510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
SUMMARY

A. 510(k) Number: K163095

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: Immco Diagnostics

F. Proprietary and Established Names: Lyme *B. burgdorferi* (IgG) MarStripe Test

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): Lyme *B. burgdorferi* (IgG) MarStripe Test is an immunoblot assay for the *in vitro* qualitative detection of human IgG antibody to individual proteins of *Borrelia burgdorferi* in human serum or plasma (K_2-EDTA) in samples which have been found positive or equivocal using an EIA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi*.
2. Indication(s) for use: Same as Intended Use
3. Special conditions for use statement(s): For prescription use only.
4. Special instrument requirements: N/A

I. Device Description: The kit is an immunoblot method to detect IgG antibodies against *B. burgdorferi* antigens. The nitrocellulose test strips are coated with purified recombinant *B. burgdorferi* antigens (10) and quality control lines (3) in specific positions. To perform the test, serum or plasma is incubated with individual *B. burgdorferi* Test Strips. In positive sera antibodies specifically bind to one or more of the test lines on the strip. The strips are washed according to the protocol, and then the pre-diluted, ready-to-use Conjugate is added to the

test strips. After incubation and wash steps, the ready-to-use Substrate is added to the strips. During a 10 minute (±4 min) incubation, conjugate and substrate binding produces visible blue/purple lines for Serum Addition Control (SAC), Conjugate Addition Control (CAC) and Cut-Off Control lines. If the sample is positive for any of the antigen coated test lines, it will show a reaction more intense than the Cut-Off line. Reactions are read visually and reported.

J. Substantial Equivalence Information:

1. Predicate device name(s): MarDx *B. burgdorferi* IgG MarBlot Strip Test System

2. Predicate 510(k) number(s): K950829

3. Comparison with predicate:

Similarities		
Item	**Lyme *B. burgdorferi* (IgG) MarStripe Test**	**MarDx *B. burgdorferi* IgG MarBlot Strip Test System (K950829)**
Intended Use	Lyme *B. burgdorferi* (IgG) MarStripe Test is an immunoblot assay for the *in vitro* qualitative detection of human IgG antibody to individual proteins of *Borrelia burgdorferi* in human serum or plasma (K_2-EDTA) in samples which have been found positive or equivocal using an EIA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi*.	A Western blot assay for the qualitative *in vitro* detection of human IgG antibody to individual proteins of Borrelia burgdorferi in human serum. The MarDx *B. burgdorferi* (lgG) MarBlot Strip Test System is intended for use in testing human serum samples which have been found positive or equivocal using an EIA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi.*
Detection of antibodies	10 antibodies directed against *Borrelia burgdorferi* IgG antigens associated with Lyme disease	10 antibodies directed against *Borrelia burgdorferi* IgG antigens associated with Lyme disease
Quantitation	Qualitative	Qualitative
Component set	Includes *B. burgdorferi* MarStripe test strips, *B. burgdorferi* IgG Positive Control, *B. burgdorferi B. burgdorferi* Negative Control, Conjugate (Anti-Human IgG), Substrate, Diluent and Wash Buffer	Includes *B. burgdorferi* Marblot strips, *B. burgdorferi* WB IgG Serum Band Locator, *B. burgdorferi* WB Weakly Reactive IgG Control, *B. burgdorferi* Negative Control, Alkaline Phosphatase Conjugate (Anti-Human IgG), Alkaline Phosphatase Developing Solution, 10x Sample Diluent Wash Solution
Positive	Anti- *B. burgdorferi* IgG antibodies	Anti- *B. burgdorferi* IgG antibodies
Screening dilution	1:101	1:101

Reading	Visual	Visual
Storage	2-8°C	2-8°C
Calibrators	Single control	Single control

Differences		
Item	**Lyme *B. burgdorferi* (IgG) MarStripe Test**	**MarDx *B. burgdorferi* IgG MarBlot Strip Test System (K950829)**
Methodology	ImmunoBlot/LIA	ImmunoBlot/Western blot
Conjugate	Horseradish peroxidase	Alkaline Phosphatase
Substrate/Chromogen	Tetramethylbenzidine (TMB)	BCIP/NBT
Cutoff	Cutoff Control line	41kD band of Weakly Reactive Control Strip
Matrix	Serum or Plasma	Serum

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: Eight specimens were tested by the Lyme *B. burgdorferi* (IgG) MarStripe Test in 4 replicates, two runs per day over 12 days for a total of 96 tests for each specimen. Samples were selected based on FDA cleared *B. burgdorferi* ELISA results, including 2 low negative samples, 2 high negative samples, 2 low positive samples and 2 moderate positive samples. Final positive or negative agreement was 100% for all specimens.

Sample	n=96	p93	p66	p58	p45	p41	p39	p30	p28	p23	p18
1	**Low Neg**										
	Band Type	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Positives	0	0	0	0	0	0	0	0	0	0
	Negatives	96	96	96	96	96	96	96	96	96	96
	% Positive	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	**Low Neg**										
	Band Type	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.
	Positives	0	0	0	0	96	0	0	0	0	0
	Negatives	96	96	96	96	0	96	96	96	96	96
	% Positive	0%	0%	0%	0%	100%	0%	0%	0%	0%	0%
3	**High Neg**										
	Band Type	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Positives	0	0	0	0	0	0	0	0	0	0
	Negatives	96	96	96	96	96	96	96	96	96	96
	% Positive	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
4	**High Neg**										
	Band Type	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.
	Positives	0	0	0	0	96	0	0	0	0	0
	Negatives	96	96	96	96	0	96	96	96	96	96
	% Positive	0%	0%	0%	0%	100%	0%	0%	0%	0%	0%
5	**Low Pos**										
	Band Type	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Pos.	Neg.
	Positives	0	0	0	0	96	0	0	0	96	0
	Negatives	96	96	96	96	0	96	96	96	0	96
	% Positive	0%	0%	0%	0%	100%	0%	0%	0%	100%	0%
6	**Low Pos**										
	Band Type	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Pos.	Neg.
	Positives	0	0	0	0	96	0	0	0	96	0
	Negatives	96	96	96	96	0	96	96	96	0	96
	% Positive	0%	0%	0%	0%	100%	0%	0%	0%	100%	0%
7	**Moderate Pos**										
	Band Type	Wpos.	Pos.	Pos.	Neg.	Pos.	Pos.	Cut.	Pos.	Pos.	Pos.
	Positives	88	96	96	0	96	96	88	96	96	96
	Negatives	8	0	0	96	0	0	8	0	0	0
	% Positive	92%	100%	100%	0%	100%	100%	92%	100%	100%	100%
8	**Moderate Pos**										
	Band Type	Pos.	Neg.	Pos.	Neg.	Pos.	Pos.	Neg.	Neg.	Pos.	Neg.
	Positives	96	0	96	0	96	96	0	0	96	0
	Negatives	0	96	0	96	0	0	96	96	0	96
	% Positive	100%	0%	100%	0%	100%	100%	0%	0%	100%	0%

Pos = positive band. Neg = negative band. Wpos = weak positive band. Cut = equivocal band.

Reproducibility: Eight specimens were tested by Lyme *B. burgdorferi* (IgG) MarStripe Test in 4 replicates, two runs per day over 9 days for a total of 72 tests for each specimen at three laboratory sites. Results were read by 2 human operators at each site, equaling a total of 432 read-outs. Samples were selected based on FDA cleared *B. burgdorferi* ELISA results, including 2 low negative samples, 2 high negative samples, 2 low positive samples and 2 moderate positive samples. Final positive or negative agreement was 100% for all specimens.

Sample		p93	p66	p58	p45	p41	p39	p30	p28	p23	p18
1	n=432										
	Low Neg	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Band Type	0	0	0	0	0	0	0	0	0	0
	Positives	432	432	432	432	432	432	432	432	432	432
	Negatives	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	% Positive										
	Low Neg	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.
	Band Type	0	0	0	0	432	0	0	0	0	0
	Positives	432	432	432	432	0	432	432	432	432	432
	Negatives	0%	0%	0%	0%	100%	0%	0%	0%	0%	0%
3	% Positive										
	High Neg	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Band Type	0	0	0	0	0	0	0	0	0	0
	Positives	432	432	432	432	432	432	432	432	432	432
	Negatives	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
4	% Positive										
	High Neg	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.
	Band Type	0	0	0	0	432	0	0	0	0	0
	Positives	432	432	432	432	0	432	432	432	432	432
	Negatives	0%	0%	0%	0%	100%	0%	0%	0%	0%	0%
5	% Positive										
	Low Pos	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Pos.	Neg.
	Band Type	0	0	0	0	432	0	0	0	432	0
	Positives	432	432	432	432	0	432	432	432	0	432
	Negatives	0%	0%	0%	0%	100%	0%	0%	0%	100%	0%
6	% Positive										
	Low Pos	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.	Pos.	Neg.
	Band Type	0	0	0	0	432	0	0	0	432	0
	Positives	432	432	432	432	0	432	432	432	0	432
	Negatives	0%	0%	0%	0%	100%	0%	0%	0%	100%	0%
7	% Positive										
	Moderate Pos	Wpos.	Pos.	Pos.	Neg.	Pos.	Pos.	Cut.	Pos.	Pos.	Pos.
	Band Type	412	432	432	0	432	432	412	432	432	432
	Positives	20	0	0	432	0	0	20	0	0	0
	Negatives	95%	100%	100%	0%	100%	100%	95%	100%	100%	100%
8	% Positive										
	Moderate Pos	Pos.	Neg.	Pos.	Neg.	Pos.	Pos.	Neg.	Neg.	Pos.	Neg.
	Band Type	432	0	432	0	432	432	0	0	432	0
	Positives	0	432	0	432	0	0	432	432	0	432
	Negatives	100%	0%	100%	0%	100%	100%	0%	0%	100%	0%
	% Positive										

Pos = positive band. Neg = negative band. Wpos = weak positive band. Cut = equivocal band.

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:* 219 sera from normal individuals (blood bank donors) representing endemic and non-endemic geographic regions of the United States were tested with Lyme *B. burgdorferi* (IgG) MarStripe Test. Analytical specificity was determined to be 99.5% (95% CI: 97.1% - 100%).

		Normal Individuals
Lyme IgG MarStripe Test	Positive	1
	Negative	218
	Total	219

Cross Reactivity: A total of 136 potentially cross-reactive specimens from individuals with other autoimmune disorders or infectious conditions were tested on Lyme *B. burgdorferi* (IgG) MarStripe Test. All positive specimens by the Lyme *B. burgdorferi* (IgG) MarStripe Test were confirmed positive when tested by the predicate IgG Western blot device.

Population	n	n Positive	% Positive
Ehrlichia chafeensis	10	0	0
Babesia microti	10	4	40
Leptospira interrogans	10	0	0
Helicobacter pylori	10	2	20
Syphilis	10	0	0
Influenza	10	0	0
Rocky Mountain Spotted Fever	10	0	0
Parvovirus B19	9	0	0
Systemic lupus erythematosus	15	0	0
Cytomegalovirus	10	0	0
Rheumatoid arthritis	16	0	0
Celiac	16	0	0
Total	136	6	4.4

Interferences: Two Lyme IgG negative and three Lyme IgG positive sera were spiked with hemoglobin (2g/L), unconjugated bilirubin (342 μmol/L), RF (100 IU/ml), triglycerides (3.7 mmol/L) and total cholesterol (13 mmol/L) and tested using Lyme *B. burgdorferi* (IgG) MarStripe Test. Samples were tested with and without interfering agents. Qualitative agreement between results of spiked and unspiked specimens was 100% for all interferents tested.

f. Assay cut-off:

Band Cutoff Determination: Cutoff was established testing a panel of Lyme positive specimens along with healthy normals and controls. The Cut-Off Control line was derived from these studies and provides a qualitative visual reference point for determination of bands as positive or negative

2. Comparison studies:

a. Method comparison with predicate device:

Prospective Study: A prospective study of FDA cleared first-step EIA specimens was performed at three geographically distinct study sites. The specimens testing positive (n=753) on a FDA cleared first-step EIA were tested with Lyme *B.*

burgdorferi (IgG) MarStripe Test and an FDA cleared immunoblot. Interpretation of immunoblot results followed the recommended criteria described by the Centers for Disease Control (CDC) and the Second National Conference on Serological Diagnosis of Lyme Disease. The results are summarized below.

		Predicate IgG WB		
		Positive	Negative	Total
Lyme IgG MarStripe Test	Positive	221	12	233
	Negative	17	503	520
	Total	238	515	753

Positive Agreement: 92.9% (221/238) (95% CI: 88.6% - 95.7%)
Negative Agreement: 97.7% (503/515) (95% CI: 95.9% - 98.7%)

The 29 discrepant specimens from the above study were tested on two additional commercially available *B. burgdorferi* IgG Western blot methods. Results of the Lyme *B. burgdorferi* (IgG) MarStripe Test were compared to the consensus (2/3 comparator) results. The 12 positives by MarStripe remained positive by consensus testing and the 17 negatives by MarStripe were found to be positives.

		Consensus Result	
		Positive	Negative
Lyme IgG MarStripe Test	Positive	12	0
	Negative	17	0

b. *Matrix comparison:* To establish equivalence of serum vs. plasma (K_2-EDTA) matrix, 10 pairs of sera/plasma (samples A-J below) were sourced from specimens tested with an FDA cleared Lyme EIA assay. Two of these were Western blot IgG positive, 8 were negative. These specimens were selectively pooled to create another set of 10 samples (samples 1-10 below), including 3 Western blot IgG positives and 7 negatives. These samples were assayed on the Lyme *B. burgdorferi* (IgG) MarStripe Test. Qualitative agreement for all pairs was 100%.

Sample ID[1]	Type[2]	p93	p66	p58	p45	p41	p39	p30	p28	p23	p18	Result	Band % Pos Agrmt	Band % Neg Agrmt
A	Serum	0	0	0	0	1	0	0	0	1	0	NEG	100	100
A	Plasma	0	0	0	0	1	0	0	0	1	0	NEG		
B	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
B	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
C	Serum	1	0	1	0	1	1	1	0	1	1	POS	100	100
C	Plasma	1	0	1	0	1	1	1	0	1	1	POS		
D	Serum	0	0	1	0	1	1	1	0	1	1	POS	100	100
D	Plasma	0	0	1	0	1	1	1	0	1	1	POS		
E	Serum	0	0	0	0	0	0	0	0	0	1	NEG	100	100
E	Plasma	0	0	0	0	0	0	0	0	0	1	NEG		
F	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
F	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
G	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
G	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
H	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
H	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
I	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
I	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
J	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
J	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
Pool 1 (A+B)	Serum	0	0	0	0	0	0	0	0	1	0	NEG	100	100
Pool 1 (A+B)	Plasma	0	0	0	0	0	0	0	0	1	0	NEG		
Pool 2 (B+C)	Serum	1	0	1	0	1	1	0	0	1	1	POS	100	100
Pool 2 (B+C)	Plasma	1	0	1	0	1	1	0	0	1	1	POS		
Pool 3 (C+D)	Serum	1	0	1	0	1	1	1	0	1	1	POS	100	100
Pool 3 (C+D)	Plasma	1	0	1	0	1	1	1	0	1	1	POS		
Pool 4 (D+E)	Serum	0	0	1	0	1	1	0	0	1	1	POS	100	100
Pool 4 (D+E)	Plasma	0	0	1	0	1	1	0	0	1	1	POS		
Pool 5 (A+E)	Serum	0	0	0	0	1	0	0	0	1	0	NEG	100	100
Pool 5 (A+E)	Plasma	0	0	0	0	1	0	0	0	1	0	NEG		
Pool 6 (F+G)	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
Pool 6 (F+G)	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
Pool 7 (G+H)	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
Pool 7 (G+H)	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
Pool 8 (H+I)	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
Pool 8 (H+I)	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
Pool 9 (I+J)	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
Pool 9 (I+J)	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		
Pool 10 (F+J)	Serum	0	0	0	0	0	0	0	0	0	0	NEG	100	100
Pool 10 (F+J)	Plasma	0	0	0	0	0	0	0	0	0	0	NEG		

[1] Pool 1 to 10 contain a 1:1 mixture of the native samples as indicated.
[2] 0 = negative band result. 1 = positive band result.

3. Clinical studies:

   a. *Clinical Sensitivity:* 94 well characterized Lyme disease clinical specimens were tested with the Lyme *B. burgdorferi* (IgG) MarStripe Test. Specimens included samples from early, early disseminated, and late phases of the disease. The sensitivity obtained was compared with that of the predicate device.

Interval	n	Lyme IgG MarStripe Test		Predicate IgG WB	
		positive	%	positive	%
Early Lyme (stage 1)	22	4	18.2	3	13.6
Early disseminated (stage 2)	44	12	27.3	11	25.0
Late Lyme (stage 3)	28	5	17.9	5	17.9
Overall	94	21	19.5	19	20.2

Sensitivity Comparison:
Lyme *B. burgdorferi* (IgG) MarStripe Test: 22.3% (21/94) (95% CI: 14.7%-32.3%)
Predicate device: 20.2% (19/94) (95% CI: 12.9%-30.0%)
Difference in proportion: 2.1%

CDC Panel Testing: A reference panel from the Center for Disease Control and Prevention (Lyme Disease Validation Panel n=10, Lyme Disease Basic Research Panel n=32) were tested on the Lyme *B. burgdorferi* (IgG) MarStripe Test and the predicate device.

Interval	n	Lyme IgG MarStripe Test		Predicate IgG WB	
		positive	%	positive	%
Controls	25	0	0	0	0
Early Lyme (stage 1)	10	1	10	1	10
Early disseminated (stage 2)	3	3	100	3	100
Late Lyme (stage 3)	4	4	100	4	100

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:
The performance of the Lyme *B. burgdorferi* (IgG) MarStripe Test was evaluated in clinical studies using sera obtained from the following: 1) patients meeting a case definition for Lyme disease based on physician diagnosis, *B. burgdorferi* culture, and

other laboratory tests; 2) patients for whom requests were made for routine *B. burgdorferi* serology tests; and 3) apparently healthy individuals from endemic and non-endemic regions for Lyme disease.

The presence of *B. burgdorferi* specific bands observed by testing with Lyme *B. burgdorferi* (IgG) MarStripe Test in different specimens is presented below.

Defined Lyme Disease Population:

		Lyme IgG MarStripe Test											
Disease Stage			Pos	p93	p66	p58	p45	p41	p39	p30	p28	p23	p18
Early Lyme (0-4 weeks), Stage 1	n	22	4	2	4	7	3	17	4	2	3	11	5
	%	100%	18.2%	9.1%	18.2%	31.8%	13.6%	77.3%	18.2%	9.1%	13.6%	50.0%	22.7%
Early disseminated, Stage 2	n	44	12	10	10	16	1	27	12	5	8	11	15
	%	100%	27.3%	22.7%	22.7%	36.4%	2.3%	61.4%	27.3%	11.4%	18.2%	25.0%	34.1%
Late Lyme, Stage 3	n	28	12	5	6	8	0	16	5	2	3	6	7
	%	100%	42.9%	17.9%	21.4%	28.6%	0.0%	57.1%	17.9%	7.1%	10.7%	21.4%	25.0%

Prospective Study Population:

		Lyme IgG MarStripe Test											
Specimen			Pos	p93	p66	p58	p45	p41	p39	p30	p28	p23	p18
Lyme EIA positive/ equivocal specimens	n	753	238	125	188	254	48	574	270	123	134	345	360
	%	100%	31.6%	16.6%	25.0%	33.7%	6.4%	76.2%	35.9%	16.3%	17.8%	45.8%	47.8%

Healthy Normal Individuals:

		Lyme IgG MarStripe Test											
Specimen			Pos	p93	p66	p58	p45	p41	p39	p30	p28	p23	p18
Normal individuals	n	219	1	4	9	2	0	12	40	1	2	7	3
	%	100%	0.5%	1.8%	4.1%	0.9%	0.0%	5.5%	18.3%	0.5%	0.9%	3.2%	1.4%

N. Software: N/A

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.