510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K172722

B. Purpose for Submission: To obtain a substantial equivalence determination and FDA clearance for a new device

C. Measurand: Anti-*Borrelia burgdorferi* (IgM) antibodies

D. Type of Test: Enzyme Immunoassay

E. Applicant: EUROIMMUN US

F. Proprietary and Established Names: Anti-Borreliaburgdorferi(IgM) antibodies

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 Anti-Borrelia burgdorferi US EUROLINE-WB (IgM) kit is a Western blot assay intended for the qualitative determination of IgM class antibodies against *Borrelia burgdorferi* in human serum and plasma (K^+-EDTA, Li^+-heparin, Na^+-citrate) samples that have been found positive or equivocal/borderline using an enzyme immunoassay (EIA) or immunofluorescence assay (IFA) test procedure for *Borrelia burgdorferi* antibodies. Results can be read manually or automated utilizing EUROLineScan. This test is used as an aid in the diagnosis of infections with *Borrelia burgdorferi* and the associated diseases, in conjunction with other laboratory and clinical findings.
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 test strips with electrophoretically separated antigen extracts of *B. burgdorferi*. Each test strip contains an additional membrane chip coated with p41 flagellin antigen. The blot strips will be blocked and incubated in the first reaction step with diluted patient samples. In the case of positive samples, specific antibodies of the class IgM will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human IgM (enzyme conjugate), catalyzing a color reaction.

J. Substantial Equivalence Information:

1. Predicate device name(s): MarDx Lyme Disease (IgM) Marblot

2. Predicate 510(k) number(s): K951709

3. Comparison with predicate:

Table 1: Similarities and Differences with the Predicate

Similarities		
Item	Device	Predicate
Intended Use	Detection of IgM antibodies to Borrelia burgdorferi	Same
Assay format	Qualitative	Same
Technology/ Procedure	Serum incubation with antigen coated strips followed by a wash step, incubation with an anti-human IgM enzyme-conjugate; wash step, incubation with substrate, wash step, air drying and visual reading.	Same
Antigens	Antigens of Borrelia Burgdorferi (strain B31) are separated in the presence of sodium dodecyl sulfate (SDS) by polyacrylamide gel electrophoresis. The resolved protein bands are then transferred to a nitrocellulose membrane.	Same
Conjugate	Alkaline phosphatase anti-human IgM	Same
Substrate	NBT/BCIP	Same

Differences		
Item	Device	Predicate
Sample	30 µl Serum or Plasma 1:51 dilution	20 µl Serum undiluted
Incubation times	30 – 30 – 10 min	30 – 15 – (4 - 12) min
Controls	Evaluation matrix with control strip (test strip incubated with a positive control serum)	Serum band locator Weakly reactive control Negative control

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: Repeatability was investigated by repeated determinations of seven characterized samples. The samples were tested on four different days with two runs per day, two replicates per run according to the package insert. Repeatability was found to be sufficient as no positive sample was found negative and vice versa.

Table 2: Precision Study

n = 16	Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)						
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**
Characterization	negative	negative	negative	negative	negative	positive	positive
% negative	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%
% positive	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%

Table 2 (continued): Detailed Precision Study Data

Band(s)	**Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)**		
	p25	**p39**	**p41**
Sample 1	0.0%	0.0%	0.0%
Sample 2	0.0%	0.0%	100.0%
Sample 3	0.0%	0.0%	100.0%
Sample 4	0.0%	0.0%	100.0%
Sample 5	0.0%	0.0%	100.0%
Sample 6	100.0%	100.0%	100.0%
Sample 7	0.0%	100.0%	100.0%

Reproducibility: Reproducibility was investigated by repeated determinations of seven characterized samples. The samples were tested on four different days with two runs per day, two replicates per run at three different sites according to the package insert. Reproducibility was found to be sufficient as no positive sample was found negative and vice versa.

Table 3: Reproducibility Study

n = 48	Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Characterization	negative	negative	negative	negative	negative	positive	positive
% negative	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%
% positive	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%

Table 3 continued: Detailed Reproducibility Data

Band(s)	Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)		
	p25	p39	p41
Sample 1	0.0%	0.0%	0.0%
Sample 2	0.0%	0.0%	100.0%
Sample 3	0.0%	0.0%	100.0%
Sample 4	0.0%	0.0%	100.0%
Sample 5	0.0%	0.0%	100.0%
Sample 6	100.0%	100.0%	100.0%
Sample 7	0.0%	100.0%	100.0%

b. Linearity/assay reportable range: N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods): N/A

d. Detection limit: N/A

e. Analytical specificity:

Normal Population Study: Testing of samples from asymptomatic population was performed in endemic and non-endemic regions with the Anti-Borrelia burgdorferi US EUROLINE-WB (IgM). A panel of 98 samples from the endemic region (Pennsylvania; 89 men and 9 women with an average age of 34 years; age range: 18 – 56 years) and a panel of 100 samples from a non-endemic region (Tennessee; 82 men and 18 women with an average age of 38 years; age range: 3 – 62 years) were tested. The results are shown below.

Table 4: Analytical Specificity Study

Panel	n	Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)	
		Positive (%)	Negative (%)
Endemic	98	5 (5.1%)	93 (94.9%)
Non-endemic	100	4 (4.0%)	96 (96.0%)

Cross-Reactivity: Cross-reactivity was investigated using characterized samples from the following groups as shown in the table below. All the samples tested were found negative with the Anti-Borrelia burgdorferi US EUROLINE-WB (IgM) according to the CDC criteria of results interpretation.

Table 5: Cross-Reactivity Study

Panel		n	Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)
			Negative (%)
EBV		15	15 (100.0%)
HSV		14	14 (100.0%)
Influenza viruses		15	15 (100.0%)
H. pylori		11	11 (100.0%)
Measles		15	15 (100.0%)
Parvovirus B19		12	12 (100.0%)
Rubella		14	14 (100.0%)
Treponema		10	10 (100.0%)
CMV		10	10 (100.0%)
Babesiosis		3	3 (100.0%)
Anaplasmosis (ehrlichiosis)		10	10 (100.0%)
Rickettsial diseases		4	4 (100.0%)
Rheumatoid arthritis		22	22 (100.0%)
Total		155	155 (100.0%)

Note: The results obtained with babesiosis (3) and ricketsial diseases (4) samples are not conclusive as enough samples were not tested.

Interferences: Hemolytic, lipemic and icteric samples showed no influence at the result up to a concentration of 500 mg/dl for hemoglobin, 2000 mg/dl for triglycerides and 40 mg/dl for bilirubin in this test system. Interferences with albumin, intralipids, and cholesterol have not been investigated.

f. Assay cut-off:

Band Cut-off Determination: The cut-off of the Anti-Borrelia burgdorferi US EUROLINE-WB (IgM) test system is defined as the lowest limit of a clearly visible band. Because visual examination by operator might be subjective, the reaction control card was developed to standardize strip evaluation. Alternatively the EUROLineScan software was established to allow for automated evaluation.

2. Comparison studies:

a. Method comparison with predicate device:

Prospective Study: A prospective study was performed at 3 different sites in the U.S. Samples were tested as per the CDC two-tiered testing for Lyme disease algorithm. All positive and equivocal samples by the first-tier EIA were tested with the Anti-Borrelia burgdorferi US EUROLINE-WB (IgM) and the predicate IgM Western blot. Results are interpreted by the CDC criteria.

Table 6: Percent Agreement with Predicate Device

n = 304		Predicate IgM WB	
		Positive	Negative
Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)	Positive	95	1
	Negative	3	205

Positive agreement	95/98 =96.9%	95% C.I.:	91.3% - 99.4%
Negative agreement	205/206 = 99.5%	95% C.I.:	97.3% - 100.0%

b. Matrix Conparison

Serum/Plasma Comparison: The use of K^+-EDTA, Li^+-heparin, Na^+-citrate plasma samples was confirmed by a correlation of 10 sample sets of serum and corresponding plasma. The sample sets were selected to cover both negative and positive results. The results of the plasma samples and the corresponding serum sample were compared and found to be sufficient as no positive sample was found negative and vice versa.

3. Clinical studies:

a. Clinical Sensitivity:

Clinical/Diagnostic Sensitivity Study: A study, consisting of 101 clinically characterized Lyme disease specimens, was conducted at the manufacturer's site with the Anti-Borrelia burgdorferi US EUROLINE-WB (IgM) test device. These specimens contain samples from early, early disseminated and late phases of the disease. The panel consisted of 35 men, 58 women and 8 unknowns. The age ranged from 16 - 87 years with a mean age of 45 years.

Table 7: Case Confirmed Lyme Disease Samples

Interval	**n**	**Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)**			**Predicate IgM WB**		
		Positive	**%**	**95% C.I.**	**Positive**	**%**	**95% C.I.**
<1 month	11	7	63.6%	30.8 - 89.1%	4	36.4%	10.9 - 69.2%
>1-3 months	23	12	52.2%	30.6 - 73.2%	9	39.1%	19.7 - 61.5%
>3-12 months	38	15	39.5%	24.0 - 56.6%	8	21.1%	9.6 - 37.3%
>12 months	29	11	37.9%	20.7 - 57.7%	4	13.8%	3.9 - 31.7%
Overall	101	45	44.6%	34.7 - 54.8%	25	24.8%	16.7 - 34.3%

CDC Panel Results: Thirty-four (34) sera of patients with clinically characterized borreliosis in different disease stages and five normals, obtained from the Centers for Disease Control and Prevention, Atlanta, GA, USA, were tested with the Anti-Borrelia burgdorferi US EUROLINE-WB (IgM) in parallel with the predicate device. The results stratified by time after onset of infection are shown below.

Table 8: Testing of CDC Lyme Disease Panel

Interval	n	Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)			Predicate IgM WB		
		Negative	%	95% C.I.	Negative	%	95% C.I.
Normals	5	5	100.0%	47.8 – 100.0%	5	100.0%	47.8 – 100.0%

Table 8 continued: Detailed Analysis of CDC Lyme Disease Panel Testing

Interval	n	Anti-Borrelia burgdorferi US EUROLINE-WB (IgM)			Predicate IgM WB		
		Positive	%	95% C.I.	Positive	%	95% C.I.
<1 month	6	3	50.0%	11.8 - 88.2%	3	50.0%	11.8 - 88.2%
>1-3 months	10	7	70.0%	34.8 - 93.3%	7	70.0%	34.8 - 93.3%
>3-12 months	12	2	16.7%	2.1 - 48.4%	2	16.7%	2.1 - 48.4%
>12 months	6	0	0.0%	0.0 - 45.9%	0	0.0%	0.0 - 45.9%
Overall	34	12	35.3%	19.7 - 53.5%	12	35.3%	19.7 – 53.5%

Note: The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

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:

Expected Values: The incidence of IgM antibodies and positivity in different populations as tested by the Anti-Borrelia burgdorferi US EUROLINE-WB (IgM) are presented below with available patient demographics.

Table 9: Expected Values

Population		n	Gender	Mean Age Years (Range)	p25	p39	p41	No. Pos. (%)
Sensitivity Study	<1 mth	11	4 men;7 women;	45 (16-70) 1 unknown	8	0	8	7 (63.6)
	>1-3 mths	23	4 men;18 women;4 unknown	47 (21-80) 5 unknown	15	4	20	12 (52.2)
	>3-12 mths	38	17 men;17 women;2 unknown	46 (18-87) 3 unknown	12	1	18	15 (39.5)
	>yr(s)	29	10 men;16 women;2 unknown	41 (18-63) 2 unknown	9	2	15	11 (37.9)
	Total	101	35 men;58 women;8 unknown	45 (16-87) 11 unknown	44	7	61	45 (44.6)
Prospective Study	Site 1	142	88 men;52 women;2 unknown	51 (4-88) 4 unknown	45	38	112	56 (39.4)
	Site 2	97	51 men;46 women	57 (20 – 75)	33	5	53	29 (29.9)
	Site 3	65	30 men;35 women	60 (19-86)	13	4	26	11 (16.9)
	Total	304	169 men;133 women;2 unknown	56 (4-88) 4 unknown	91	47	191	96 (31.6)
Normal Population Study	Endemic	98	89 men;9 women	34 (18-56)	3	4	84	5 (5.1)
	Non-Endemic	100	82 men;18 women	38 (3-62)	2	2	87	4 (4.0)
	Total	198	171 men;27 women	36 (3-62)	5	6	171	9 (4.5)

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 Parts 801 and 809, as applicable.

O. Conclusion:

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