510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K203295

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 and to obtain a substantial equivalence determination and FDA clearance for a modified intended use for a previously cleared medical device. This regulatory filing follows the FDA guidance document titled “Bundling Multiple Devices or Multiple Indications in a Single Submission”1. For these devices, bundling is appropriate since the device review presented scientific and regulatory issues that were most efficiently addressed during a single review. In determining whether a bundled submission was appropriate FDA considered that: (i) the supporting data are similar; (ii) primarily one review division/group will be involved; and (iii) the devices or indications for use are similar.

B Measurand:

Anti-*Borrelia burgdorferi* antibodies

C Type of Test:

Enzyme-linked immunosorbent assay (ELISA)

III Intended Use/Indications for Use:

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

B Indication(s) for Use:
Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit

The Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit is intended as a qualitative test for the detection of IgM antibodies to B. burgdorferi sensu stricto in human serum from symptomatic patients or people suspected of infection. When used as the first-tier screening test, positive and equivocal results must be supplemented through additional testing by one of the following methods:

a) Standard two-tier test methodology (STTT) using an IgM blot test following current interpretation guidelines, OR

b) Modified two-tier test methodology (MTTT) using the Gold Standard Diagnostics *Borrelia burgdorferi* VlsE-OspC IgG/IgM ELISA Test

The assay can also be used as a second-tier confirmation test using the MTTT methodology when used with when used with the Gold Standard Diagnostics *Borrelia burgdorferi* VlsE-OspC IgG/IgM ELISA Test as the first-tier screening test.

Positive test results by either the STTT or MTTT methodology are supportive evidence for the presence of antibodies and exposure to *Borrelia burgdorferi*, the cause of Lyme disease. A diagnosis of Lyme disease should be made based on the presence of *Borrelia burgdorferi* antibodies, history, symptoms, and other laboratory findings.

Gold Standard Diagnostics *Borrelia burgdorferi* VlsE-OspC IgG/IgM ELISA Test Kit

The Gold Standard Diagnostics *Borrelia burgdorferi* VlsE-OspC IgG/IgM ELISA Test Kit is intended as a qualitative test for the detection of IgG and IgM class antibodies to VlsE and OspC antigens from *Borrelia burgdorferi* sensu stricto in human serum from symptomatic patients or people suspected of having Lyme disease. When used as the first-tier screening test, positive and equivocal results must be confirmed through additional testing by one of the following methods:

a) Standard two-tier test methodology (STTT) using an IgG and/or IgM blot testing following current interpretation guidelines, OR

b) Modified two-tier test methodology (MTTT) using one or more of the following three ELISA based assays: Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test, Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test, Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test.

The assay can also be used as a second-tier confirmation test using the MTTT methodology when used with one or more of the following three ELISA based assays: Gold Standard Diagnostics *Borrelia burgdorferi* IgG/IgM ELISA Test, Gold Standard Diagnostics *Borrelia burgdorferi* IgG ELISA Test, Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test.

Positive test results by either the STTT or MTTT methodology are supportive evidence for the presence of antibodies and exposure to *Borrelia burgdorferi*, the cause of Lyme disease. A diagnosis of Lyme disease should be made based on the presence of *Borrelia burgdorferi* antibodies, history, symptoms, and other laboratory findings.

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

D Special Instrument Requirements:
N/A

IV Device/System Characteristics:

A Device Description:
The Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit is an Enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgM antibodies to *Borrelia burgdorferi* in human serum.

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 from *B. burgdorferi* sensu stricto strain B31. The lysates use spirochetes growing in BSK-H complete medium until mid-exponential phase. The recombinant VlsE protein is produced in E. coli SURE2 cells and purified by affinity chromatography. The purity of each antigen is assayed by SDS-PAGE followed by Coomassie staining and/or western blotting.

The kit includes 12 x 8 well Antigen Coated strips, Conjugate, Substrate, Stop Solution, Wash Buffer, Diluent, Negative Control, Positive Control, and a Cutoff Control. The controls are provided to determine if the assay is functioning properly and to determine the antibody level. The reagents are sufficient for 96 determinations.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit, *Borrelia burgdorferi* Igm Blot Test

B Predicate 510(k) Number(s):
K200023

C Comparison with Predicate(s):

Device & Predicate Device(s):	K203295	K200023	K113846
Device Trade Name	Device 1: Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit Device 2: Gold Standard Diagnostics *Borrelia burgdorferi* VlsE-OspC IgG/IgM ELISA Test Kit	Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test Kit	Gold Standard Diagnostics *Borrelia burgdorferi* IgM Blot Test Kit
General Device Characteristic Similarities			
Intended Use/Indications For Use	These are two ELISA assays for the qualitative detection of *B. burgdorferi* antibodies in human serum. For a complete description of the Intended Use please see Item III B. above	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.	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.*
Sample Matrix	Same	Serum	Same

Sample Processing	Same	Dilute samples 1:100 in Diluent	Same
Controls Provided	Same	Positive, Cutoff, Negative	Same
Assay Type	Same	Qualitative	Same
General Device Characteristic Differences			
Antigens	*B. burgdorferi* B31 strain, *B. burgdorferi* 2591 strain, *B. burgdorferi* recombinant VlsE protein from B31 strain Recombinant VlsE and OspC from *B. burgdorferi* strain B31	*B. burgdorferi* B31 strain, *B. burgdorferi* 2591 strain, *B. burgdorferi* recombinant VlsE protein from B31 strain	*B. burgdorferi* B31 strain
Assay Format	Same	Antigen coated microtiter plate – 96 wells	Nitrocellulose strips
Technology	Same	ELISA	Immunoblot
Reagents Provided	Same	Diluent, Wash, Conjugate, Substrate, Stop Solution	Diluent/Wash, Conjugate, Substrate
Volumes	Same	100 µL sample, 50 µL substrate, 50 µL stop solution	1500ul sample, 1500ul substrate
Incubation	Same	15/15/15 minutes at room temperature	30/30/10-13 minutes at room temperature
Reported Results	Same	Positive, Equivocal, Negative	Positive, Negative
Interpretation	Same	Optical density readings from Spectrophotometer	Visual
Results interpretation	Same	Convert to units. Negative <9 Equivocal 9.0-11.0 Positive >11.0	Compare to cutoff band

VI Standards/Guidance Documents Referenced:

None.

VII Performance Characteristics (if/when applicable):

Note: *This clearance is for a modified use for a previously cleared IVD, the Gold Standard Diagnostics Borrelia burgdorferi IgM ELISA Test Kit (K200023). Representative analytical study data are presented below.*

A Analytical Performance:

1. Precision/Reproducibility:

To determine the precision of the Gold Standard Diagnostics *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. 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 Results

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 Results

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:

   Not Applicable.

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 Study

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

Cross-reactivity Study: A study using 277 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* IgM ELISA Test. The results are summarized in the following table:

Table 4. Cross-reactivity Study Results

Organism/Disease State	Samples Tested (N)	# Positive / (%)
Tick borne Relapsing Fever IgM	21	0 / 0%

Treponemal Infections	29	0/ 0%
Rickettsia IgM	23	0 / 0%
Ehrlichiosis IgM	10	6 / 60%*
Babesiosis IgM	16	10 / 63%*
Leptospirosis IgM	10	8 / 80%*
Epstein-Barr Virus IgM	14	0 / 0%
H. pylori IgM	10	0 / 0%
Fibromyalgia	25	0 / 0%
Rheumatoid Arthritis	12	0 / 0%
Autoimmune Disease	46	0 / 0%
Multiple Sclerosis	22	0 / 0%
Severe Periodontitis	23	0 / 0%

* Samples were also positive by the predicate device

Interfering Substances Study: The effect of potential interfering substances on samples using the Gold Standard Diagnostics *Borrelia burgdorferi* 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. Interference Testing Results

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:

   Not Applicable.

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

   Not Applicable.

6. Detection Limit:

   Not Applicable.

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:

Gold Standard Diagnostics MTTT-IgM ELISA Method Comparison: The Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test was utilized in a MTTT (2-ELISA) protocol with the Gold Standard Diagnostics *Borrelia burgdorferi* VlsE-OspC IgG/IgM ELISA Test. The MTTT (2-ELISA) results were compared to the standard two-tier testing (STTT) using the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA followed by testing all positive and equivocal results on the predicate IgM blot test.

Comparison studies were conducted at three sites (one internal and two external reference laboratories) using prospective samples submitted for Lyme serology testing. Four hundred eighty-one (481) serum samples were tested on the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test. A total of 68 positive and equivocal samples were obtained.

In the STTT protocol the samples that were positive or equivocal (n=68) were tested with the predicate *B. burgdorferi* IgM blot test. In the MTTT protocol the samples (n=68) were tested on a second ELISA, the Gold Standard Diagnostics *Borrelia burgdorferi* VlsE-OspC IgG/IgM ELISA Test. In the second-tier ELISA test, positive and equivocal results were considered positive. These testing strategies are summarized in the two figures below.

Figure 1. STTT-IgM Western Blot Algorithm (WB-STTT [IgM])

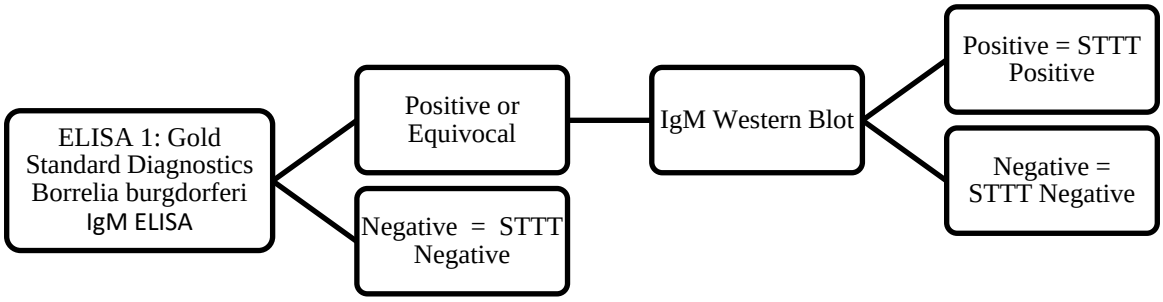
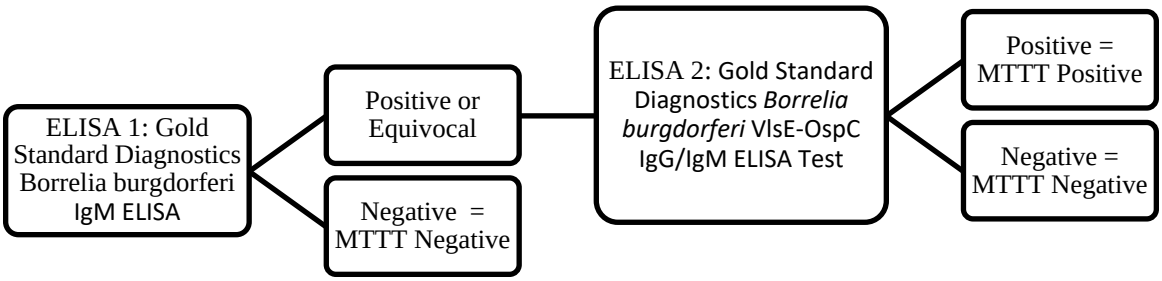


Figure 2. MTTT-IgM ELISA Algorithm (ELISA-MTTT [IgM])



Performance of the secont-tier Gold Standard Diagnostics VlsE-OspC ELISA was assessed by comparing results to second-tier western blot testing on only those samples positive by the first-tier Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA.

Table 6. Second-tier Performance Summary First-tier Positives Only

		Predicate Western Blot [IgM]		
		Positive	**Negative**	**Total**
Gold Standard Diagnostics VlsE-OspC ELISA	**Positive**	20	20	40
	Negative	0	28	28
Total		20	48	68

PPA: 100% (20/20) 95% CI: 83.2-100.0%
NPA: 58.3% (28/48) 95% CI: 43.2-72.4%

The results of the MTTT when compared to the STTT, including all samples that were part of the prospective study (n=481), are summarized in the following table:

Table 7. Performance Summary - ELISA-MTTT [IgM] compared to WB-STTT [IgM]

		WB-STTT [IgM]		
		Positive	**Negative**	**Total**
Gold Standard Diagnostics ELISA-MTTT [IgM]	**Positive**	20	20	40
	Negative	0	441	441
Total		20	461	481

PPA: 100% (20/20) 95% CI: 83.2-100%
NPA: 95.7% (441/461) 95% CI: 93.4-97.3%

The above performance table artificially inflates the negative percent agreement of the second-tier test since a large number of negatives are negative by the first tier test.

2. Matrix Comparison:

Not Applicable.

C Clinical Studies:

1. Clinical Sensitivity:

Sensitivity Study: A sensitivity study was performed on 125 clinically characterized samples. The samples encompass early, disseminated, and late stages of Lyme disease. The samples were tested on both the Gold Standard Diagnostics ELISA-MTTT [IgM] algorithm and the predicate WB-STTT [IgM] algorithm. The results are summarized in the following table:

Table 8. Sensitivity Study Results - Comparison of ELISA-MTTT [IgM] and WB-STTT [IgM] algorithms

	N	Gold Standard Diagnostics ELISA-MTTT [IgM]		Predicate WB-STTT [IgM]	
Disease Stage		**Positive/Eqv.**	**% Agreement with Clinical Diagnosis**	**Positive/Eqv.**	**% Agreement with Clinical Diagnosis**
Early	62	39	62.9%	40	64.5%
Disseminated	22	22	100%	22	100%
Late	41	34	82.9%	9	22.0%

CDC Reference 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 MTTT-IgM and on the predicate STTT-IgM. The results are summarized in the following table:

Table 9. CDC Reference Panel Results - Comparison of ELISA-MTTT [IgM] and WB-STTT [IgM] algorithms

Sample Category	Gold Standard Diagnostics ELISA-MTTT [IgM]		Predicate WB-STTT [IgM]	
	Pos.	**% Agreement with Clinical Diagnosis**	**Pos.**	**% Agreement with Clinical Diagnosis**
Early Lyme (N = 60)	46	76.7%	30	50.0%
Cardiac Lyme (N = 3)	2	66.7%	2	66.7%
Neurological Lyme (N = 7)	7	100%	7	100%

Late Lyme (N = 20)	16	80%	7	35%
Healthy Controls (N = 100)	0	100%	1	99%
Disease Controls (N – 90)	3	96.7%	2	97.8%

2. Clinical Specificity:

   Not Applicable.

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

   Not Applicable.

D Clinical Cut-Off:

Not Applicable.

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 of the Gold Standard Diagnostics *Borrelia burgdorferi* IgM ELISA Test

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%

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.