI: 94.3-96.1%
*Includes Positive and Equivocal results combined

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

Table 12. Second-Tier Testing Results

Test System	Tier 1+ or Eqv.	Western Blot IgG +	Western Blot IgG -
Predicate Assay	301	109	192
LIAISON Lyme IgG	276	109	167
Predicate and LIAISON Lyme IgG	166	97	69

Despite a larger number of initially positive specimens on the predicate device, both the predicate and the LIAISON Lyme IgG test yielded the same number of samples positive by second tier western blot testing. Furthermore, of the 135 specimens positive by the predicate device and negative by the LIAISON Lyme IgG test (Table 11), 123 (91.1%) were negative by second tier western blot.

Performance agreement of the Lyme IgG assay when utilized as a first-tier test in a standard two-tiered testing algorithm (STTT) is summarized below and compared to results for the predicate assay.

Table 13. Second-Tier Agreement – Standard Two-tiered Testing Algorithm

	Predicate Assay IgG - STTT		
LIAISON Lyme IgG - STTT	**Positive**	**Negative**	**Total**
Positive	97	12[b]	109
Negative	12[a]	2500	2512
Total	109	2512	2621

[a] All 12 discordant samples were negative when evaluated by another FDA cleared immunoblot assay
[b] 3/12 discordant samples were positive when evaluated by another FDA cleared immunoblot assay

Second-Tier Tier Positive % Agreement: 89.0% (97/109) 95% CI: 81.7-93.6%
Second-Tier Negative % Agreement: 99.5% (2500/2512) 95% CI: 99.2-99.7%

Cumulatively, these data support equivalence of the LIAISON Lyme IgG test to the predicate device as a first-tier test for Lyme disease.

Performance of the LIAISON Lyme IgG was also evaluated when utilized as a second-tier test as part of a Modified Two-tiered Testing (MTTT) algorithm. Specifically, the 2621 prospective specimens were tested initially with the cleared assay, LIAISON Lyme Total Antibody Plus. Positive or equivocal results on the LIAISON Lyme Total Antibody Plus were subsequently evaluated by the LIAISON Lyme IgG assay with positive or equivocal results considered MTTT positive. Agreement of the MTTT algorithm was also assessed against use of the cleared chemiluminescent immunoassay (CLIA) LIAISON Lyme Total Antibody Plus followed by a cleared western blot test as part of an STTT protocol. These schemes are summarized below.

Figure 1. STTT IgG Western Blot Algorithm (WB-STTT [IgG])

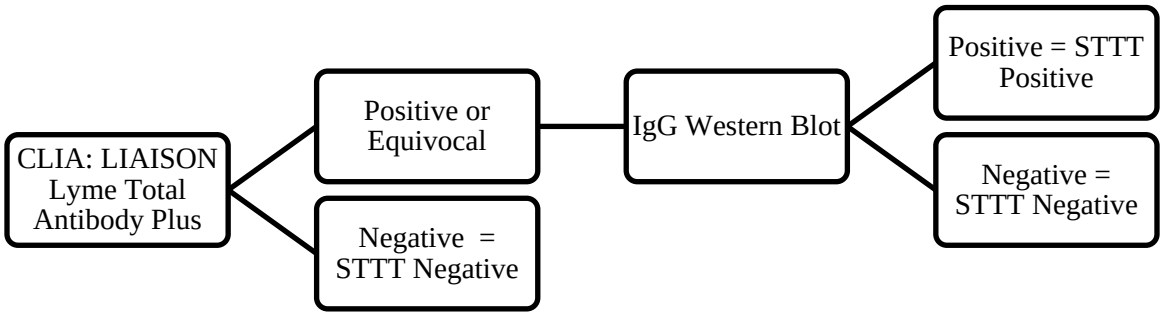
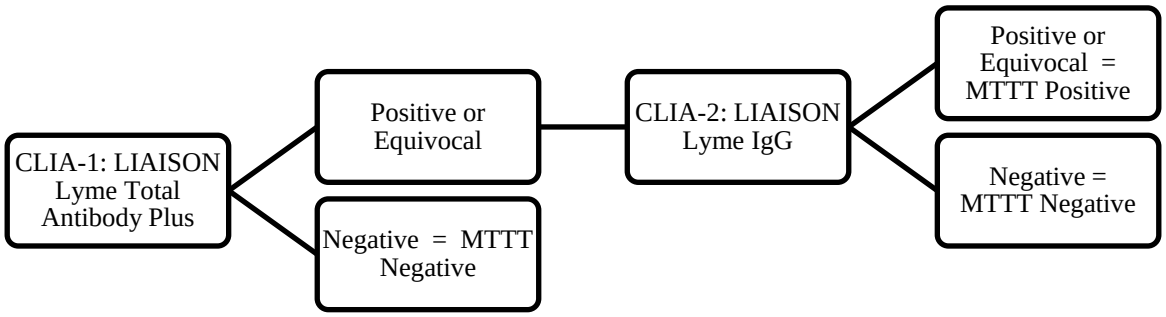


Figure 2. MTTT IgG CLIA Algorithm (CLIA-MTTT [IgG])



Performance of the Lyme IgG assay as second tier test was considered on only those specimens positive by the first-tier test. A total of 225 specimens exhibited positive or equivocal results when evaluated with the Lyme Total Antibody Plus assay as a first-tier test.

Table 14. Performance of the Lyme IgG MTTT Algorithm compared to STTT using WB IgG

		WB-STTT (IgG)		
		Positive	**Negative**	**Total**
CLIA-MTTT (IgG)	**Positive**	93	90	183
	Negative	3	39	42
Total		96	129	225

Positive % Agreement: 96.9% (93/96) 95% CI: 91.2-98.9%
Negative % Agreement: 30.2% (39/129) 95% CI: 23.0-38.6%

Retrospective Cohort Testing: A retrospective cohort was also evaluated that consisted of 280 panel members obtained from CDC. The retrospective panel contained 90 specimens from patients known to have Lyme disease, 90 specimens from patients with diseases other than Lyme disease, and 50 specimens each from healthy controls in both endemic and non-endemic regions.

The CDC Lyme panel was tested internally at DiaSorin on the LIAISON Lyme IgG assay and the predicated device (ZEUS ELISA *B. burgdorferi* IgG assay). No repeat testing of equivocal samples was performed on the ZEUS IgG assay due to limited sample volume. No western blots were performed on any of the positive or equivocal results. Summary of agreement is included in the table below.

Table 15. First-Tier Performance – CDC Reference Panel

Sample Category	LIAISON Lyme IgG					ZEUS *B. Burgdorferi* IgG				
	Pos.	**Eqv.**	**Neg.**	**PPA/ NPA**	**95% CI**	**Pos.**	**Eqv.**	**Neg.**	**PPA/ NPA**	**95% CI**
Acute	28	1	10[a]	74.4%	58.9-85.4%	23	1	15	61.5%	45.9-75.1%
Convalescent	29	0	2[b]	93.5%	79.3-98.2%	22	3	6	80.6%	63.7-90.8%
Late	20	0	0	100%	83.9-100%	20	0	0	100%	83.9-100%
Healthy Controls (N = 100)	3[c]	0	97	97%	91.6-99.0%	1	1	98	98%	93.0-99.5%
Disease Controls (N = 90)	9[d]	0	81	90%	82.1-94.7%	9	4	77	85.6%	76.8-91.4%

[a] Of the 10 negative discordant specimens, all were Early Lyme-EM and all were CDC 2-tier IgG Negative
[b] Of the 2 negative discordant specimens, both were Early Lyme-EM, both were CDC 2-tier IgG negative
[c] Of the 3 discordant healthy controls, 2 were non-endemic controls and 1 was an endemic negative control
[d] The 9 discordant look-alike diseases were the following specimens: 4 Syphilis, 2 Mononucleosis, 1 Periodontis, 1 Rheumatoid arthritis, and 1 Fibromyalgia

These results establish that LIAISON Lyme IgG assay performance is equivalent to the predicate ZEUS *B. Burgdorferi* IgG test for the pre-determined CDC Reference Panel cohort classifications.

Performance of the Lyme IgG assay was also evaluated against the same specimens when utilized as a second-tier test as part of a MTTT algorithm. The STTT and MTTT testing algorithms utilized for this study are the same as identified above in Figures 1 and 2. Results from this comparison are summarized in the below table. One sample from the endemic negative control group did not have sufficient volume for testing; therefore, a total of 279 retrospective samples were evaluated.

Table 16. Performance Comparisons between EIA-MTTT (IgG) and WB-STTT (IgG) methods – CDC Panel Results

	Stage I (N = 60)		Stage II (N = 10)		Stage III (N = 20)		Healthy Controls (N=99)		Disease Controls (N = 90)	
	WB-STTT (IgG)	CLIA-MTTT (IgG)	WB-STTT (IgG)	CLIA-MTTT (IgG)	WB-STTT (IgG)	CLIA-MTTT (IgG)	WB-STTT (IgG)	CLIA-MTTT (IgG)	WB-STTT (IgG)	CLIA-MTTT (IgG)
Positive	18	48	5	9	20	20	0	0	0	2
Negative	42	12	5	1	0	0	99	99	90	88
Sensitivity or PPA	30%	80%	50%	90%	100%	100%	N/A	N/A	N/A	N/A
Specificity or PPA	N/A	N/A	N/A	N/A	N/A	N/A	100%	100%	100	97.8

Based on the above results, use of the LIAISON Lyme IgG assay as a second-tier test, successfully detects a greater proportion of early Lyme disease cases. Use of the LIAISON Lyme IgG assay is therefore considered acceptable for use with the LIAISON Lyme Total Antibody Plus assay following the Modified Two-Tiered Testing Methodology.

Analytical Specificity: The LIAISON Lyme IgG assay was used to test 300 samples from apparently healthy individuals in the U.S. This population was 57.3% female, 42.7% male with a mean age of 50 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 17. Analytical Specificity Study Results

Population	N	% Positivity
Endemic	150	6.7% (10/150)
Non-endemic	150	1.3% (2/150)
All specimens	300	4.0% (12/300)

2. Matrix Comparison:

Matrix Equivalency Study: Thirty-two (32) matched patient sets of serum, SST serum, K2-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 & Bablok regression. All sample types met acceptance criteria for use in the LIAISON Lyme IgG assay. A summary of the results are shown in the following table.

Table 18. Matrix Equivalency Study Results

Comparison to Serum	Bias	95% CI
SST Serum Constant	-0.01	-0.04 – 0.00
SST Serum Proportional	1.04	1.01 – 1.10
K_2-EDTA Constant	0.00	-0.01– 0.03
K_2-EDTA Proportional	0.96	0.93 – 1.01
Lithium Heparin Constant	0.01	-0.04 – 0.04
Lithium Heparin Proportional	0.97	0.91 – 1.03

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 2,621 masked specimens prospectively collected from patients between the ages of 2-103 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 19.

Table 19. Expected Values

Populations	Number Tested	Gender[a]		Age Range	Positive/Tested
		Male	Female		
Prospective	2,621	1,156	1,459	2-103	276/2,621
Endemic	150	49	101	18-87	10/150
Non-Endemic	150	79	71	19-71	2/150

[a] Six specimens did not include gender information

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.