

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K142038

**B. Purpose for Submission:**

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

**C. Measurand:**

IgG/IgM class antibodies against *Borrelia burgdorferi*

**D. Type of Test:**

Enzyme immunoassay

**E. Applicant:**

EUROIMMUN US Inc.

**F. Proprietary and Established Names:**

EUROIMMUN Lyme ELISA (IgG/IgM)

**G. Regulatory Information:**

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

**H. Intended Use:**

1. Intended use(s): The EUROIMMUN Lyme ELISA (IgG/IgM) test kit is intended for the qualitative determination of IgG and/or IgM class antibodies against *Borrelia burgdorferi* in human serum and plasma (K<sup>+</sup>-EDTA, Li<sup>+</sup>-heparin) from symptomatic patients or people suspected of *B. burgdorferi* infection. It is used as an aid in the diagnosis of Lyme disease, in conjunction with other laboratory and clinical findings. All positive and borderline results should be supplemented by a second step testing method such as Western blot.
2. Indication(s) for use: Same as Intended Use
3. Special conditions for use statement(s): N/A

4. Special instrument requirements: N/A

**I. Device Description:**

The test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with *Borrelia burgdorferi* antigens. In the first reaction step, diluted patient samples, calibrator and controls are incubated in the wells. Anti-*Borrelia burgdorferi* antibodies will bind to the antigens coated in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, anti-human IgG/IgM HRP enzyme conjugate (rabbit/goat) is added to each well. The enzyme conjugate will bind to any wells that have human IgG and/or IgM binding to the *B. burgdorferi* antigens. The wells are washed to remove any unbound HRP enzyme conjugate and 3,3',5,5'-tetramethylbenzidine (TMB) enzyme substrate is added. If the HRP enzyme is present in the well (positive reaction), the HRP enzyme will react with the TMB substrate and produce a blue color. After an additional incubation time to allow the color development, a stop solution is added which turns the blue color yellow and inhibits further color development to allow for a stable spectrophotometric reading. The test strips are placed in a microplate reader and the optical density of the color is measured. The amount of antigen specific bound antibody is proportional to the color intensity.

**J. Substantial Equivalence Information:**

1. Predicate device name(s): Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™
2. Predicate 510(k) number(s): K003754
3. Comparison with predicate:

**Similarities**

Item	Device	Predicate
<b>Intended Use</b>	Detection of IgG and IgM antibodies to <i>Borrelia burgdorferi</i>	Same
<b>Technology</b>	ELISA	Same
<b>Assay Platform</b>	96-well microtiter plates	Same
<b>Conjugate</b>	Anti-human IgG/IgM labelled with horseradish peroxidase	Same
<b>Substrate</b>	TMB	Same
<b>Wash Buffer</b>	10x concentrate	Same
<b>Stop Solution</b>	0.5 M/1 N sulphuric acid	Same
<b>Reagent Preparation</b>	Reagents, calibrator & controls are ready for use, except for the wash buffer.	Same
<b>Procedure</b>	Sample incubation with micro-well antigen coated plate, followed by a wash step, incubation with an anti-human IgG/IgM enzyme conjugate; wash step, incubation with substrate; stopping of the reaction with stop solution, photometric reading.	Same

## Differences

Item	Device	Predicate												
Antigen	<i>B. burgdorferi</i> sensu stricto B31 US strain VlsE and OspC antigens	Synthetic peptide (VlsE protein derived)												
Assay Format	Qualitative	“Presumptive detection” as stated on FDA Indications for Use form												
Samples Type	Serum or plasma (K+-EDTA, Li+-heparin)	Serum												
Sample Dilution	1:101	1:21												
Calibrators and Controls	Prediluted. 1 calibrator (cut-off) 2 controls: 1 positive, 1 negative	Not prediluted. 1 calibrator (cut-off) 2 controls: 1 positive, 1 negative												
Reported Results	Ratio	Index												
Cut off levels	<table style="margin-left: auto; margin-right: auto;"> <tr> <td><i>Ratio</i></td> <td><i>Result</i></td> </tr> <tr> <td>≥0.8 to &lt;1.1</td> <td>borderline</td> </tr> <tr> <td>≥1.1</td> <td>positive</td> </tr> </table>	<i>Ratio</i>	<i>Result</i>	≥0.8 to <1.1	borderline	≥1.1	positive	<table style="margin-left: auto; margin-right: auto;"> <tr> <td><i>Index</i></td> <td><i>Result</i></td> </tr> <tr> <td>0.91 to 1.09</td> <td>equivocal</td> </tr> <tr> <td>≥ 1.10</td> <td>positive</td> </tr> </table>	<i>Index</i>	<i>Result</i>	0.91 to 1.09	equivocal	≥ 1.10	positive
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≥1.1	positive													
<i>Index</i>	<i>Result</i>													
0.91 to 1.09	equivocal													
≥ 1.10	positive													

**K. Standard/Guidance Document Referenced (if applicable):** N/A

**L. Test Principle:** Enzyme Immunoassay

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

**Precision:** Repeatability was investigated using samples with values at different concentrations. The intra-assay repeatability is based on 20 determinations and the inter-assay repeatability is based on 40 determinations performed in 20 different runs on 10 different days (with 2 runs per day and 2 replicates per run). The results are shown below.

Sample No.	Mean Ratio	Within-Run		Within-Day		Between-Days		Total	
		SD	%CV	SD*	%CV	SD	%CV	SD	%CV
1	0.1	0.006	4.1	0.00	0.0	0.009	6.6	0.01	7.8
2	0.2	0.008	3.9	0.00	0.0	0.007	3.5	0.01	5.2
3	0.5	0.022	4.2	0.00	0.0	0.031	6.0	0.04	7.3
4	0.8	0.051	6.4	0.00	0.0	0.051	6.4	0.07	9.1
5	1.1	0.029	2.5	0.00	0.0	0.058	5.2	0.06	5.8
6	1.9	0.065	3.5	0.00	0.0	0.133	7.2	0.15	8.0
7	3.0	0.103	3.4	0.00	0.0	0.223	7.4	0.25	8.2
8	3.7	0.113	3.0	0.00	0.0	0.247	6.6	0.27	7.3

\*When the estimate of within-day SD is negative, it is set to 0 [CLSI EP-5: Evaluation of Precision Performance of Quantitative Measurements Methods, Repeatability Estimate.]

**Reproducibility:** Reproducibility was investigated using samples with values at different concentrations, which are based on 30 determinations performed with 3

different lots with 1 run per lot and 10 replicates per run according to the package insert. Site-to-site testing was done in 48 determinations per sample performed at 3 different sites for 4 days with 2 runs per day and 2 replicates per run. The results are shown below.

Sample No.	Mean Ratio	Within-Run		Within-Day		Between-Days		Between-Sites		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.6	0.056	8.7	0.014	2.1	0.014	2.1	0.020	3.0	0.030	4.6
2	0.8	0.047	6.0	0.074	9.4	0.074	9.4	0.051	6.5	0.057	7.3
3	1.0	0.099	9.8	0.017	1.7	0.017	1.7	0.094	9.3	0.070	6.9
4	5.9	0.367	6.2	0.587	9.9	0.587	9.9	0.138	2.3	0.364	6.2
5	6.4	0.268	4.2	0.769	12.0	0.769	12.0	0.227	3.5	0.421	6.6
6	7.1	0.218	3.1	0.941	13.2	0.941	13.2	0.310	4.3	0.490	6.9
7	9.2	0.373	4.0	1.136	12.3	1.136	12.3	0.307	3.3	0.605	6.5
8	10.1	0.606	6.0	1.182	11.7	1.182	11.7	0.316	3.1	0.701	6.9

b. *Linearity/assay reportable range:* N/A

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

d. *Detection limit:* N/A

e. *Analytical specificity:*

**Analytical Specificity Study:** The levels of anti-*Borrelia burgdorferi* antibodies from asymptomatic populations were analyzed with the EUROIMMUN Lyme ELISA (IgG/IgM) in a panel of 98 samples from an endemic region (Pennsylvania; 89 men and 9 women; age range: 18 – 56 y) and in another panel of 100 samples from a non-endemic region (Tennessee; 82 men and 18 women; age range: 2 –76 y). The results are shown below.

Sample Type	N	Negative	Equivocal	Positive	% Equivocal + Positive
Endemic	98	95	0	3	3.1%
Non-endemic	100	95	3	2	5.0%

**Cross Reactivity:** Cross reactivity was investigated using 886 serologically characterized sera positive for antibodies against different disease conditions and the results obtained are shown in the table below.

No.	Panel	n	Lyme ELISA (IgG/IgM)	
			Negative	%
1	Anti- <i>Treponema pallidum</i>	88	87*	98.9
2	Anti-Adenovirus	50	50	100.0
3	Anti- <i>Bordetella pertussis</i> toxin	50	50	100.0
4	Anti-Bordetella FHA	50	50	100.0
5	Anti-CMV	50	50	100.0
6	Anti-EBV-CA	50	47	94.0
7	Anti- <i>Helicobacter pylori</i>	50	50	100.0

8	Anti-HSV-1	50	50	100.0
9	Anti-Influenza virus type A	50	50	100.0
10	Anti-Influenza virus type B	50	50	100.0
11	Anti-Measles virus	50	50	100.0
12	Anti-Mumps virus	50	50	100.0
13	Anti- <i>Mycoplasma pneumoniae</i>	50	50	100.0
14	Anti-Parainfluenza virus types 1-4	50	50	100.0
15	Anti-RSV	50	50	100.0
16	Anti-Parvovirus B19	35	34	97.1
17	ANA	14	14	100.0
18	Rheumatoid arthritis	17	16	94.1
19	Fibromyalgia	18	18	100.0
20	Multiple sclerosis and other neurological diseases	22	22	100.0

\*1 Borderline sample counted as positive.

Specimens from tick-borne relapsing fever, rickettsial diseases, ehrlichiosis, babesiosis, and leptospirosis have not been tested; therefore the performance of this device is unknown with these pathologies.

**Interferences:** Hemolytic, lipemic and icteric samples showed no influence on the result up to a concentration of 1000 mg/dl for hemoglobin, 2000 mg/dl for triglycerides and 40 mg/dl for bilirubin in testing with the EUROIMMUN Lyme ELISA (IgG/IgM). Interferences with albumin, intralipids and cholesterol on the assay have not been investigated.

*f. Assay cut-off:*

**Determination of Assay Cut-off:** The assay cut-off recommendation is based on a ROC analysis from the results of 81 reference samples obtained from the Centers for Disease Control and Prevention in Atlanta, GA.

2. Comparison studies:

*a. Method comparison with predicate device:*

**Method Comparison Study:** A prospective study was performed with clinical samples collected from various locations in the Northeastern US. The samples were tested with the EUROIMMUN Lyme ELISA (IgG/IgM) in parallel with the predicate device. The panel consisted of 173 men, 243 women and 2 unknowns with the age range from 19 - 76 years. The table below shows the results from the prospective studies.

n = 418		Predicate ELISA		
		Positive	Equivocal	Negative
EUROIMMUN ELISA	Positive	103	2	20
	Borderline	4	0	11
	Negative	2	2	274

Positive Agreement = 96.5% (109/113)      95% C.I. 91.2 - 99.0%  
 Negative Agreement = 89.8% (274/305)      95% C.I. 85.9 - 93.0%

Note: Borderline/equivocal counted as positives

*b. Matrix comparison:*

**Serum/Plasma Comparison:** The usability of plasma was investigated using sample pairs each of serum and corresponding plasma (K<sup>+</sup>-EDTA, Li<sup>+</sup>-heparin). Passing-Bablok regression was calculated for the comparison of serum to plasma. The regression equation is near the ideal correlation (intercept 0; slope 1.0) indicating equivalence of concentrations between serum and the corresponding plasma matrices. Coefficients of determination were found to be above 0.975 and % recovery compared to serum was in the range of 87 to 109 % (serum = 100 %).

	K <sup>+</sup> -EDTA Plasma	Li <sup>+</sup> -Heparin Plasma
<b>N</b>	20	20
<b>Concentration Range (serum)</b>	Ratio 0.4 – 4.3	Ratio 0.4 – 4.3
<b>Concentration Range (plasma)</b>	Ratio 0.4 – 4.2	Ratio 0.4 – 4.2
<b>Regression Equation</b> (y = plasma, x = serum)	y = -0.00 + 0.97 x	y = 0.04 + 0.96 x
<b>95% C.I. of Intercept</b>	-0.05 – 0.05	-0.03 – 0.07
<b>95% C.I. of Slope</b>	0.92 – 1.01	0.94 – 1.00
<b>Coefficient of Determination R<sup>2</sup></b>	0.9944	0.9961
<b>Mean %Recovery</b>	97 %	100 %
<b>Range of %Recovery</b>	87 – 103 %	93 – 109 %

3. Clinical studies:

*a. Clinical Sensitivity:*

**Sensitivity Study:** A study, consisting of 100 clinically characterized Lyme disease specimens, was conducted at the manufacturer’s site with the EUROIMMUN Lyme ELISA (IgG/IgM) test device in parallel with the predicate device. These specimens contain samples from early, early disseminated and late phases of the disease. The panel consisted of 36 men, 52 women and 12 unknowns. The age ranged from 16 - 80 years.

Disease Stage	n	EUROIMMUN Lyme ELISA (IgG/IgM)		Predicate ELISA	
		Positive or Borderline	Sensitivity (%) 95% C.I.	Positive or Borderline	Sensitivity (%) 95% C.I.
<b>Acute</b> (EM or culture positive, <3 months after onset)	46	44	95.7 85.2 - 99.5%	35	76.1 61.2 - 87.4%
<b>Convalescent</b> (EM or culture positive, 3-12 months after onset)	30	27	90.0 73.5 - 97.9%	25	83.3 65.3 - 94.4%

<b>Late</b> (Lyme disease with presentations other than EM, onset unknown or >1 year)	24	24	100.0 85.8 - 100.0%	22	91.7 73.0 - 99.0%
<b>Total</b>	100	95	95.0 88.7 - 98.4%	82	82.0 73.1 - 89.0%

**CDC Panel Testing:** Forty (40) samples of various reactivity were acquired from the Centers for Disease Control and Prevention in Atlanta, GA and evaluated internally. Of the 40 samples, 5 samples were from normal blood donors and 35 samples were from patients diagnosed with Lyme disease (clinically characterized borreliosis stratified by disease stage). All samples were tested with the EUROIMMUN Lyme ELISA (IgG/IgM) in parallel with the predicate device. Note: The results of the testing are presented here as a means of conveying further information on the performance of this assay with a characterized serum panel and does not imply an endorsement of the assay by the CDC.

Agreement to clinical diagnosis:

Disease Stage	n	EUROIMMUN Lyme ELISA (IgG/IgM)		Predicate ELISA	
		Positive or Borderline	Agreement with Clinical Diagnosis	Positive or Borderline	Agreement with Clinical Diagnosis
<b>Normals</b>	5	0	100.0%	0	100.0%
<b>&lt; 1 month</b>	6	6	100.0%	6	100.0%
<b>&gt; 1 - 3 months</b>	11	10	90.9%	10	90.9%
<b>&gt; 3 - 12 months</b>	11	9	81.8%	9	81.8%
<b>&gt; 12 months</b>	7	7	100.0%	7	100.0%
<b>Total</b>	40	32	80.0%	32	80.0%

b. *Clinical specificity:* N/A

c. *Other clinical supportive data (when a. and b. are not applicable):* N/A

4. Clinical cut-off: N/A

5. Expected values/Reference range:

The range of values and positivity of different populations among different studies with the EUROIMMUN Lyme ELISA (IgG/IgM) test kit are presented below with available patient demographics.

Population	n	Sex	Age Range	Ratio Results			Qualitative Results	
				Mean	Range	Std. Dev.	Positive or Borderline	%
<b>Prospective Study</b>	418	173 men, 243 women, 2 unknown	19-76 y; 2 unknown	1.5	0.0 - 9.8	2.29	140	33.5%
<b>Sensitivity Study</b>	100	36 men, 52 women, 12 unknown	16-80 y; 12 unknown	5.1	0.2 - 10.7	3.38	95	95.0%
<b>CDC Panel (Lyme Disease)</b>	35	unknown	unknown	4.2	0.3 - 8.5	2.79	32	80.0%
<b>Normal Endemic</b>	98	89 men, 9 women	18-56 y	0.4	0.1 - 5.8	0.85	3	3.1%
<b>Normal Non-Endemic</b>	100	82 men, 18 women	2-76 y	0.3	0.1 - 3.8	0.40	5	5.0%

**Note:** It is recommended that each laboratory determine its own normal range based on the population and equipment used.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

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