

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
MEMORANDUM**

A. 510(k) Number: K161513

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

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

D. Type of Test: Enzyme Immunoassay

E. Applicant: EUROIMMUN US

F. Proprietary and Established Names: EUROIMMUN Anti-*Borrelia burgdorferi* US Westernblot (IgG)

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 Anti-*Borrelia burgdorferi* US Westernblot (IgG) kit is a Western blot assay intended for the qualitative determination of IgG 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 *B. 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*. 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 IgG (and IgA, IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate), catalyzing a color reaction.

J. Substantial Equivalence Information:

1. Predicate device name(s): MarDx Lyme Disease (IgG) Marblot Strip Test System
2. Predicate 510(k) number(s): K950829
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate device
Intended Use	Detection of IgG antibodies to <i>Borrelia burgdorferi</i>	Same
Assay format	Qualitative	Same
Technology/ Procedure	Serum incubation with antigen coated strips followed by a wash step, incubation with an anti-human IgG enzyme conjugate; wash step, incubation with substrate, wash step, air drying and visual reading.	Same
Antigens	Antigens of <i>Borrelia Burgdorferi</i> (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 IgG	Same
Substrate	NBT/BCIP	Same

Differences		
Item	New Device	Predicate device
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 7 native characterized samples. [Negative (samples 1 and 2), low positive (sample 3), and moderate positive (samples 4, 5, 6 and 7)]. The samples were tested on 4 different days with 2 runs per day, 2 reps per run according to the package insert. No positive sample was found negative and vice versa.

n = 16	EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Characterization	negative	negative	positive	positive	positive	positive	positive
% negative	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%
% positive	0.0%	0.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Band(s)	EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)									
	p18/21	p25	p28	p30	p39	p41	p45	p58	p66	p83/93
Sample 1	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%
Sample 2	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	81.3%	0.0%
Sample 3	75.0%	0.0%	0.0%	0.0%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%
Sample 4	87.5%	81.3%	0.0%	0.0%	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%
Sample 5	100.0%	87.5%	75.0%	0.0%	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%
Sample 6	81.3%	81.3%	0.0%	0.0%	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%
Sample 7	100.0%	0.0%	68.8%	50.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%

Reproducibility: Reproducibility was investigated by repeated determinations of 7 native characterized samples. [Negative (samples 1 and 2), low positive (sample 3), and moderate positive (samples 4, 5, 6 and 7)]. The samples were tested on 4 different days with 2 runs per day, 2 reps per run at 3 different sites according to the package insert. No positive sample was found negative and vice versa.

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

Band(s)	EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)									
	p18/21	p25	p28	p30	p39	p41	p45	p58	p66	p83/93
Sample 1	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%
Sample 2	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	81.3%	0.0%
Sample 3	87.5%	0.0%	0.0%	0.0%	100.0%	100.0%	97.9%	0.0%	100.0%	100.0%
Sample 4	91.7%	85.4%	0.0%	0.0%	97.9%	100.0%	100.0%	97.9%	100.0%	0.0%
Sample 5	93.8%	87.5%	81.3%	0.0%	100.0%	100.0%	100.0%	97.9%	100.0%	0.0%
Sample 6	89.6%	85.4%	0.0%	0.0%	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%
Sample 7	100.0%	0.0%	81.3%	50.0%	97.9%	100.0%	0.0%	100.0%	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 an asymptomatic population in both endemic and non-endemic regions was performed. The levels of anti-*B. burgdorferi* antibodies were analyzed with the EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG) in a panel of 98 samples from an endemic region (Pennsylvania; 89 men and 9 women with an average age of 34 y; age range: 18 - 56 y) and in a panel of 100 samples from a non-endemic region (Tennessee; 82 men and 18 women with an average age of 38 y; age range: 3 - 62 y). 3.1% of the samples from the endemic region were found positive for anti-*B. burgdorferi* (IgG), whereas none of the samples from the non-endemic region were positive.

Panel	n	EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)	
		positive (%)	negative (%)
Endemic: Pennsylvania	98	3	97 (96.9%)
Non-endemic: Tennessee	100	0	100 (100%)

Cross Reactivity: Cross reactivity was investigated using characterized samples from the following groups shown in the table below. All the samples tested were found negative with the EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG) according to the CDC criteria.

Panel	n	EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)
		negative (%)
EBV	15	15 (100.0%)
HSV	14	14 (100.0%)
Influenza viruses	15	15 (100.0%)
<i>H. pylori</i>	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%)

Panel	n	EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)
		negative (%)
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 rickettsial 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:

The cut-off of the EUROLINE-WB test system is defined as the lowest limit of a clearly visible band. Because visual examination by the 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. All samples were initially tested with a FDA cleared first-step EIA. All ELISA positive and equivocal/borderline samples were then tested with the EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG) and the predicate IgG Western blot. Results from the test device and the predicate Western blot are interpreted by the CDC criteria.

n = 304		Predicate IgG WB	
		positive	negative
EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)	positive	233	6
	negative	8	57

Negative agreement: 57/63 = 90.5%

95% C.I.: 80.4% - 96.4%

Positive agreement: 233/241 = 96.7%

95% C.I.: 93.6% - 98.6%

b. Matrix comparison:

Serum/Plasma Comparison: The use of K⁺-EDTA, Li⁺-heparin and Na⁺-citrate plasma samples were confirmed by a correlation of 20 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 equivalent as no positive sample was found negative and vice versa.

3. Clinical studies:

a. Clinical Sensitivity:

Sensitivity Study: A study, consisting of 101 clinically characterized Lyme disease specimens, was conducted at the manufacturer's site with the EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG) test device in parallel with the predicate device. The clinically characterized specimens encompassed samples from early, early disseminated, and late phases of the disease. (Initial (acute) and Convalescent samples from patients with documented erythema migrans (EM); and of known Lyme disease patients with presentations other than EM, e.g., neuro-, arthritic, etc.) 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.

Interval	n	EUROIMMUN Anti-Borrelia US Westernblot (IgG) burgdorferi		Predicate IgG WB	
		positive	%	positive	%
<1 month	11	6	54.5%	5	45.5%
>1-3 months	23	13	56.5%	9	39.1%
>3-12 months	38	25	65.8%	7	18.4%
>12 months	29	12	41.4%	9	31.0%
Overall	101	56	55.4%	30	29.7%

Clinical Sensitivity:

EUROIMMUN Westernblot (IgG): 56/101 = 55.4% 95% C.I.: 45.2% - 65.3%
Predicate IgG WB: 30/101 = 29.7% 95% C.I.: 21.0% - 39.6%

CDC Panel Testing: 34 sera of patients with clinically characterized borreliosis in different disease stages and 5 normals, obtained from the Centers for Disease Control and Prevention, Atlanta, GA, USA, were tested with the EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG) in parallel with the predicate device.

Interval	n	EUROIMMUN Anti-Borrelia US Westernblot (IgG) burgdorferi		Predicate IgG WB	
		positive	%	positive	%
Normals	5	0	0.0%	0	0.0%
<1 month	6	3	50.0%	3	50.0%
>1-3 months	10	2	20.0%	2	20.0%
>3-12 months	12	5	41.7%	5	41.7%
>12 months	6	6	100.0%	6	100.0%
Overall	39	16	41.0%	16	41.0%

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.

EUROLineScan vs. Visual Read

The use of the EUROLineScan reader and software compared to visual reading was investigated using 111 characterized samples. Evaluation was performed both visually using the reaction control card and automatically using the EUROLineScan software. The results of the EUROLineScan interpretation and of the visual evaluation by three different technicians are shown in the tables below. Results of the two evaluation methods were found to be in line. Overall correlation between Readers 1 & 3 was 100% and 97.3 % between Reader 1 and 3 to Reader 2. Evaluation of the results is performed according to the CDC criteria.

n = 111		EUROIMMUN Anti-Borrelia burgdorferi US EUROLINE-WB (IgG) Visual Interpretation					
		Reader 1		Reader 2		Reader 3	
		pos	neg	pos	neg	pos	neg
EUROIMMUN Anti-Borrelia burgdorferi US EUROLINE-WB (IgG) EUROLineScan Interpretation	pos	50	0	50	0	50	0
	neg	0	61	3	58	0	61

Reader 1 vs EUROLINE Scan

Negative agreement 61 / 61 = 100.0% 95% C.I.: 92.3% - 100.0%
Positive agreement 50 / 50 = 100.0% 95% C.I.: 91.5% - 100.0%
Overall agreement 111 / 111 = 100.0% 95% C.I.: 96.0% - 100.0%

Reader 2 vs EUROLINE Scan

Negative agreement 58 / 58 = 100.0% 95% C.I.: 92.6% - 100.0%
Positive agreement 50 / 53 = 94.3% 95% C.I.: 84.0% - 98.7%
Overall agreement 108 / 111 = 97.3% 95% C.I.: 92.0% - 99.4%

Reader 3 vs EUROLINE Scan

Negative agreement 61 / 61 = 100.0% 95% C.I.: 92.3% - 100.0%
Positive agreement 50 / 50 = 100.0% 95% C.I.: 91.5% - 100.0%
Overall agreement 111 / 111 = 100.0% 95% C.I.: 96.0% - 100.0%

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:

Expected Values: The range of values and positivity of different populations among different studies are presented below with available patient demographics.

Population	n	Sex	Mean Age & Range	EUROIMMUN Anti-Borrelia burgdorferi US Westernblot (IgG)										
				p18/21	p25	p28	p30	p39	p41	p45	p58	p66	p83/93	Anti-B. burgd. positive (%)

Prospective Study

EIA Positive	304	169 men, 133 women, 2 unknown	56 yrs 4 - 88 yrs 4 unknown	239	197	111	130	251	297	184	190	176	190	233 (76.6%)
---------------------	-----	-------------------------------------	-----------------------------------	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	----------------

Lyme Disease

Sensitivity Study	101	35 men, 58 women, 8 unknown	45 yrs 16 - 87 yrs 9 unknown	47	33	5	31	49	96	27	28	49	28	56 (55.4%)
--------------------------	-----	-----------------------------------	------------------------------------	----	----	---	----	----	----	----	----	----	----	---------------

Normal Population Study

Endemic	98	89 men, 9 women	34 yrs 18 - 56 yrs	10	3	1	7	4	84	6	14	14	10	3 (3.1%)
Non-Endemic	100	82 men, 18 women	38 yrs 3 - 62 yrs	9	2	0	13	2	87	4	5	16	8	0 (0.0%)

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

N. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes or No

O. Proposed Labeling:

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

P. Conclusion:

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