510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K113846

B. Purpose for Submission: New device

C. Measurand: IgM antibodies to *Borrelia burgdorferi* proteins

D. Type of Test: Western blot immunoassay

E. Applicant: Gold Standard Diagnostics

F. Proprietary and Established Names: *Borrelia burgdorferi* B31 IgM Line Blot Test Kit

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: 83 Microbiology

H. Intended Use:

1. Intended use(s):
The Gold Standard Diagnostics *Borrelia burgdorferi* B31 IgM Line Blot Test Kit is intended for the qualitative detection of IgM antibodies to *B. burgdorferi sensu stricto* (B31) in human serum. This test is intended for use in testing human serum samples which have been found positive or equivocal using an ELISA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi.*

2. Indication(s) for use:
The Gold Standard Diagnostics *Borrelia burgdorferi* B31 IgM Line Blot Test Kit is intended for the qualitative detection of IgM antibodies to *B. burgdorferi sensu stricto* (B31) in human serum. This test is intended for use in testing human serum samples which have been found positive or equivocal using an ELISA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi.*

3. Special conditions for use statement(s): For prescription use

4. Special instrument requirements: None

I. Device Description:

The Gold Standard Diagnostics *Borrelia burgdorferi* B31 IgM test is a line blot assay. The 3 antigenic proteins specific for *B. burgdorferi (sensu stricto)* are either purified or cloned and expressed in the host *E.coli.* The purified individual proteins are transferred to a nitrocellulose membrane using a spraying micro-dispensing method. Positions of the lines are defined on the filter and the antigen bands are assigned in the following order: 23, 39, and 41 kDa.

During the test procedure, antibodies to *Borrelia burgdorferi* B31 *(sensu stricto)* present in the human serum sample will bind to the antigens coated onto the nitrocellulose strips. After removing serum and unbound antibodies by washing, the nitrocellulose strip is incubated with an antihuman IgM antibody-enzyme conjugate. After the unbound conjugate has been removed by a washing step, visualization of the antigen-antibody-antibody complex is accomplished by the addition of a substrate which forms a blue-violet precipitate at each site (antigen bands). The reaction is stopped by washing the nitrocellulose strip with distilled or deionized water. Depending on the observed band pattern one can interpret the presence or absence of specific IgM antibodies to *B. burgdorferi* infection.

J. Substantial Equivalence Information:

1. Predicate device name(s): *B. burgdorferi* (IgM) Marblot Strip Test System from Trinity Biotech

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

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	The Gold Standard Diagnostics *Borrelia burgdorferi* B31 IgM Line Blot Test Kit is intended for the qualitative detection of IgM antibodies to *B. burgdorferi sensu stricto* (B31) in human serum. This test is intended for use in testing human serum samples which have been found positive or equivocal using an ELISA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi.*	MarDx *B. burgdorferi* (IgM) Marblot Strip Test System is a Western blot assay for the qualitative *in vitro* detection of human IgM antibody to individual proteins of *B. burgdorferi* in human serum. The MarDx *B. burgdorferi* (IgM) Marblot Strip Test System is intended for use in testing human samples which have been found positive or equivocal using an EIA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi.*
Specimen type	Serum	Serum
Method	Qualitative	Qualitative
Assay	Immunoblot	Immunoblot

Differences		
Item	Device	Predicate
Antigens	Individually purified or cloned proteins of *B. burgdorferi*	Whole cell extract of *B. burgdorferi* antigens
Assay	Line blot	Western blot

K. Standard/Guidance Document Referenced (if applicable): Not applicable (N/A)

L. Test Principle: Enzyme-immunoassay on a blot

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility of the assay was done by testing a negative sample, a high negative sample, a low positive sample, and a moderate positive sample in triplicate for five days, twice a day, at three sites with two technicians per site giving a total of 90 data points per sample. Results of band reproducibility and sample reproducibility are shown below:

Band Reproducibility:

Sample/kDa	41	39	23	Number of Bands
Negative	0	0	0	0 significant bands
High Negative	0	0	79	≤1 significant bands
Low Positive	86	0	90	2 significant bands
Moderate Positive	90	90	90	3 significant bands

Sample Reproducibility:

	Band Reproducibility	Final Interpretation	
		Positive	Negative
Sample			
Negative	100% (0/0)		100% (90/90)
High Negative	87.8% (79/90)		100% (90/90)
Low Positive	97.8% (176/180)	95.6% (86/90)	
Moderate Positive	100% (270/270)	100% (90/90)	

*A low positive sample is expected to yield a positivity of 95%.

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:*

Analytical Specificity Study: For the determination of analytical specificity for the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot Test, testing of 234 asymptomatic samples (blood donors) from both endemic and non-endemic regions was performed. The results are summarized in the following table:

Region	Number of Samples	Number Positive	Analytical Specificity
Endemic	115	0	100%
Non-endemic	119	0	100%

Cross Reactivity: A cross reactivity study was performed on 215 specimens known to contain potentially cross reactive antibodies to *B.burgdorferi*. Sera from patients with infections and sera from patients with diagnoses that can be confused with the late manifestations of Lyme disease were tested. The results are summarized in the following table:

Infection / Diagnosis	Number of Sera Tested	# Positive / (%)
Tick-borne Relapsing Fever / Rickettsial Diseases	23	1 / (4%)
Treponemal Infections	12	0 / (0%)
Ehrlichiosis	20	1 / (5%)
Babesiosis	20	2 / (10%)
Leptospirosis	1	0 / (0%)
Parvovirus B19	9	0 / (0%)
Epstein-Barr Virus	11	0 / (0%)

Cytomegalovirus	32	0 / (0%)
H. pylori	12	0 / (0%)
Fibromyalgia	10	0 / (0%)
Rheumatoid Arthritis	12	0 / (0%)
Herpes Simplex Virus	16	0 / (0%)
Varicella Zoster Virus	12	0 / (0%)
Autoimmune Disease	25	0 / (0%)

Two of the 20 Babesiosis samples and one each of the 23 Tick-borne relapsing fever / Rickettsial Disease and 20 Ehrlichiosis specimens were positive on the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot Test. These samples when tested on the predicate device also gave positive results.

Interfering Substances: The effect of potential interfering substances on samples using the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot Test was evaluated. High levels of hemoglobin, bilirubin, cholesterol and intralipids in serum samples were tested on six sera (two positives, one low positive, one high negative, and two negatives). The recommended concentrations in the guideline "Interference Testing in Clinical Chemistry" from the Clinical and Laboratory Standards Institute were used. The interferents at the concentrations tested did not have any influence on the band pattern. A small variability in band intensity was seen that is in the normal range of deviation and did not change the final interpretation. The results are summarized in the following table:

Substance	Concentration	Interference
Hemoglobin	2 g/l	None detected
Bilirubin	342 µmol/l	None detected
Cholesterol	13 mmol/l	None detected
Intralipids	37 mmol/l	None detected

f. Assay cut-off:

Determination of Cutoff: The cutoff was determined by testing a total of 365 sera of known anti-*B. burgdorferi* IgM positives and negatives. The cutoff concentration was determined by testing several dilutions of each antigen and choosing the concentration that met the sample criteria best.

2. Comparison studies:

a. Method comparison with predicate device:

Method Comparison Study: The performance of the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot test was determined by conducting a prospective clinical study at three different geographic sites (Pennsylvania, North Carolina, and California) in the U.S. The patient samples were tested by an anti-*B. burgdorferi* total antibody ELISA test first and the resulting equivocal and positive specimens were tested on the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot test and a commercially available *B. burgdorferi* IgM Blot test. The results are summarized in the following table:

		Predicate Device IgM Blot	
		Positive	Negative
Gold Standard Diagnostics IgM Line Blot	Positive	154	1
	Negative	1	154

Positive Percent Agreement = 99.4% (154/155) [95% CI: 96.5-100%]
Negative Percent Agreement = 99.4% (154/155) [95% CI: 96.5-100%]

The discrepant samples were tested on a second commercially available assay. On the one Line Blot negative sample that was positive by the predicate, the second assay gave a positive result. Of the one Line Blot positive and predicate negative samples, the second assay called the sample positive.

The 310 samples were also tested by the Gold Standard *B. burgdorferi IgG* Line Blot test and 36.8% (114/310) were found to be positive and 63.2% (196/310) were found to be negative.

Sensitivity Study: A sensitivity study for the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot Test was performed on 100 clinically characterized samples. The samples encompassed early, disseminated, and late stages of Lyme disease. The results are summarized in the following table:

Stage	**Number of Samples**	**Line Blot Sensitivity with 95% CI**	**Commercially Available Device Sensitivity with 95% CI**
Early	40	87.5% (35/40) [73.2%-95.8%]	77.5% (31/40) [61.6%-89.2%]
Disseminated	20	95.0% (19/20) [75.1%-99.9%]	85% (17/20) [62.1%-96.8%]
Late	40	27.5% (11/40) [14.6%-43.9%]	22.5% (9/40) [10.8%-38.5%]

CDC Reference Panel: A standard panel of positive and negative specimens provided by the Centers of Disease Control (CDC) for Lyme disease detection

was tested both on the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot Test and on the predicate device. The results are summarized in the following table:

Stage	Total	GSD Line Blot % Agreement	Commercially Available Device % Agreement
Healthy	5	100% (5/5)	100% (5/5)
Early (0-2 months)	15	86.7% (13/15)	73.3% (11/15)
Intermediate (3-12 months)	13	92.3% (12/13)	84.6% (11/13)
Late (>1 year)	7	57.1% (4/7)	57.1% (4/7)

b. Matrix comparison: N/A

3. Clinical studies:

a. Clinical Sensitivity: N/A

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 immune response to *B. burgdorferi* appears to follow a normal response pattern. IgM antibodies can be detected in some patients within days after onset, while IgG antibodies can be detected as early as two weeks after onset. IgM antibodies often decrease some weeks to months after convalescence, but it is also possible that IgM antibody titers remain constant up to some years. Band patterns will differ from sample to sample due to differences in patient immune responses and the stage to which the disease has progressed. The following table summarizes the frequency of bands observed with the Gold Standard Diagnostics *B. burgdorferi* B31 IgM Line Blot Test.

Stage / kDa	41	39	23
Early Lyme (n=55)	81.8%	25.5%	89.0%

Disseminated (n=33)	70.0%	39.0%	72.8%
Late Lyme (n=47)	45.0%	9.0%	21.0%
Prospective Samples (Not-staged) (n=310)	53.5%	24.5%	60.6%
Normal (n=115) (Non-Endemic)	3.0%	1.0%	7.0%
Normal (n=119) (Endemic)	1.0%	2.0%	4.0%

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.