510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K183446

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

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

D. Type of Test: Enzyme Immunoassay

E. Applicant: Bio-Rad Laboratories

F. Proprietary and Established Names: BioPlex 2200 Lyme Total

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 BioPlex 2200 Lyme Total kit is a multiplex flow immunoassay intended for the qualitative detection of total (IgM/IgG) antibodies to *Borrelia burgdorferi* in human serum and plasma (EDTA, heparin). This assay should be used to test patients with history and/or symptoms of infection with *B. burgdorferi*. The BioPlex 2200 Lyme Total kit is intended for use with the Bio-Rad BioPlex 2200 System. All reactive and equivocal specimens should be tested with a second tier test such as Lyme IgG and IgM Western blot assays. Positive second tier results are supportive evidence of infection with *B. burgdorferi*. Diagnosis of Lyme borreliosis should be made based on the presence of *B. burgdorferi* antibodies, history, symptoms, and other laboratory data. Non-reactive first tier or negative second tier results should not be used to exclude borreliosis.

2. Indication(s) for use: Same as Intended Use

3. Special conditions for use statement(s): N/A

4. Special instrument requirements: BioPlex 2200 System

I. Device Description:

The BioPlex 2200 Lyme Total kit employs fluoromagnetic, dyed beads which are coated with recombinant p58, OspCB or synthetic peptide FVlsE (consisting of FlaB and VlsE sequences). Unique fluorescent signatures identify the presence of total (IgG/IgM) antibodies to *B. burgdorferi* in a two-step assay format.

The BioPlex 2200 System combines an aliquot of patient sample, sample diluent, and bead reagent in a reaction vessel. The mixture is incubated at 37°C. After a wash cycle, a mixture of murine monoclonal anti-human IgG and murine monoclonal anti-human IgM antibody conjugated to phycoerythrin (PE) is added to the dyed beads, and this mixture is incubated at 37°C. The excess conjugate is removed in another wash cycle and the beads are re-suspended in sheath fluid. The bead mixture then passes through the detector. The identity of the dyed beads is determined by the fluorescence of the dyes, and the amount of antibody captured by the antigen is determined by the fluorescence of the attached PE. Raw data is calculated in relative fluorescence intensity (RFI).

J. Substantial Equivalence Information:

1. Predicate device name(s): Immunetics C6 *B. burgdorferi* (Lyme) ELISA Kit

2. Predicate 510(k) number(s): K003754

3. Comparison with predicate:

Table 1: Similarities and Differences with the Predicate

Similarities		
Item	Device BioPlex 2200 Lyme Total	Predicate C6 *B. burgdorferi* (Lyme) ELISA Kit (K003754)
Intended Use	The BioPlex 2200 Lyme Total kit is a multiplex flow immunoassay intended for the qualitative detection of total (IgM/IgG) antibodies to *Borrelia burgdorferi* in human serum and plasma (EDTA, heparin). This assay should be used to test patients with history and/or symptoms of infection with *B. burgdorferi*. The BioPlex 2200 Lyme Total kit is intended for use with the Bio-Rad BioPlex 2200 System. All reactive and equivocal specimens should be tested with a second tier test such as Western blot. Positive second tier results are supportive evidence of infection	The Immunetics C6 *B. burgdorferi* (Lyme) ELISA Kit is intended for use in the presumptive detection of lgG and lgM antibodies to *B. burgdorferi* in human serum. The assay should be used only on samples from patients with clinical history, signs or symptoms consistent with *B. burgdorferi* infection, including individuals who have received the licensed recombinant OspA Lyme disease vaccine (Lymerix). Positive or equivocal results should be supplemented by testing with a standardized Western Blot (second step) method. Positive Western Blot results provide evidence for exposure

Similarities		
Item	Device BioPlex 2200 Lyme Total	Predicate C6 *B. burgdorferi* (Lyme) ELISA Kit (K003754)
	with *B. burgdorferi*. Diagnosis of Lyme borreliosis should be made based on the presence of *B. burgdorferi* antibodies, history, symptoms, and other laboratory data. Non-reactive first tier or negative second tier results should not be used to exclude borreliosis.	to or infection with *B. burgdorferi*. The diagnosis of Lyme 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.
Measured Analyte	Anti-*Borrelia burgdorferi* (IgM and IgG) antibodies	Same
Assay Type	Qualitative	Same

Differences		
Item	Device	Predicate
Assay Technology	Automated multiplexed flow immunoassay	ELISA (enzyme- linked immunosorbent assay)
Antigen	Recombinant p58, OspC type B (OspCB) and synthetic peptide FVlsE	Synthetic peptide (C6 peptide)
Signal Detection	Fluorescence	Colorimetric
Solid Phase	Antigen-coated paramagnetic microbeads	Antigen-coated microwell
Conjugate	Murine monoclonal anti- human IgG, murine monoclonal anti-human IgM and phycoerythrin conjugated murine monoclonal anti-human FXIII antibody	Horseradish peroxidase- conjugated (HRP) goat anti-human IgG/IgM conjugate
Sample Size	5µL	10µL
Sample Handling/ Process	Automated	Manual
Unit of Measure	Antibody Index (AI)	Lyme Index (LI)
Sample Matrix	Serum and plasma	Serum
Instrumentation	BioPlex 2200 System	None (Manual)

K. Standard/Guidance Document Referenced (if applicable): N/A

L. Test Principle: Immunofluorescence

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

Precision: Precision testing of the BioPlex 2200 Lyme Total kit was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) EP5-A3 guideline. The serum samples were tested in duplicate, two runs per day, over 20 days (2 replicates x 2 runs x 20 days = 80 replicates per panel member) using one lot of the BioPlex 2200 Lyme Total Reagent Pack, Calibrator Set and Control Set. The results are summarized in the table below.

Table 2: Precision Study

			Within Run (Repeatability)		Between Run		Between Day		Total	
Sample Description	**N**	**Mean (AI)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
High Negative 1	80	0.7	0.032	4.5%	0.000	0.0%	0.015	2.1%	0.035	4.9%
High Negative 2	80	0.9	0.050	5.4%	0.000	0.0%	0.010	1.1%	0.051	5.5%
Cut-Off	80	1.0	0.040	3.9%	0.000	0.0%	0.030	2.8%	0.050	4.8%
Low Positive	80	1.5	0.040	2.7%	0.032	2.1%	0.031	2.1%	0.060	4.0%
Mid Positive	80	2.8	0.081	2.9%	0.071	2.5%	0.039	1.4%	0.114	4.1%
High Positive	80	4.7	0.288	6.1%	0.000	0.0%	0.139	3.0%	0.320	6.8%

Reproducibility: To assess reproducibility of the BioPlex 2200 Lyme Total kit, a reproducibility panel was prepared at Bio-Rad Laboratories. The panel contained members with varying levels of antibodies to the analytes in the BioPlex 2200 Lyme Total kit, and a positive control (antibody reactive for all analytes). Reproducibility testing was performed at 3 clinical trial sites. One lot of BioPlex 2200 Lyme Total Reagent Packs, BioPlex 2200 Lyme Total Calibrator Sets and BioPlex 2200 Lyme Total Control Sets was used to evaluate reproducibility. Each of the panel members and a positive and negative control were tested in quadruplicate on 2 runs per day over 5 days at each of 3 sites (4 replicates x 2 runs x 5 days = 40 replicates per panel member per site = 120 total replicates for 3 sites). The data was analyzed for intra-assay and inter-assay reproducibility according to the CLSI guidance EP15-A3. Results are shown in the table below.

Table 3: Reproducibility Study

Lyme Total Panel Member	N	Mean (AI)	Repeatability		Between Run		Between Day		Between Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Negative	120	0.4	0.020	5.1%	0.000	0.0%	0.004	1.0%	0.006	1.5%	0.022	5.4%
High Negative	120	0.7	0.035	5.1%	0.013	1.8%	0.023	3.3%	0.032	4.5%	0.055	7.8%
Low Positive	120	1.1	0.040	3.6%	0.016	1.5%	0.027	2.4%	0.075	6.7%	0.091	8.1%
Mid Positive	120	1.8	0.049	2.7%	0.000	0.0%	0.024	1.3%	0.111	6.2%	0.124	7.0%
High Positive	120	4.7	0.108	2.3%	0.116	2.5%	0.121	2.6%	0.131	2.8%	0.239	5.1%

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: BioPlex 2200 Lyme Total kit analytical specificity was evaluated against 836 apparently healthy individuals from endemic and non-endemic regions and excluded pre-screened blood donors. Results are summarized in the following table.

Table 4: Analytical Specificity Study

	N	BioPlex 2200 Lyme Total (Negative percent agreement with expected result)	Legally marketed Lyme IgM/IgG Immunoassay (Negative percent agreement with expected result)
Normal Non-Endemic	416	96.4%	95.4%
Normal Endemic	420	96.4%	94.8%

Cross-Reactivity: A cross-reactivity study was performed to determine if samples from various disease states and other potentially cross-reacting agents interfere with test results when tested with the BioPlex 2200 Lyme Total kit. Samples known to be positive for each cross-reactant listed in the table below were evaluated with the BioPlex 2200 Lyme Total kit. All samples were pre-tested by a commercially available Lyme assay and only those that tested negative by the commercially available assay were further tested by the BioPlex 2200 Lyme Total kit. Percent negative agreement for each potential cross reactant is shown below.

Table 5: Cross-Reactivity Study

Potential Cross-Reactant	**Non-Reactive BioPlex 2200 Lyme Total kit Results/ Negative Commercial Lyme IgM/IgG Immunoassay Results**	
	N	Negative Agreement
Anti-nuclear antibody (ANA)	13	100.0%
Babesiosis	10	100.0%
Chronic Fatigue Syndrome	14	100.0%
Cytomegalovirus	13	100.0%
Epstein-Barr Virus	49	91.8%
Ehrlichiosis	11	90.9%
HAMA	13	100.0%
Helicobacter Pylori	12	100.0%
HIV	13	100.0%
Influenza virus	15	93.3%
Leptospirosis	34	97.1%
Multiple sclerosis	14	100.0%
Parvovirus B19	14	100.0%
Periodontal	35	97.1%
Rheumatoid arthritis	14	100.0%
Rheumatoid Factor positive	13	100.0%
Rickettsial diseases	16	100.0%
Rubella	14	100.0%
Syphilis	48	97.9%
Systemic Lupus Erythematosus	15	100.0%
Toxoplasmosis	29	100.0%
Varicella Zoster Virus	14	100.0%

Interfering Substances: An interfering substances study was conducted to evaluate the potential interference of specific endogenous and exogenous substances with the BioPlex 2200 Lyme Total kit according to CLSI EP07-A2 guideline. No interference was observed with any of the substances tested. The substances and the maximum levels tested are shown in the table below:

Table 6: Interfering Substances

Substance	Concentration
Hemoglobin	1000 mg/dL
Bilirubin (unconjugated)	20 mg/dL
Bilirubin (conjugated)	30 mg/dL
Triglycerides	3300 mg/dL
Total Protein	12 g/dL
Cholesterol	500 mg/dL
Ascorbic Acid	6 mg/dL
EDTA	800 mg/dL
Sodium Heparin	8000 units/dL
Lithium Heparin	8000 units/dL

f. Assay cut-off:

A total of 1,372 normal sera were used to determine the percentile cut-off in increments of 1/1000th for each analyte. Based on a retrospective selection of around the 98th percentile, the relative fluorescence intensity (RFI) corresponding to the chosen cutoff was assigned a value of 1.0 antibody index (AI), and subsequently evaluated for performance against clinically diagnosed samples. The statistics show that the 98th percentile cut-off meets the minimum agreement specifications while maximizing the clinical specificity. The assay employs an equivocal zone that brackets the cut-off (0.9-1.0 AI), with samples ≥1.1 AI reported as reactive and ≤0.8 AI reported as non-reactive.

2. Comparison studies:

a. Method comparison with predicate device:

Prospective Study: Performance of the BioPlex 2200 Lyme Total kit was evaluated using prospectively collected serum samples from patients submitted for Lyme disease testing originating from various geographically distinct locations in the U. S. A total of 792 serum samples were evaluated at three (3) U. S. clinical testing sites. Equivoal (Eqv.) samples are added to positives for calculating agreement results since they will be tested by the second tier Western blot. Results are shown in the table below.

Table 7: Percent Agreement with Predicate Device - 1st Tier

		Commercial Lyme IgM/IgG Immunoassay				
		Pos	**Eqv.**	**Neg**	**% Agreement**	**95% CI**
BioPlex 2200 Lyme Total	**Reactive**	65	0	30	**PPA** = 70.1% (68/97)*	60.0 - 79.0%
	Eqv.	2	1	4		
	Non-Reactive	21	8	661	**NPA** = 95.1% (661/695)*	93.2 - 96.6%
Total		88	9	695		

* PPA=positive percent agreement NPA= negative percent agreement

Second Tier Testing: All reactive/positive and equivocal samples by the BioPlex 2200 Lyme Total kit and the commercially available predicate device were tested by FDA-cleared IgG and IgM Western blot assays. Shown below are the second tier Western blot results (combined IgG and IgM) for samples that were reactive/ positive and equivocal in the prospective sample study.

Table 8: Percent Agreement with Predicate Device – 2nd Tier PPA

	Tier 1 + or Eqv.	**WB +**	**WB -**
Commercial Lyme IgM/IgG Immunoassay	97	47	50
BioPlex 2200 Lyme Total	102	49	53
Commercial Lyme IgM/IgG Immunoassay + BioPlex 2200 Lyme Total	68	46	22

1st Tier PPA (95% CI)	70.1% (60.0-79.0%)	68/97
2nd Tier PPA (95% CI)	97.9% (88.7-99.9%)	46/47

b. Matrix comparison:

Matched serum and plasma (EDTA and heparin) samples drawn from the same donor were acquired through a vendor. Paired serum and plasma samples were prepared and values within the measurement range of the assay were analyzed. Samples were spiked with high titer Lyme samples to obtain concentrations that span the assay range. Samples were assayed in replicates of two with the second replicate run in reverse order. Mean plasma AI values were compared to matched mean serum AI values. Linear regression analysis was used to determine the presence of a matrix effect when compared to serum. The regression correlation parameters for slope, intercept and correlation coefficient (r) are shown below.

Table 9: Matrix Comparison Study

Matrix Comparison	N	Slope (95% CI)	Intercept (95% CI)	Correlation (r)
K_2-EDTA vs. Serum	77	1.02 (0.95 to 1.08)	0.17 (-0.09 to 0.44)	0.960
K_3-EDTA vs. Serum	74	1.01 (0.93 to 1.08)	0.14 (-0.14 to 0.42)	0.951
Sodium Heparin vs. Serum	77	0.97 (0.91 to 1.02)	0.09 (-0.13 to 0.30)	0.970
Lithium Heparin vs. Serum	78	0.97 (0.92 to 1.01)	0.05 (-0.12 to 0.22)	0.981

3. Clinical studies:

a. Clinical Sensitivity:

Sensitivity Study: Sensitivity of the BioPlex 2200 Lyme Total kit was evaluated using 105 clinically characterized samples from an archived collection containing early, convalescent and late phases of Lyme disease. The table below shows the sensitivity of the BioPlex 2200 Lyme Total kit for each of the disease phases along with a commercial Lyme IgM/IgG immunoassay.

Table 10: Sensitivity with Respect to Disease Stage

Clinical Stage	N	BioPlex 2200 Lyme Total				Commercial Lyme IgM/IgG Immunoassay			
		Reactive	Eqv.	Non-Reactive	Sensitivity 95% CI	Pos (+)	Eqv.	Neg (-)	Sensitivity 95% CI
Acute (< 3 months)	72	50	0	22	69.4% (50/72) 57.5 - 79.8%	42	0	30	58.3% (42/72) 46.1 - 69.9%
Convalescent (< 12 months)	26	16	0	10	61.5% (16/26) 40.6 - 79.8%	17	1	8	69.2% (18/26) 48.2 - 85.7%
Late (> 12 months)	7	6	0	1	85.7% (6/7) 42.1 - 99.6%	5	0	2	71.4% (5/7) 29.0 – 96.3%
Total	105	72	0	33	68.6% (72/105) 58.8 - 77.3%	64	1	40	61.9% (65/105) 51.9 - 71.2%

Correlation with CDC Panel: A panel of 280 positive and negative specimens from the Centers for Disease Control and Prevention (CDC) was tested for the presence of Lyme antibodies using the BioPlex 2200 Lyme Total kit. The results are presented as a means to convey further information on the performance of the BioPlex 2200 Lyme Total kit with a masked, characterized serum panel. This does not imply an endorsement of the BioPlex 2200 Lyme Total

kit by the CDC. Results are summarized in Table 11 below.

Table 11: Testing of CDC Lyme Reference Sera

Sample Category	N (280)	BioPlex 2200 Lyme Total			
		Reactive	Equivocal	Non-Reactive	% Agreement with Clinical Diagnosis
Acute	39	33	0	6	84.6%
Convalescent	31	29	0	2	93.5%
Late	20	20	0	0	100%
Look-alike Diseases	90	1	2	87	96.7%
Healthy Controls	100	1	2	97	97.0%

The BioPlex 2200 Lyme Total kit was further evaluated using the CDC panel and a commercially available predicate device. The results are summarized in the Table 12 below:

Table 12: Comparative Testing of CDC Reference Sera

Sample Category(N=280)		N	BioPlex 2200 Lyme Total				Commercial Lyme IgM/IgG Immunoassay			
			R(+)	Eqv.	NR (-)	% Agreement	Pos (+)	Eqv.	Neg (-)	% Agreement
Early EM	**Acute**	30	24	0	6	80.0%	19	0	11	63.3%
	Convalescent	30	28	0	2	93.3%	27	0	3	90.0%
Cardiac Lyme	**Acute**	3	3	0	0	100%	3	0	0	100%
Neurological Lyme	**Acute**	6	6	0	0	100%	6	0	0	100%
	Convalescent	1	1	0	0	100%	1	0	0	100%
Lyme Arthritis/ Neuro.	**Late**	20	20	0	0	100%	20	0	0	100%
Fibromyalgia	**Look-alike Diseases**	15	0	1	14	93.3%	0	0	15	100%
Rheumatoid arthritis		15	0	0	15	100%	0	0	15	100%
Multiple sclerosis		15	0	0	15	100%	0	0	15	100%
Mononucleosis		15	0	2	13	86.7%	2	0	13	86.7%
Syphilis		15	0	0	15	100%	1	0	14	93.3%

Sample Category(N=280)		N	BioPlex 2200 Lyme Total				Commercial Lyme IgM/IgG Immunoassay			
			R(+)	Eqv.	NR (-)	% Agreement	Pos (+)	Eqv.	Neg (-)	% Agreement
Periodontitis		15	0	0	15	100%	0	0	15	100%
Endemic Negative Controls	**Healthy Normals**	50	0	2	48	96.0%	0	0	50	100%
Non-Endemic Negative Controls		50	1	0	49	98.0%	1	0	49	98.0%

R= Reactive, NR= Nonreactive

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:

Expected Values: The BioPlex 2200 Lyme Total kit was used to test 836 serum samples from apparently healthy subjects from both endemic and non-endemic regions and 792 test ordered samples from endemic regions in the U. S. The asymptomatic endemic population included 158 males ranging in age from 16 to 89 yrs. and 262 females ranging in age from 11 to 85 yrs. The asymptomatic non-endemic population included 139 males ranging in age from 3 to 91 yrs. and 275 females ranging in age from 13 to 95 yrs. with 2 samples of unknown age and gender. The test ordered endemic population included samples with de-identified patient data. The test results are presented in the following table.

Table 13: Expected Values

Population	N	BioPlex 2200 Lyme Total Qualitative Results		
		Reactive	Equivocal	Non-Reactive
Asymptomatic Non-Endemic	416	11 (2.6%)	4 (1.0%)	401 (96.4%)
Asymptomatic Endemic	420	11 (2.6%)	4 (1.0%)	405 (96.4%)
Test Ordered Endemic	792	95 (12.0%)	7 (0.9%)	690 (87.1%)

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.