510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172254

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:

Immco Diagnostics

F. Proprietary and Established Names:

Lyme *B. burgdorferi* (IgM) 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* (IgM) MarStripe Test is an immunoblot assay for the in vitro qualitative detection of human IgM 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:

   Not applicable (N/A)

I. Device Description:

The kit is an immunoblot method to detect IgM antibodies against *B. burgdorferi* antigens. The test kit contains:

- **Test strips.** Purified *B. burgdorferi* antigens (3) and quality control lines (3) on nitrocellulose arepresent in specific positions
- **Diluent.** Provided for specimen dilutions
- **Positive Control**
- **Negative Control**
- **Conjugate.** IgM-HRP Conjugate binds reactive antibodies to the Substrate
- **Substrate.** Provides colorimetric reaction for visual read of bound antibodies
- **Wash Buffer.** Removes reagents and unbound antibodies after incubation steps

J. Substantial Equivalence Information:

1. Predicate device name(s):

   MarDx *B. burgdorferi* IgM MarBlot Strip Test System

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

   K951709

3. Comparison with predicate:

Parameter	**New device** **Lyme *B. burgdorferi* (IgM) MarStripe Test**	**Predicate device** **MarDx *B. burgdorferi* IgM MarBlot Strip Test System (K951709)**
Similarities		
Intended Use	Lyme *B. burgdorferi* (IgM) MarStripe Test is an immunoblot assay for the in vitro qualitative detection of human IgM antibody to individual proteins of *Borrelia burgdorferi* in human serum or plasma (K2-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*.	MarDx *B. burgdorferi* (IgM) MarBlot Strip Test System is a immunoblot assay for the *in vitro* qualitative detection of human IgM 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*.
Detection of antibodies	3 antibodies directed against *Borrelia burgdorferi* IgM antigens associated with Lyme disease	3 antibodies directed against *Borrelia burgdorferi* IgM antigens associated with Lyme disease
Quantitation	Qualitative	Qualitative
Component set	Includes *B. burgdorferi* MarStripe test strips, *B. burgdorferi* IgM Positive Control, *B. burgdorferi* Negative Control, Conjugate (Anti- Human IgM), Substrate, Diluent and Wash Buffer	Includes *B. burgdorferi* Marblot strips, *B. burgdorferi* WB IgM Serum Band Locator, *B. burgdorferi* WB Weakly Reactive IgM Control, *B. burgdorferi* Negative Control, Alkaline Phosphatase Conjugate (Anti-Human IgM), Alkaline Phosphatase Developing Solution, 10x Sample Diluent Wash Solution
Positive control	Anti- *B. burgdorferi* IgM antibodies	Anti- *B. burgdorferi* IgM antibodies
Screening dilution	1:101	1:101
Reading	Visual	Visual
Storage	2-8°C	2-8°C
Calibrators	Single control	Single control
Differences		
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):

FDA Guidance for Industry and Food and Drug Administration Staff - Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of Antibodies to *Borrelia burgdorferi*, published July, 2013.

CLSI M34-A: Western Blot Assay for Antibodies to *Borrelia burgdorferi*

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures

CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance

CLSI EP7-A2: Interference Testing in Clinical Chemistry

CLSI EP9-A2: Method Comparison Bias Estimation Using Patient Samples

L. Test Principle:

Enzyme Immunoassay

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision. A panel of eight specimens were tested. Each specimen was tested in 4 replicates on each run, 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, 1 cutoff sample, 1 low positive sample and 2 moderate positive samples. The cutoff specimen presented reactions near the cutoff control for two bands.

Qualitative agreement was 100% for the results of seven specimens; the cutoff specimen produced a positive result in 78/96 replicates (81.3%). A summary of results by antibody line is provided in Table 1below:

Table 1**. Summary of Precision Results by Antibody Line**

Sample		EIA Value	p41	p39	p23
1	Low Negative	0.22			
	Band Type		Neg.	Neg.	Neg.
	Positives		0	0	0
	Negatives		96	96	96
	% Positive		0.0%	0.0%	0.0%
2	Low Negative	0.20			
	Band Type		Neg.	Neg.	Neg.
	Positives		0	0	3
	Negatives		96	96	93
	% Positive		0.0%	0.0%	3.1%
3	High Negative	0.80			
	Band Type		Neg.	Neg.	Neg.
	Positives		0	0	0
	Negatives		96	96	96
	% Positive		0.0%	0.0%	0.0%
4	High Negative	0.87			
	Band Type		Neg.	Neg.	Cut.
	Positives		0	0	3
	Negatives		96	96	93
	% Positive		0.0%	0.0%	3.1%
5	Low Positive	1.31			
	Band Type		Pos.	Neg.	Pos.
	Positives		96	0	96
	Negatives		0	96	0
	% Positive		100.0%	0.0%	100.0%
6	Cutoff	1.37			
	Band Type		Cut.	Neg.	Wpos.
	Positives		88	0	79
	Negatives		8	96	17
	% Positive		91.7%	0.0%	82.3%
7	Moderate Positive	3.36			
	Band Type		Pos.	Neg.	Pos.
	Positives		96	0	96
	Negatives		0	96	0
	% Positive		100.0%	0.0%	100.0%
8	Moderate Positive	2.92			
	Band Type		Pos.	Pos.	Pos.
	Positives		96	96	96
	Negatives		0	0	0
	% Positive		100.0%	100.0%	100.0%

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

Reproducibility. A panel of eight specimens was tested at three laboratories (2 external, 1 internal). At each site, each specimen was tested in 4 replicates per run, two runs per day over 12 days for a total of 288 tests. Results were read by 2 human operators at each site, equaling a total of 576 read-outs. Samples were selected based on FDA cleared *B. burgdorferi* ELISA results The cutoff specimen was chosen so that two bands were near the cutoff control.

Final positive or negative agreement was 100% for both low negative samples, one high negative and one moderate positive sample. One high negative sample produced a 99.7 % negative agreement. One low positive and one moderate positive sample produced a 99.3% positive agreement. The cut off sample produced a 68.4% positive agreement. A summary of results by antibody line is provided in Table 2 below.

Table 2**. Summary of Reproducibility Results by Antibody Line**

	Sample	EIA Value	p41	p39	p23
1	Low Negative	0.22			
	Band Type		Neg.	Neg.	Neg.
	Positives		0	0	1
	Negatives		576	576	575
	% Positive		0.0%	0.0%	0.2%
2	Low Negative	0.20			
	Band Type		Neg.	Neg.	Neg.
	Positives		0	0	26
	Negatives		576	576	550
	% Positive		0.0%	0.0%	4.5%
3	High Negative	0.80			
	Band Type		Neg.	Neg.	Neg.
	Positives		0	0	0
	Negatives		576	576	576
	% Positive		0.0%	0.0%	0.0%
4	High Negative	0.87			
	Band Type		Neg.	Neg.	Cut.
	Positives		1	2	58
	Negatives		575	574	518
	% Positive		0.2%	0.3%	10.1%
5	Low Positive	1.31			
	Band Type		Pos.	Neg.	Pos.
	Positives		572	0	576
	Negatives		4	576	0
	% Positive		99.3%	0.0%	100.0%
6	Cutoff	1.37			
	Band Type		Cut.	Neg.	WPos.
	Positives		442	0	504
	Negatives		134	576	72
	% Positive		76.7%	0.0%	87.5%
7	Moderate Positive*	3.36			
	Band Type		Pos.	Neg.	Pos.
	Positives		573	0	572
	Negatives		1	574	2
	% Positive		99.8%	0.0%	99.7%
8	Moderate Positive	2.92			
	Band Type		Pos.	Pos.	Pos.
	Positives		576	572	576
	Negatives		0	4	0
	% Positive		100.0%	99.3%	100.0%

* Two replicates of this run are missing due to lack of sample.

b. *Linearity/assay reportable range:* Not applicable (N/A)

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

d. Detection limit: N/A

e. Analytical specificity:

Sera from 220 normal individuals, with no history of physician-diagnosed Lyme disease representing endemic and non-endemic geographic regions of the United States were tested with the Lyme *B. burgdorferi* (IgM) Marstripe Test. Analytical specificity was deterermined to be 99.5% (95% CI: 97.1% - 100%). See Tables 3 and 4 below.

Table 3**. Analytical Specificity Results by Band**

Specimen			Pos	p41	p39	p23
Normal Individuals	n	220	1	2	1	4
	%		0.5%	0.9%	0.5%	1.8%

Table 4. **Analytical Specificity Results**

		Population
		Normal Indivduals
Lyme IgM MarStripe Test	Positive	1
	Negative	219
	Total	220

Cross-Reactivity:

A total of 136 potentially cross-reactive specimens from individuals with other autoimmune disorders or infectious conditions, including *Ehrlichia chafeensis* (10), *Babesia microti* (10), *Leptospira interrogans* (10), *Helicobacter pylori* (10), Syphilis (10), Influenza (10), Rocky Mountain spotted fever (10), parvovirus B19 (9), CMV (10) systemic lupus erythematosus (15), rheumatoid arthritis (16), and celiac disease (16) were tested. Positive results and breakdown of antibody reactions by line are provided below in Table 5.

Table 5. **Cross-Reactivity Results by Antibody Band**

		Positive specimens/reactive antibody lines - n(%)			
Population	n	Positive	p41	p39	p23
E. chafeensis	10	3 (30)*	3 (30)	5 (50)	4 (40)
B. microti	10	2 (20)*	2 (20)	1 (10)	1 (10)
L. interogans	10	1 (10)*	1 (10)	1 (10)	1 (10)
H. pylori	100	1 (1)*	1 (1)	1 (1)	1 (1)
Syphilis	10	0 (0)	0 (0)	0 (0)	0 (0)
Influenza	10	0 (0)	0 (0)	1 (10)	0 (0)
Epstein-Barr Virus	22	0 (0)	1 (4.5)	0 (0)	0 (0)
Rocky Mountain Spotted fever	10	0 (0)	0 (0)	0 (0)	0 (0)
Parvovirus B19	9	0 (0)	0 (0)	2 (22.2)	1 (11.1)
Systmatic lupus erythematosus	15	0 (0)	0 (0)	0 (0)	0 (0)
Cytomegalovirus	10	0 (0)	0 (0)	0 (0)	0 (0)
Rheumatoid arthritis	15	0 (0)	0 (0)	0 (0)	0 (0)
Celiac disease	15	0 (0)	0 (0)	0 (0)	0 (0)

* Positive results were also observed with an FDA cleared western blot

Interferences:

Five (5) specimens (2 negative, 3 positive) were spiked with hemoglobin (2g/L), unconjugated bilirubin (342 μmol/L), rheumatoid factor (100 IU/ml), triglycerides (3.7 mmol/L) and total cholesterol (13 mmol/L), and then tested for *Borrelia burgdorferi* IgM following guidance of CLSI EP7-A2. Samples were tested with and without interfering agents. Qualitative agreement between results, spiked and unspiked was 100% for all interferents tested.

f. Assay cut-off:

Cut-off was established by testing a panel of Lyme positive specimens along with healthy normal 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:

Specimens that were determined positive using an FDA cleared first-step EIA assay were tested prospectively at three geographically distinct study sites. The specimens testing positive (n=676) on a FDA cleared first-step EIA were tested with Lyme B. burgdorferi (IgM) 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 in Table 6.

Table 6. **Results of Lyme *B. burgdorferi* (IgM) MarStripe Test and Predicate – Prospective Specimens**

		FDA Cleared Immunoblot		
		Positive	Negative	Total
Lyme IgM MarStripe Test	Positive	302	23	325
	Negative	21	330	351
	Total	323	353	676

Positive Percent Agreement: 93.5% (95% CI: 90.1% - 95.8%)
Negative Percent Agreement: 93.5% (95% CI: 90.2% - 95.7%)

b. Matrix comparison:

To establish equivalence of serum vs. plasma matrices, 20 pairs of sera/plasma (samples A-J below) were sourced from specimens tested on an FDA cleared Lyme EIA assay. These specimens included 3 Western Blot IgM positives and 17 negatives. These samples were assayed on the Lyme *B. burgdorferi* (IgM) MarStripe Test. AQL was 100% agreement between serum and plasma. See Table 7 below for results.

Table7. **Serum vs. Plasma – Matrix Comparisson Study**

Sample ID	Type	p41*	p39*	p23*	Result	Band % Pos Agrmt**	Band % Neg** Agrmt
A	Serum	0	0	0	NEG	100	100
A	Plasma	0	0	0	NEG		
B	Serum	1	0	1	POS	100	100
B	Plasma	1	0	1	POS		
C	Serum	0	0	0	NEG	100	100
C	Plasma	0	0	0	NEG		
D	Serum	0	0	1	NEG	100	100
D	Plasma	0	0	1	NEG		
E	Serum	1	0	1	POS	100	100
E	Plasma	1	0	1	POS		
F	Serum	0	0	1	NEG	100	100
F	Plasma	0	0	1	NEG		
G	Serum	0	0	1	NEG	100	100
G	Plasma	0	0	1	NEG		
H	Serum	0	0	0	NEG	100	100
H	Plasma	0	0	0	NEG		
I	Serum	0	0	0	NEG	100	100
I	Plasma	0	0	0	NEG		
J	Serum	1	0	1	POS	100	100
J	Plasma	1	0	1	POS		
K	Serum	0	0	0	NEG	100	100
K	Plasma	0	0	0	NEG		

Sample ID	Type	p41*	p39*	p23*	Result	Band % Pos Agrmt**	Band % Neg** Agrmt
L	Serum	0	0	0	NEG	100	100
L	Plasma	0	0	0	NEG		
M	Serum	0	0	0	NEG	100	100
M	Plasma	0	0	0	NEG		
N	Serum	0	0	0	NEG	100	100
N	Plasma	0	0	0	NEG		
O	Serum	0	0	0	NEG	100	100
O	Plasma	0	0	0	NEG		
P	Serum	0	0	1	NEG	100	100
P	Plasma	0	0	1	NEG		
Q	Serum	0	0	0	NEG	100	100
Q	Plasma	0	0	0	NEG		
R	Serum	0	0	0	NEG	100	100
R	Plasma	0	0	0	NEG		
S	Serum	0	0	1	NEG	100	100
S	Plasma	0	0	1	NEG		
T	Serum	0	0	0	NEG	100	100
T	Plasma	0	0	0	NEG		

*0 = negative band result, 1 = positive band result
**Refers to agreement across all three bands

3. Clinical studies:

a. Clinical Sensitivity:

87 well characterized Lyme disease clinical specimens were tested with the Lyme *B. burgdorferi* (IgM) MarStripe Test. Specimens included samples from early, early disseminated, and late phases of the disease. The performance of the Lyme *B. burgdorferi* (IgM) MarStripe Test was compared with that of an FDA cleared device.

Table 8. **Lyme *B.burgdorferi* (IgM) MarStripe Test and Predicate Results for Well-Characterized Samples**

Interval	n	Lyme IgM MarStripe		FDA cleared IgM WB	
		Positive	%	Positive	%
Early Lyme (stage 1)	19	8	42.1	9	47.4
Early disseminated (stage 2)	43	5	11.6	6	14
Late Lyme (stage 3)	25	3	12	2	8
Overall	87	16	18.4	17	19.5

Sensitivity Comparison:

Lyme *B. burgdorferi* (IgM) MarStripe Test: 18.4% (16/87) (95% CI: 11.2% - 28.4%)
Predicate device: 19.5% (17/87) (95% CI: 12.1% - 29.7%)

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) was tested with the Lyme *B. burgdorferi* (IgM) MarStripe Test and the predicate device.

Table 9. **Lyme *B.burgdorferi* (IgM) MarStripe Test and Predicate Results for CDC Panel**

Interval	**n**	**Lyme IgM MarStripe Test**		**Predicate IgM WB**	
		positive	**%**	**positive**	**%**
Controls	25	0	0	2	8
Early Lyme (stage 1)	10	6	60	6	60
Early disseminated (stage 2)	3	3	100	3	100
Late Lyme (stage 3)	4	1	25	2	50
Overall	42	10	23.8	13	31.0

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.

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* (IgM) 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 first table summarizes the presence of *B. burgdorferi* specific bands in specimens from documented cases of Lyme disease. Specimens have been grouped by the time after onset of symptoms.

Table 10. **Lyme *B. burgdorferi* (IgM) MarStripe Test results in defined Lyme disease populations**

Disease Stage		MarStripe Results				
		n	Pos	p41	p39	p23
Early Lyme, Stage 1	n	19	8	9	7	9
	%		42.1%	47.4%	36.8%	47.4%
Early disseminated, Stage 2	n	43	5	8	6	10
	%		11.6%	18.6%	14.0%	23.3%
Late Lyme, Stage 3	n	25	3	2	2	5
	%		12.0%	8.0%	8.0%	20.0%

Table 11. **Lyme *B. burgdorferi* (IgM) MarStripe Test results in the prospective study**

Specimen		MarStripe Results				
		n	Pos	p41	p39	p23
Lyme EIA positive / equivocal specimens	n	676	325	362	218	365
	%		48.1%	53.6%	32.2%	54.0%

Table 12. **Lyme *B. burgdorferi* (IgM) MarStripe Test results in healthy normal individuals**

Specimen		MarStripe Results				
		n	Pos	p41	p39	p23
Normal individuals	n	220	1	2	1	4
	%		0.5%	0.9%	0.5%	1.8%

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.