BioPlex 2200 Lyme Total
510(k) Summary

Bio-Rad Laboratories hereby submits this 510(k) in accordance with the requirements of SMDA 1990 and 21 CFR 807.92. This summary of 510(k) safety and effectiveness information provides detail as a basis for a determination of substantial equivalence for the BioPlex 2200 Lyme Total kit.

510(k) Number:
K183446

Summary Preparation Date:
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Applicant:
Bio-Rad Laboratories

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

Type of Test:
Quantitative multiplexed flow immunoassay

Proprietary and Established Names:
BioPlex 2200 Lyme Total

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 (83)

Intended Use:

Intended use(s):

The BioPlex 2200 Lyme Total kit is a multiplex flow immunoassay intended for the qualitative detection of total (lgM/IgG) antibodies to *Borrelia burgdorferi* in human serum and plasma (EDTA, heparin). This assay should be used to test patients with a history and/ or symptoms of infection with *B. burgdorferi*. The BioPlex 2200 Lyme Total assay 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 IgM and IgG 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.

Device Description:
BioPlex 2200 Lyme Total kit includes the following components:

- One (1) 10 mL vial of Bead Set , containing dyed beads coated with recombinant p58, OspC type B (OspCB) and synthetic peptide FVlsE (consisting of FlaB and VlsE sequences), an Internal Standard bead (ISB) and a Serum Verification bead (SVB) in MOPS (3-[N-Morpholino] propane sulfonic acid) buffer containing bovine proteins with protein stabilizers. BND (5-bromo-5-nitro-1,3-dioxan) (≤ 0.1%), ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) are added as preservatives.
- One (1) 5 mL vial of Conjugate, containing phycoerythrin conjugated murine monoclonal anti-human IgG, and murine monoclonal anti-human IgM, and phycoerythrin conjugated murine monoclonal anti-human FXIII antibody in phosphate buffer, supplemented with murine and bovine protein stabilizers. ProClin 300 (≤ 0.3%) and sodium azide (< 0.1%) are added as preservatives.
- One (1) 10 mL vial of Sample Diluent, containing bovine and murine proteins in triethanolamine buffer. ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) are added as preservatives.

Additional materials required but not supplied include BioPlex 2200 Sheath Fluid containing Phosphate Buffered Saline (PBS) with ProClin 300 (0.03%) and sodium azide (<0.1%) added as preservatives; and the BioPlex 2200 Wash Solution containing Phosphate Buffered Saline (PBS) and Tween 20 with ProClin 300 (0.03%) and sodium azide (<0.1%) added as preservatives.

Substantial Equivalence Information:

1. <u>Predicate device name(s)</u>:
   Immunetics C6 *B. burgdorferi* (Lyme) ELISA Kit, K003754

2. <u>Comparison with predicate:</u>

Device Similarities		
Characteristics	New Device BioPlex 2200 Lyme Total	Predicate Device C6 *B. burgdorferi* (Lyme) ELISA Kit
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 assay 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 IgM and IgG 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.	The lmmunetics 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 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	Qualitative

Device Differences		
Characteristics	New Device BioPlex 2200 Lyme Total	Predicate Device Immunetics C6 *B. burgdorferi* (Lyme) ELISA Kit, K003754
Assay Technology	Automated multiplex flow competitive immunoassay	ELISA (enzyme-linked immunosorbent assay)
Antigen	Recombinant p58, OspC type B (OspCB) and synthetic peptide FVlsE	Synthetic peptide (C6 peptide)
Signal Detection	Fluorescence	Optical density readings from Spectrophotometer
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	10 µL
Sample Handling/Process	Automated	Manual
Unit of Measure	Antibody Index (AI)	Lyme Index (LI)
Sample Matrix	Serum and plasma	Serum
Instrumentation	Bio-Rad BioPlex 2200 System	None (Manual)

Test Principle: The BioPlex 2200 Lyme Total kit employs fluoromagnetic, dyed beads which are coated with recombinant p58, OspCB or synthetic peptide FVlsE. Unique fluorescent signatures identify the presence of total (IgM/IgG) 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).

Two additional dyed beads, an Internal Standard Bead (ISB) and a Serum Verification Bead (SVB), are present in each reaction mixture to verify detector response and the addition of serum or plasma to the reaction vessel respectively. Refer to the BioPlex 2200 System Operation Manual for more information.

The system is calibrated using a set of three (3) distinct calibrator vials supplied separately by Bio-Rad Laboratories. One vial contains negative sample, and the other two vials contain human *B. burgdorferi sensu stricto* antibodies, which are used for qualitative calibration of the assays. The qualitative results

are expressed in an antibody index (AI). The assay results are reported as nonreactive, equivocal or reactive.

Performance Characteristics

Non-Clinical Studies

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 AI, and subsequently evaluated for performance against clinically diagnosed samples. The statistics show that the 98th percentile cutoff 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.

Precision Study: 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.

BioPlex 2200 Lyme Total Precision

				Within Run (Repeatability)		Between Run		Between Day		Total	
Sample Type	**Sample Description**	**N**	**Mean (AI)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Serum	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 Study: 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.

BioPlex 2200 Lyme Total Reproducibility

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%

Analytical Specificity: Analytical specificity of the BioPlex 2200 Lyme Total kit was evaluated against 836 samples from asymptomatic individuals from endemic and non-endemic regions. These samples were obtained from an asymptomatic population and excluded pre-screened blood donors. The results, expressed as % negatives, are presented in the following table.

BioPlex 2200 Lyme Total Analytical Specificity

	N	BioPlex 2200 Lyme Total	Commercial Lyme IgM/IgG Immunoassay
Asymptomatic Non-Endemic	416	96.4%	95.4%
Asymptomatic Endemic	420	96.4%	94.8%

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:

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

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.

Results of Cross-Reactivity Study

Potential Cross Reactant	Negative BioPlex 2200 Results/ Negative Commercial Lyme IgM/IgG Immunoassay Results	
	N	%Negative Agreement
Anti-nuclear antibody (ANA)	13/13	100%
Babesiosis	10/10	100%
Chronic Fatigue Syndrome	14/14	100%
Cytomegalovirus	13/13	100%
Epstein-Barr Virus	45/49	92%
Ehrlichiosis	10/11	91%
HAMA	13/13	100%
Helicobacter pylori	12/12	100%
HIV	13/13	100%
Influenza virus	14/15	93%
Leptospirosis	33/34	97%
Multiple sclerosis	14/14	100%
Parvovirus B19	14/14	100%
Periodontal	34/35	97%
Rheumatoid arthritis	14/14	100%
Rheumatoid Factor positive	13/13	100%
Rickettsial diseases	16/16	100%
Rubella	14/14	100%
Syphilis	47/48	98%
Systemic Lupus Erythematosus (SLE)	15/15	100%
Toxoplasmosis	29/29	100%
Varicella Zoster Virus	14/14	100%

Matrix comparison: Matched serum and plasma (EDTA and heparin) samples drawn from the same donor were acquired through a vendor. A minimum of 40 sets of 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.

BioPlex 2200 Lyme Total Matrix Comparison

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

Clinical Studies

Method Comparison 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. Equivocal (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.

BioPlex 2200 Lyme Total 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.

Second Tier Testing and PPA Analysis

	Tier 1 + or ±	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

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.

BioPlex 2200 Lyme Sensitivity with respect to Disease Stage

Clinical Stage	N	BioPlex 2200 Lyme Total				Commercial Lyme IgM/IgG Immunoassay			
		Reactive	Equivocal	Non-Reactive	Sensitivity 95% CI	Positive	Equivocal	Negative	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%

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 the table below.

BioPlex 2200 Lyme Total Testing of CDC Reference Sera

(N=280)	N	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 CDC panel was further evaluated against the BioPlex 2200 Lyme Total kit and a commercially available predicate device. The results are summarized in the table below.

BioPlex 2200 Lyme Total 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%

Sample Category (N=280)		N	BioPlex 2200 Lyme Total				Commercial Lyme IgM/IgG Immunoassay			
			R (+)	Eqv	NR (-)	% Agreement	Pos (+)	Eqv	Neg (-)	% Agreement
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%
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

Conclusion: The submitted information in this 510(k) Premarket Notification will support a determination of substantial equivalence for BioPlex 2200 Lyme Total. The clinical and non-clinical tests demonstrate that this device is safe and effective and performs as well as the legally marketed predicate device.