510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K200023

B Applicant

Gold Standard Diagnostics

C Proprietary and Established Names

Gold Standard Diagnostics Borrelia burgdorferi IgM ELISA Test Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LSR	Class II	21 CFR 866.3830 - Treponema Pallidum Treponemal Test Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination and FDA clearance for a new device.

B Measurand:

Anti-*Borrelia burgdorferi* IgM antibodies

C Type of Test:

Manual ELISA

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The Gold Standard Diagnostics Borrelia burgdorferi IgM ELISA Test Kit is intended as a qualitative presumptive (first-step) test for the detection of IgM antibodies to B. burgdorferi sensu stricto in human serum from symptomatic patients or people suspected of infection. Positive and equivocal results must be supplemented by testing with a second-step Western blot assay.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements: None

IV Device/System Characteristics:

A Device Description:
During the test procedure, antibodies to *B. burgdorferi* (*sensu stricto*) if present in the human serum sample will bind to the antigens coated onto the wells forming antigen-antibody complexes. Excess antibodies are removed by washing. A conjugate of goat anti-human IgM antibodies conjugated with horseradish peroxidase is then added, which binds to the antigen-antibody complexes. Excess conjugate is removed by washing. This is followed by the addition of a chromogenic substrate, tetramethylbenzidine (TMB). If specific antibodies to the antigen are present in the patients' serum, a blue color will develop. The enzymatic reaction is then stopped with a stopping solution causing the contents of the well to turn yellow. The wells are read photometrically with a microplate reader at 450nm.

The antigens used in the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test kit is a combination of *B. burgdorferi sensu stricto* strain B31 lysate, *B. burgdorferi sensu stricto* strain 2591 lysate, and a recombinant VlsE protein from *B. burgdorferi sensu stricto* strain B31 grown in *E. coli* SURE2 cells.

B Principle of Operation:

ELISA

V Substantial Equivalence Information:

A Predicate Device Name(s):
Mardx Lyme Disease EIA (IgM) Test System

B Predicate 510(k) Number(s):
K894293

C Comparison with Predicate(s):

Device & Predicate Device(s):	K200023	K894293
Device Trade Name	Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit	Trinity Biotech MarDx *Borrelia burgdorferi* EIA IgM Test Kit
General Device Characteristic Similarities		
Intended Use/Indications for Use	The Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit is intended as a qualitative presumptive (first-step) test for the detection of IgM antibodies to *B. burgdorferi* sensu stricto in human serum from symptomatic patients or people suspected of infection. Positive and equivocal results must be supplemented by testing with a second-step Western blot assay.	Trinity Biotech MarDx *Borrelia burgdorferi* EIA IgM Test System is a qualitative test intended for use in the presumptive detection of human IgM antibodies to *Borrelia burgdorferi* in human serum. This EIA system should be used to test serum from patients with a history and symptoms of infection with *B. burgdorferi.* All positive and equivocal specimens should be retested with a highly specific, second-tier test such as Western blot. Positive second-tier results are supportive evidence of infection with *B. burgdorferi.* The diagnosis of Lyme disease should be made based on history and symptoms (such as erythema migrans), and other laboratory data, in addition to the presence of antibodies to *B. burgdorferi.* Negative results (either first or second-tier) should not be used to exclude Lyme disease.
Technology	ELISA	Same
Sample Matrix	Human serum	Same

General Device Characteristic Differences		
Antigens	*B. burgdorferi* B31 strain, *B. burgdorferi* 2591 strain, *B. burgdorferi* recombinant VlsE protein from B31 strain	*B. burgdorferi* B31 strain

VI Standards/Guidance Documents Referenced:

N/A

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision: To determine the precision of the *Borrelia burgdorferi* IgM ELISA Test, a within-lab precision study was conducted. A precision panel consisting of a negative sample, a high negative sample, a low positive sample, and a moderate positive sample, along with the kit controls, was tested in-house. Each of the panel members was tested in duplicate, twice per day, for 12 days. The sample panel was masked and randomized. The results are summarized in the following table:

Table 1: Precision Study

Sample	N	Mean Units		Within-Run	Between-Run	Between-Day	Total
Moderate Positive	48	20.6	SD	1.234	0.476	0.423	1.222
			CV	6.0%	2.3%	2.1%	5.9%
Low Positive	48	12.4	SD	0.849	0.728	0.405	0.834
			CV	6.9%	5.9%	3.3%	6.7%
High Negative	48	5.7	SD	0.740	0.495	0.349	0.727
			CV	13.1%	8.7%	6.2%	12.8%
Negative	48	2.5	SD	0.328	0.103	0.061	0.324
			CV	13.3%	4.2%	2.5%	13.1%
Positive Control	48	25.2	SD	1.197	0.337	0.492	1.183
			CV	1.3%	1.3%	2.0%	4.7%
Cutoff Control	48	9.9	SD	0.317	0.238	0.305	0.287
			CV	3.2%	2.4%	3.1%	2.9%
Negative Control	48	0.7	SD	0.124	0.052	0.017	0.123
			CV	18.1%	736%	2.5%	17.9%

Reproducibility: A reproducibility panel consisting of a negative sample, a high negative sample, a low positive sample, and a moderate positive sample, along with the kit controls,

was tested at three different sites. The sample panel was masked and randomized. Each of the panel members was tested in triplicate, twice per day, for five days. The Within-Run, Between-Run, Between-Days, and Between-Sites Standard Deviation and Coefficients of Variation (CV) were calculated. The results are summarized in the following table:

Table 2: Reproducibility Study

Sample	N	Mean Units		Within-Run	Between-Run	Between-Day	Between-Sites	Total
Moderate Positive	90	54.4	SD	3.26	1.37	2.49	1.38	3.73
			CV	6.0%	2.5%	4.6%	2.5%	6.9%
Low Positive	90	17.1	SD	1.13	0.46	0.98	0.78	1.29
			CV	6.6%	2.8%	5.7%	4.6%	7.6%
High Negative	90	6.5	SD	0.60	0.34	0.48	0.56	0.75
			CV	9.2%	5.2%	7.4%	8.6%	11.4%
Negative	90	1.8	SD	0.31	0.25	0.29	0.32	0.34
			CV	17.0%	14.3%	15.9%	17.5%	18.6%
Positive Control	30	24.2	SD	1.69	1.10	1.28	0.34	1.64
			CV	7.0%	7.0%	5.3%	3.5%	6.8%
Cutoff Control	60	9.9	SD	0.36	0.17	0.22	1.33	0.34
			CV	3.6%	1.7%	2.2%	5.5%	3.4%
Negative Control	30	0.7	SD	0.09	0.03	0.04	0.04	0.72
			CV	13.3%	5.1%	5.1%	5.0%	13.1%

2. Linearity: N/A

3. Analytical Specificity/Interference:

Analytical Specificity: The analytical specificity was determined by testing 208 asymptomatic individuals' samples from endemic and non-endemic regions. The Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test results are summarized in the following table:

Table 3: Analytical Specificity

	Number of Samples	Number Positive/Equivocal	Analytical Specificity
Endemic Region	103	4	96.1%
Non-endemic Region	105	10	90.5%

Cross Reactivity: A study using 277 samples was conducted to evaluate potential cross reactivity from different infections and disease conditions. The samples were obtained from serum vendors who confirmed their positivity for each respective marker. The samples were tested on the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test. The results are summarized in the following table:

Table 4: Cross Reactivity

Infection / Diagnosis	Number of Sera Tested	# Positive / (%)
Tick-borne Relapsing Fever IgM	21	0 / (0%)
Treponemal Infections (TPPA)	29	0 / (0%)
Rickettsia IgM	23	0 / (0%)
Ehrlichiosis IgM	10	6 / (60%)*
Babesiosis IgM	16	10 / (63%)*
Leptospirosis IgM	10	8/ (80%)*
H. pylori IgM	10	0 / (0%)
Epstein-Barr Virus IgM	14	0 / (0%)
Varicella Zoster Virus	16	6 / (38%)
Fibromyalgia	25	0 / (0%)
Rheumatoid Arthritis	12	0 / (0%)
Autoimmune Disease	46	0 / (0%)
Multiple Sclerosis	22	0 / (0%)
Severe Periodontitis	23	0 / (0%)

Interfering Substances: The effect of potential interfering substances on samples using the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test was evaluated. Three samples, a high negative, an equivocal and a low positive were spiked with high levels of interferants and were tested along with serum without spiked interferants. The recommended concentrations from the guideline "Interference Testing in Clinical Chemistry" EP07-A3 from the Clinical and Laboratory Standards Institute were used (see table below). The tested substances did not affect the performance of the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test.

Table 5: Interfering Substances

Substance	Concentration	Interference
Albumin	60 mg/ml	None detected
Bilirubin	0.4 mg/ml	None detected
Cholesterol	4.0 mg/ml	None detected
Hemoglobin	10 mg/ml	None detected
Triglycerides	15 mg/ml	None detected

4. Assay Reportable Range: N/A

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods): N/A

6. Detection Limit: N/A

7. Assay Cut-Off:
The cutoff was determined by testing a total of 208 normal sera which consisted of 103 sera from an endemic region of Lyme disease and 105 sera from a non-endemic region of Lyme disease. The mean plus two standard deviations was used to determine the assay cutoff. A known positive sample was then diluted to produce a ready to use cutoff control. An

additional 194 samples consisting of 114 samples from different phases of Lyme disease, 8 negative healthy samples, 72 negative Lyme disease samples but do have other diseases that may cause serologic cross-reactivity, were tested. A receiver operating characteristics (ROC) analysis was performed to evaluate the performance of the assay and confirm that the chosen cutoff provided the best compromise between sensitivity and specificity.

B Comparison Studies:

1. Method Comparison with Predicate Device:
Comparison studies were conducted at three sites (one internal and two external reference laboratories) using prospective samples submitted for Lyme serology testing. 531 serum samples were tested on both the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test and on the predicate *B. burgdorferi* IgM ELISA Test. The results are summarized in the following table:

Table 6: Method Comparison

		Predicate IgM ELISA			
		Positive	Equivocal*	Negative	**Total**
Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit	Positive	72	9	5	**86**
	Equivocal*	4	8	5	**17**
	Negative	1	9	418	**428**
	Total	**77**	**26**	**428**	**531**

*Equivocal samples counted as positive

Positive percent agreement = 90.3% (93/103) 95% CI (82.9% - 95.5%)
Negative percent agreement = 97.7% (418/428) 95% CI (95.8% - 99%)

Second Tier Testing: All positive and equivocal samples by the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test and by the Predicate IgM ELISA were tested by an FDA cleared IgM Western blot assay. The results are summarized in the following table:

Table 7: Comparison by Second Tier Testing

	Tier 1 Positive or Equivocal	**IgM Blot Positive**	**IgM Blot Negative**
Predicate IgM ELISA	103	59	44
Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit	103	61	42
Predicate IgM ELISA + Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit	93	58	35

2nd Tier Percent Agreement		
2nd Tier PPA (95% CI)	98.3% (90.9% - 99.9%)	58/59

2. Matrix Comparison: N/A

C Clinical Studies:

1. Clinical Sensitivity:
   A sensitivity study was performed on 114 clinically characterized samples. The samples encompass early, disseminated, and late stages of Lyme disease. The samples were tested on both the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test and on the predicate *B. burgdorferi* IgM ELISA Test. The results are summarized in the following table:

Table 8: Clinical Sensitivity

Disease Stage	**n**	**Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit**	**Predicate IgM ELISA**
Early	58	75.9% (44/58)	77.6% (45/58)
Disseminated	17	100.0% (17/17)	100.0% (17/17)
Late	39	89.7% (35/39)	84.6% (33/39)

CDC Panel: A panel of 280 positive and negative specimens from the Center of Disease Control (CDC) for Lyme disease detection was tested on both the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test and on the predicate device. The results are presented as a means to convey further information on the performance of the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test with a masked characterized serum panel. This does not imply an endorsement of the assay by the CDC. The results are summarized in the following table:

Table 9: CDC Panel Testing

Disease Stage	n	Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit		Predicate IgM ELISA	
		Positive or Equivocal	% Agreement with Clinical Diagnosis	Positive or Equivocal	% Agreement with Clinical Diagnosis
Healthy	100	7	93.0%	14	86.0%
Early Lyme	60	46	76.7%	48	80.0%
Cardiac Lyme	3	2	66.7%	2	66.7%
Neurological Lyme	7	7	100%	7	100%
Late	20	17	85.0%	17	85.0%
Look-alike Disease	90	17	81.1%	25	72.2%

2. Clinical Specificity: N/A

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable): N/A

D Clinical Cut-Off:

N/A

E Expected Values/Reference Range:

The range of values and positivity rate among different studies and population for the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test are as follows:

Table 10: Expected Values

Population	# Samples	Unit Results			Qualitative Results	
		Mean	Range	Std. Dev.	# Positive/ Equivocal	% Positive/ Equivocal
Normal Endemic	103	3.5	0.4. – 10.7	2.341	4	3.9%
Normal Non-Endemic	105	4.2	0.0 – 15.5	3.136	10	9.5%
Prospective Study	531	7.7	0.0 – 76.6	11.742	103	19.4%
Sensitivity Study	114	32.6	0.0 – 81.0	21.138	96	84.2%

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

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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