510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K180264

B. Purpose for Submission: To obtain a substantial equivalence determination and FDA clearance for a new device.

C. Measurand: Anti-*Borrelia burgdorferi* (IgM and IgG) antibodies

D. Type of Test: Enzyme Immunoassay

E. Applicant: Gold Standard Diagnostics

F. Proprietary and Established Names: Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA 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: Microbiology

H. Intended Use:

1. Intended use(s): The Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test Kit is intended as a qualitative presumptive (first-step) test for the detection of IgG and 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.

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 Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM test consists of antigenic proteins specific for *B. burgdorferi* (sensu stricto) which are either purified or cloned and expressed in the surrogate host *E.coli*. The individual antigens are transferred to polystyrene 96 well microtiter plates.

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/IgM antibodies conjugated with horseradish peroxidase are 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.

J. Substantial Equivalence Information:

1. Predicate device name(s): Trinity Biotech Captia *Borrelia burgdorferi* IgG/IgM ELISA Test Kit.

2. Predicate 510(k) number(s): K033070

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (K033070)
Intended Use	The Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test Kit is intended as a qualitative presumptive (first-step) test for the detection of IgG and 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 Captia *Borrelia burgdorferi* IgG/IgM ELISA Test Kit is intended for the qualitative presumptive (first-step) detection of total (IgG/IgM) antibodies to *B. burgdorferi* in human serum. This ELISA should only be used for patients with signs and symptoms that are consistent with Lyme disease. Equivocal or positive results must be supplemented by testing with a standardized Western blot (second step) procedure. Positive supplemental (second-step) results are supportive evidence of exposure to *B. burgdorferi* and can be used to support a clinical diagnosis of Lyme disease. The diagnosis of Lyme

Similarities		
Item	Device	Predicate (K033070)
		disease must be made based on history, signs (such as erythema migrans), symptoms, and other laboratory data, in addition to the presence of antibodies to *B. burgdorferi.* Negative results (either first or second step) should not be used to exclude Lyme disease.
Assay Format	Antigen coated microtiter plate – 96 wells.	Same
Technology	ELISA	Same
Sample Matrix	Human serum	Same
Controls Provided	Positive, Cut-off, Negative	Same
Reagents Provided	Diluent, Wash, Conjugate, Substrate, Stop Solution	Same
Reported Results	Positive, Equivocal, Negative	Same
Interpretation	Optical density readings from Spectrophotometer	Same

Differences		
Item	Device	Predicate
Sample Processing	Dilute Samples 1:100 in Diluent	Dilute Samples 1:20 in Diluent
Volumes	100ul sample, 50ul substrate, 50ul stop solution	100ul sample, 100ul substrate, 100ul stop solution
Incubation	15/15/15 minutes at room temperature	25/25/10-15 minutes at room temperature
Antigens	*B. burgdorferi* B31 strain, *B. burgdorferi* 2591 strain, *B. burgdorferi* recombinant VlsE, B31 strain	*B. burgdorferi* B31 strain
Results Interpretation	Convert to units. Negative: <9 Equivocal: 9.0-11.0 Positive: >11.0	Convert to units. Negative: ≤0.90 Equivocal: 0.91-1.09 Positive: ≥1.10

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: To determine the precision of the Gold Standard Diagnostics *Borrelia burgdorferi* IgG/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. 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	19.6	SD	0.815	1.534	1.472	1.737
			CV	4.2%	7.8%	7.5%	8.9%
Low Positive	48	12.1	SD	0.267	1.417	1.248	1.442
			CV	2.2%	11.7%	10.3%	11.9%
High Negative	48	6.1	SD	0.211	0.662	0.642	0.695
			CV	3.4%	10.8%	10.5%	11.4%
Negative	48	1.7	SD	0.113	0.164	0.151	0.199
			CV	6.6%	9.6%	8.8%	11.6%

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	21.1	SD	1.117	1.543	1.434	0.386	1.905
			CV	5.3%	7.3%	6.8%	1.8%	9.0%
Low Positive	90	13.8	SD	0.591	1.155	1.026	0.404	1.297
			CV	4.3%	8.4%	7.5%	2.9%	9.4%

High Negative	90	6.4	SD	0.323	0.756	0.715	0.237	0.822
			CV	5.0%	11.7%	11.1%	3.7%	12.8%
Negative	90	1.6	SD	0.145	0.335	0.312	0.347	0.365
			CV	9.1%	21.1%	19.6%	21.8%	23.0%

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: The analytical specificity was determined by testing 210 asymptomatic individuals' samples from endemic (Pennsylvania) and non-endemic (Arizona) regions. The Gold Standard Diagnostics *Borrelia burgdorferi* lgG/IgM ELISA Test results are summarized in the following table.

Table 3: Analytical Specificity Study

	Number of Samples	Number Positive/Equivocal	Analytical Specificity
Endemic Region	110	3	97.3%
Non-endemic Region	100	2	98.0%

Cross Reactivity: A study using 221 samples was conducted to evaluate potential cross reactivity from different 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* IgG/IgM ELISA Test. The results are summarized in the following table.

Table 4: Cross-Reactivity Study

Infection / Diagnosis	**Number of Sera Tested**	**# Positive / (%)**
Tick-borne Relapsing Fever	26	2 / (7.7%)
Treponemal Infections	23	2* / (8.7%)
Rickettsia	10	0 / (0%)
Ehrlichiosis	10	0 / (0%)
Babesiosis	11	0 / (0%)
Leptospirosis	11	0 / (0%)
Parvovirus B19	12	0 / (0%)
Influenza A&B	10	0 / (0%)
Epstein-Barr Virus	10	0 / (0%)
Cytomegalovirus	19	0 / (0%)
H. pylori	11	0 / (0%)

Fibromyalgia	10	1/ (10%)
Rheumatoid Arthritis	11	0 / (0%)
Herpes Simplex Virus	13	0 / (0%)
Varicella Zoster Virus	12	0 / (0%)
Autoimmune Disease	22	0 / (0%)

*Also positive on the predicate device

Interfering Substances: The effect of potential interfering substances on samples using the Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test was evaluated. Three samples, a negative, a low positive, and a moderate 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 EP7-A2" 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/IgM ELISA Test.

Table 5: Interfering Substances Study

Substance	Concentration	Interference
Albumin	120 g/L	None detected
Bilirubin	342 µmol/L	None detected
Cholesterol	13 mmol/L	None detected
Hemoglobin	2 g/L	None detected
Triglycerides	37 mmol/L	None detected

f. Assay cut-off:

Determination of the Assay Cut-off: The cut-off was determined by testing a total of 200 normal sera which consisted of 100 sera from an endemic region of Lyme disease and 100 sera from a non-endemic region. The mean plus two standard deviations was used to determine the assay cut-off. After the cut-off was determined 125 characterized Lyme disease samples were tested. An ROC analysis was then generated with the 325 samples (200 normal and 125 characterized samples) to verify the chosen cut-off. The analysis confirmed that the assay cut-off provided an optimal level of sensitivity and specificity.

2. Comparison studies:

a. Method comparison with predicate device:

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. Five hundred and twenty (520) serum samples were tested on both the Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test and on the predicate *B. burgdorferi* IgG/IgM ELISA Test. The results are summarized in the following table.

Table 6: Percent Agreement with Predicate Device

		Predicate IgG/IgM ELISA			
		Positive	Equivocal*	Negative	**Total**
Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test Kit	Positive	55	7	2	**64**
	Equivocal	8	2	9	**19**
	Negative	2	1	434	**437**
	Total	**65**	**10**	**445**	**520**

*Equivocal samples counted as positive

Positive percent agreement = 96.0% (72/75) 95% CI (88.8% - 99.2%)
Negative percent agreement = 97.5% (434/445) 95% CI (95.6% - 98.8%)

b. Matrix comparison: N/A

3. Clinical studies:

a. Clinical Sensitivity:

Sensitivity Study: A sensitivity study was performed on 89 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/IgM ELISA Test and on the predicate *B. burgdorferi* IgG/IgM ELISA Test. The results are summarized in the following table.

Table 7: Case Confirmed Lyme Disease Samples

Disease Stage	**n**	**Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test Kit**	**Predicate IgG/IgM ELISA**
Early	38	76.3% (29/38)	78.9% (30/38)
Disseminated	15	100.0% (15/15)	100.0% (15/15)
Late	36	97.2% (35/36)	94.4% (34/36)

CDC Panel: A standard panel of positive and negative specimens provided by the Center of Disease Control (CDC) for Lyme disease detection was tested on both the Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test and on the predicate device. The results are summarized in the following table.

Table 8: Testing of CDC Lyme Disease Panel

Disease Stage	n	Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test Kit		Predicate IgG/IgM ELISA	
		Positive or Equivocal	% Agreement with Clinical Diagnosis	Positive or Equivocal	% Agreement with Clinical Diagnosis
Healthy	5	0	100%	0	100%
Early (0-2 months)	15	13	86.7%	12	80.0%
Convalescent (3-12 months)	13	7	53.8%	7	53.8%
Late (>1 year)	7	7	100%	7	100%

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 range of values and positivity rate among different studies and populations for the Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test are as follows.

Table 9: Expected Values

Population	#Samples	Unit Results			Qualitative Results	
		Mean	Range	Std. Dev.	#Positive/ Equivocal	%Positive/ Equivocal
Normal Endemic	110	5.8	06.- 11.3	2.075	3	2.7%
Normal Non-Endemic	100	4.2	0.9- 12.3	2.077	2	2.0%
Prospective Study	520	6.3	0.70-32.3	5.407	83	16.0%
Sensitivity Study	89	25.0	3.6-34.9	8.449	79	88.8%

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

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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