510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K200025

B Applicant

Gold Standard Diagnostics

C Proprietary and Established Names

Gold Standard Diagnostics *Borrelia burgdorferi* IgG 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* IgG 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* IgG ELISA Test Kit is intended as a qualitative presumptive (first-step) test for the detection of IgG 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 IgG 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* IgG 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 (IgG) Test System

B Predicate 510(k) Number(s):
K894224

C Comparison with Predicate(s):

Device & Predicate Device(s):	K200025	K894224
Device Trade Name	Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test Kit	Trinity Biotech MarDx *Borrelia burgdorferi* EIA IgG Test Kit
General Device Characteristic Similarities		
Intended Use/Indications for Use	The Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test Kit is intended as a qualitative presumptive (first-step) test for the detection of IgG 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 IgG Test System is a qualitative test intended for use in the presumptive detection of human IgG 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:

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision: To determine the precision of the *Borrelia burgdorferi* IgG 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. The sample panel was masked and randomized. Each of the panel members was tested in duplicate, twice per day, for 12 days. 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	20.3	SD	1.488	1.312	1.257	1.439
			CV	7.3%	6.5%	6.2%	7.1%
Low Positive	48	11.5	SD	0.845	0.718	0.718	0.816
			CV	7.4%	6.2%	6.2%	7.1%
High Negative	48	8.3	SD	0.880	0.646	0.615	0.857
			CV	10.6%	7.8%	7.4%	10.4%
Negative	48	0.8	SD	0.116	0.049	0.076	0.113
			CV	14.2%	6.5%	10.0%	14.8%
Positive Control	48	17.2	SD	0.947	0.649	0.739	.932
			CV	5.5%	3.8%	4.3%	5.4%
Cutoff Control	48	10.1	SD	0.241	0.115	0.285	0.264
			CV	2.7%	1.1%	2.8%	2.6%
Negative Control	48	0.4	SD	0.052	0.424	0.144	0.051
			CV	12.9%	10.6%	11.0%	12.7%

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 sample panel was masked and randomized. The results are summarized in the following table:

Table 2: Reproducibility Study

Sample	N	Mean Units		Within-Run	Between-Run	Between-Day	Between-Site	Total
Moderate Positive	90	21.0	SD	1.54	0.40	1.07	0.91	1.29
			CV	7.3%	1.9%	5.1%	4.3%	6.1%
Low Positive	90	13.7	SD	0.72	0.34	1.09	1.24	1.28
			CV	5.5%	2.6%	8.0%	9.1%	9.3%
High Negative	90	6.6	SD	0.76	0.27	0.46	0.68	0.67
			CV	11.7%	4.1%	7.0%	10.3%	10.2%
Negative	90	3.0	SD	0.33	0.56	0.49	0.56	0.55
			CV	21.1%	18.7%	16.4%	18.8%	18.3%
Positive Control	30	19.1	SD	0.65	0.67	0.67	0.63	0.62
			CV	3.5%	3.5%	3.5%	3.3%	3.2%
Cutoff Control	60	10.0	SD	0.25	0.22	0.23	0.22	0.22
			CV	2.4%	2.2%	2.3%	2.2%	2.2%
Negative Control	30	0.5	SD	0.08	0.06	0.06	0.50	0.50
			CV	11.0%	11.0%	11.0%	9.5%	9.6%

2. Linearity: N/A

3. Analytical Specificity/Interference:
The analytical specificity was determined by testing 208 asymptomatic individuals' samples from endemic and non-endemic regions. The Gold Standard Diagnostics *Borrelia burgdorferi* IgG 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	0	100%

Cross Reactivity: A study using 377 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 or clinical diagnosis. The samples were tested on the Gold Standard Diagnostics *Borrelia burgdorferi* IgG 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 IgG	21	0 / (0%)
Treponemal Infections (TPPA)	23	0 / (0%)
Rickettsiosis IgG	25	6 / (24%)
Ehrlichiosis IgG	10	2 / (20%)
Babesiosis IgG	12	0 / (0%)

Infection / Diagnosis	Number of Sera Tested	# Positive / (%)
H. pylori IgG	11	0 / (0%)
Parvovirus B19 IgG	12	0 / (0%)
Influenza A&B IgG	12	0 / (0%)
Epstein-Barr Virus IgG	34	1 / (3%)
Cytomegalovirus IgG	31	0 / (0%)
Herpes Simplex Virus IgG	21	0 / (0%)
Varicella Zoster Virus	16	1 / (6%)
Fibromyalgia	32	0 / (0%)
Rheumatoid Arthritis	12	0 / (0%)
Autoimmune Disease	59	0 / (0%)
Multiple Sclerosis	23	0 / (0%)
Severe Periodontitis	23	0 / (0%)

Interfering Substances: The effect of potential interfering substances on samples using the Gold Standard Diagnostics *Borrelia burgdorferi* IgG 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. The tested substances did not affect the performance of the Gold Standard Diagnostics *Borrelia burgdorferi* IgG 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 210 normal sera which consisted of 105 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. 523 serum samples were tested on both the Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test and on the predicate *B. burgdorferi* IgG ELISA Test. The results are summarized in the following table:

Table 6: Method Comparison

		Predicate IgG ELISA			
		Positive	Equivocal*	Negative	**Total**
Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test Kit	Positive	40	5	2	**47**
	Equivocal*	3	7	0	**10**
	Negative	2	4	460	**466**
	Total	**45**	**16**	**462**	**523**

*Equivocal samples counted as positive

Positive percent agreement = 90.2% (55/61) 95% CI (79.8% - 96.3%)
Negative percent agreement = 99.6% (460/462) 95% CI (98.5% - 99.9%)

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

Table 7: Comparison by Second Tier Testing

	Tier 1 Positive or Equivocal	**IgG Blot Positive**	**IgG Blot Negative**
Predicate IgG ELISA	61	37	24
Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test Kit	57	37	20
Predicate IgG ELISA + Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test Kit	55	37	18

2nd Tier Percent Agreement		
2nd Tier PPA (95% CI)	100% (92.2% - 100%)	37/37

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* IgG ELISA Test and on the predicate *B. burgdorferi* IgG ELISA Test. The results are summarized in the following table:

Table 8: Clinical Sensitivity

Disease Stage	n	Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test Kit	Predicate IgG ELISA
Early	58	46.6% (27/58)	27.6% (16/58)
Disseminated	17	82.4% (14/17)	52.9% (9/17)
Late	39	97.4% (38/39)	97.4% (38/39)

CDC Panel: A panel of 280 positive and negative specimens from the Centers of Disease Control (CDC) for Lyme disease detection was tested on both the Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test and on the predicate device. The results are presented to convey further information on the performance of the Gold Standard Diagnostics *Borrelia burgdorferi* IgG 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* IgG ELISA Test Kit		Predicate IgG ELISA	
		Positive or Equivocal	Agreement with Clinical Diagnosis	Positive or Equivocal	Agreement with Clinical Diagnosis
Healthy	100	1	99.0%	1	99.0%
Early Lyme	60	41	68.3%	21	35.0%
Cardiac Lyme	3	2	66.7%	3	100%
Neurological Lyme	7	6	85.7%	3	42.9%
Late	20	20	100%	20	100%
Look-alike Disease	90	11	87.8%	10	88.9%

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* IgG 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	3.7	0.5 – 14.3	2.452	4	3.9%
Normal Non-Endemic	105	3.9	0.6 – 8.9	2.002	0	0.0%
Prospective Study	523	4.3	0.1 – 22.2	4.409	57	10.9%
Sensitivity Study	114	13.6	0.9 – 40.4	7.994	81	71.1 %

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.