510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:
k063866

B. Purpose for Submission:
Addition of Syphilis IgG assay on the BioPlex™ 2200

C. Measurand:
Syphilis IgG

D. Type of Test:
Multiplex Flow Immunoassay (multiplex fluoromagnetic bead assay)

E. Applicant:
BIO-RAD LABORATORIES

F. Proprietary and Established Names:
BioPlex™ 2200 Syphilis IgG Kit on the BioPlex™ 2200 Multi-Analyte Detection System

G. Regulatory Information:

1. Regulation section:
   CFR 866.3830 *Treponema pallidum* Treponemal test reagents

2. Classification:
   II

3. Product code:
   LIP Enzyme-linked immunoabsorption assay, *Treponema pallidum*

4. Panel:
   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The Bio-Rad Syphilis IgG kit is a multiplex flow immunoassay intended for the qualitative detection of *Treponema pallidum* IgG antibodies in human serum. This test system is also indicated for use for confirming reactive test results from non-treponemal based screening assays.

2. Indication(s) for use:

The test system, when used in conjunction with non-treponemal based assays, provides serological evidence of infection with *T. pallidum*.

3. Special conditions for use statement(s):

   For prescription use only

   The BioPlex™ 2200 Syphilis IgG Kit is not intended for use in screening blood or plasma donors.

   Warning: A positive result is not useful for establishing a diagnosis of syphilis. In most situations, such a result may reflect prior treated infection; a negative result can exclude a diagnosis of syphilis except for incubating or early primary disease.

4. Special instrument requirements:

The Syphilis IgG kit is intended for use with the Bio-Rad BioPlex™ 2200 Multi-Analyte Detection System.

I. Device Description:

The BioPlex™ 2200 System consists of the BioPlex™ 2200 Instrument, the BioPlex™ 2200 family of multiplexed assays, and the BioPlex™ 2200 software.The BioPlex™ 2200 Instrument is a fully automated, floor-standing, self contained, immunoassay analyzer for multiplex assays that can generate results on demand in less than one hour. Reactions occur on the surface of fluoromagnetic beads and all reagents needed are contained within panel specific reagent packs that are stored onboard with refrigeration. A series of control beads is evaluated in each reaction to ensure reliable results for each sample. The analyzer incorporates a dedicated software package for instrument control, data collection, results analysis, calibration, quality control, and service software.

KIT COMPONENTS

The reagent pack contains supplies sufficient for 100 tests.

1. Bead Set which contains dyed beads coated with *T. pallidum*, Internal Standard (ISB), Serum Verification (SVB), and a Reagent Blank (RBB)
2. Conjugate
3. Sample Diluent

The BioPlex™ 2200 syphilis IgG assay combines an aliquot of patient sample, sample diluent, and bead set reagent into a reaction vessel. The mixture is incubated at 37 ^{0}C. After a wash cycle, anti-human IgG antibody, conjugated to the fluorescent protein phycoerythrin, is added to the dyed beads (r15, r17, and r47) and this mixture is incubated at 37 ^{0}C. The excess is removed in another wash cycle and the beads are re-suspended in wash buffer. 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 phycoerythrin. Raw data are calculated in relative fluorescence intensity (RFI).

Three additional dyed beads, Internal Standard Bead (ISB), Serum Verification Bead (SVB), and a Reagent Blank Bead (RBB) are present in each reaction mixture to verify detector response, the addition of serum to the reaction vessel, and the absence of significant non-specific binding in serum, respectively.

The syphilis IgG assays are calibrated using a set of four calibrator vials. For the IgG assays, the calibrator sets represent different antibody concentrations and are used for qualitative calibration. Results are calculated for each of the three antibodies and are compared against their own respective cut-off. Results are expressed as an antibody index (AI). A result of ≤ 0.9 AI is considered negative, ≥ 1.1 positive, and 0.9 to 1.1 indeterminate. For specimens that are indeterminate, a patient sample can be re-collected in the future for additional testing. A single test result is reported after completing a composite analysis of all the antibodies (the highest AI value determined for the three syphilis antigens is reported and used for interpretation).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Trep-Check Anti-Treponema IgG EIA (Phoenix Bio-Tech Corp.)

2. Predicate 510(k) number(s):
   k001552

3. Comparison with predicate:

	Similarities	
between Components / Materials	**BioPlex™ 2200 Syphilis IgG Kit**	**Predicate EIA**
Intended Use	Qualitative detection of IgG antibodies to *Treponema pallidum*.	Qualitative detection of IgG antibodies to *Treponema pallidum*.
Reagents	Wash Buffer, Sample Diluent	Sample Diluent, Wash Buffer
Calibrator(s)	Calibrators	Calibrator
Controls	Negative Control and multi-analyte Positive Control	Negative Control and Positive Control
	Differences	
Solid Phase	Bead reagent - dyed antigen coated beads.	96 well microplate – antigen coated microwells.
Reagents	Conjugate: Anti-human IgG / Phycoerythrin.	Conjugate: goat anti-human IgG / horseradish peroxidase,

		Substrate (TMB), Stop Solution.
Sheath Fluid	Sheath Fluid is used to suspend the bead reagent and introduce it into the detector.	Not similar; not utilized in EIA's.
Analyte Detection	Multi-analyte detection of human IgG antibodies to *Treponema pallidum*.	Detection of human IgG antibodies to *Treponema pallidum*.
Matrices	Serum.	Serum and Plasma.

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

Guidance

Review Criteria for In Vitro Diagnostic Devices for Detection of IGM Antibodies to Viral Agents http://www.fda.gov/cdrh/ode/527.pdf

Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff
http://www.fda.gov/cdrh/oivd/guidance/1588.html

L. Test Principle:

BioPlex™ 2200 immunoassays use heterogeneous (multiplex) sets of magnetic beads to simultaneously detect multiple analytes. The beads are infused with varying ratios of two fluorescent classification dyes, creating unique bead sets. Beads within each set are coated with a ligand (i.e., antigen, antibody, analyte, etc.) specific to a particular assay, allowing the capture and detection of specific analytes from a sample. Target analytes are captured on bead surfaces and probed with a corresponding fluorescent conjugate. With excitation and emission spectra distinct from those of the classification dyes used to identify analyte and control beads, the conjugate serves as the "reporter" fluorescence signal. Three additional dyed beads, an Internal Standard Bead (ISB), a Serum Verification Bead (SVG), and a Reagent Blank Bead (RBB) are present in each reaction mixture to verify detector response, the addition of serum to the reaction vessel, and the absence of significant non-specific binding in serum.

After the reaction, bead fluorescence is measured by the instrument in a manner similar to the way fluorescently-labeled cells are evaluated in a standard flow cytometer. The beads flow single file through a flow cell in a sheath of fluid to be interrogated by two lasers. As each bead travels through the interrogation zone, light scatter and fluorescence are measured. Signals from one laser are used to determine bead classification (specific analyte or control), and signals from the other laser are used to measure reporter fluorescence (sample reactivity). System software converts the reporter signal to a Relative Fluorescence Intensity (RFI) value, which is then converted to a fluorescence ratio (FR) using the inherent reporter fluorescence of the Internal Standard Bead (ISB). Fluorescence Ratio is compared to an assay-specific calibration curve to

determine analyte concentration in antibody index (AI) or other appropriate units, or to score the result qualitatively as reactive, equivocal, or non-reactive.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility testing was performed at three US testing facilities on a total of three (3) lots of the Syphilis IgG kit, three (3) lots of the Syphilis IgG Calibrator Set, and three (3) lots of the Syphilis IgG Control Set. The panel was tested in duplicate (x2) on two (2) runs per day for three (3) days using one (1) lot of BioPlex™ 2200 Syphilis IgG Reagent Pack and one (1) lot of Syphilis IgG Calibrator Set (2 times x 2 runs x 3 days x 3 sites = 36 replicates per panel member and controls). The data were analyzed for intra-assay and inter-assay reproducibility and results as shown in the table below.

The reproducibility panel consisted of seven (7) panel members for each of the three (3) recombinant proteins associated with *T. pallidum* (15kD, 17kD, and 47kD); two high positive, two positive antibody levels near the cutoff, two high negative, and one low negative for each analyte. Additionally, one positive control for 15kD and 47kD, one positive control for 17kD, and a negative control (antibody negative for all analytes) were also tested.

Table V. Reproducibility; BioPlex 2200 Syphilis IgG

Syphilis IgG Panel Members		Sample N	Grand Mean AI	Within-Run		Between-Run		Between-Day		Between-Site*		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
T. pallidum recombinant 15kD	High Positive 1	36	3.0	0.1	3.3%	0.1	4.4%	0.1	2.3%	0.3	10.4%	0.4	12.0%
	High Positive 2	36	3.3	0.1	2.2%	0.1	2.4%	0.2	4.7%	0.2	7.2%	0.3	9.2%
	Low Positive 1	36	1.4	0.0	3.0%	0.1	4.7%	0.1	10.1%	0.2	15.5%	0.3	19.3%
	Low Positive 2	36	1.3	0.0	2.2%	0.0	3.6%	0.0	3.1%	0.2	14.0%	0.2	15.0%
	Positive Control	36	2.2	0.1	2.9%	0.1	2.2%	0.0	0.0%	0.5	23.3%	0.5	23.6%
T. pallidum recombinant 17kD	High Positive 1	36	3.2	0.1	3.1%	0.2	4.8%	0.0	0.0%	0.3	7.9%	0.3	9.7%
	High Positive 2	36	2.8	0.1	2.8%	0.1	4.2%	0.1	2.5%	0.2	8.2%	0.3	9.9%
	Low Positive 1	36	1.3	0.0	3.7%	0.0	1.9%	0.1	5.5%	0.1	6.2%	0.1	9.3%
	Low Positive 2	36	1.3	0.0	2.5%	0.0	3.7%	0.1	6.0%	0.1	5.6%	0.1	9.4%
	Positive Control	36	2.3	0.1	3.2%	0.0	1.5%	0.1	3.5%	0.3	13.3%	0.3	14.2%
T. pallidum recombinant 47kD	High Positive 1	36	3.8	0.1	2.3%	0.1	1.9%	0.2	5.2%	0.3	7.8%	0.4	9.9%
	High Positive 2	36	3.6	0.1	2.4%	0.1	2.3%	0.0	0.0%	0.3	7.5%	0.3	8.2%
	Low Positive 1	36	1.2	0.0	3.8%	0.0	2.4%	0.1	5.6%	0.1	11.1%	0.2	13.2%
	Low Positive 2	36	1.3	0.0	2.9%	0.0	3.2%	0.1	4.1%	0.2	11.9%	0.2	13.3%
	Positive Control	36	2.3	0.1	4.0%	0.0	0.0%	0.0	0.0%	0.5	19.5%	0.5	19.9%

**Between site variance includes between lot variance.*

Precision Study

Precision testing was performed at one site using one lot of the Syphilis IgG kit, one lot of the Syphilis IgG Calibrator Set and one lot of the Syphilis IgG Control Set. The panel was tested in duplicate (x2) on two (2) runs per day for twenty (20) days using one (1) lot of Syphilis IgG kit, one (1) lot of Syphilis IgG Calibrator Set and one (1) lot of Syphilis IgG Control Set (2 times x 2 runs x 20 days = 80 replicates per panel member). The data were analyzed for intra-assay and inter-assay precision as shown in the tables below.

The precision panel consisted of nine (9) panel members for each of the three (3) recombinant proteins associated with *T. pallidum* (15kD, 17kD and 47kD) where in two (2) had high reactive levels, two (2) had low reactive levels, and two (2) had antibody levels near the cut-off. Additionally, there were two (2) high negative panel members and one (1) low negative panel member for each analyte.

Table W. Precision Results; BioPlex 2200 Syphilis IgG 15kD

Syphilis 15kD Panel Members	Sample N	Mean	Within-Run		Between-Day		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	80	0.0	0.02	N/A	0.00	N/A	0.00	N/A	0.02	N/A
High Negative 1	80	0.5	0.01	2.3%	0.03	6.6%	0.03	6.6%	0.04	8.3%
High Negative 2	80	0.5	0.02	4.0%	0.03	6.7%	0.00	0.0%	0.05	9.6%
Near Cut-off 1	80	1.0	0.04	3.5%	0.07	7.0%	0.03	3.1%	0.09	8.4%
Near Cut-off 2	80	1.0	0.04	3.8%	0.06	6.5%	0.03	3.3%	0.08	7.8%
Low Positive 1	80	1.4	0.05	3.9%	0.12	8.6%	0.05	4.0%	0.14	10.4%
Low Positive 2	80	1.3	0.05	3.5%	0.10	7.9%	0.05	4.1%	0.13	9.6%
High Positive 1	80	3.1	0.11	3.7%	0.22	7.3%	0.11	3.6%	0.27	9.0%
High Positive 2	80	3.4	0.07	2.1%	0.17	5.0%	0.18	5.2%	0.26	7.6%

N/A = No precision specification established for samples below cut-off.

Table X. Precision Results; BioPlex 2200 Syphilis IgG 17kD

Syphilis 17kD Panel Members	Sample N	Mean	Within-Run		Between-Day		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	80	0.0	0.00	N/A	0.00	N/A	0.00	N/A	0.00	N/A
High Negative 1	80	0.5	0.04	7.0%	0.03	6.0%	0.03	6.0%	0.06	10.8%
High Negative 2	80	0.5	0.03	6.0%	0.04	8.4%	0.03	6.0%	0.06	11.5%
Near Cut-off 1	80	1.0	0.03	3.3%	0.07	6.9%	0.03	3.1%	0.08	7.9%
Near Cut-off 2	80	1.0	0.03	3.1%	0.05	5.3%	0.04	4.3%	0.08	7.3%
Low Positive 1	80	1.3	0.06	4.3%	0.09	7.5%	0.04	3.5%	0.12	9.4%
Low Positive 2	80	1.3	0.04	3.3%	0.08	6.3%	0.03	2.4%	0.10	7.4%
High Positive 1	80	3.2	0.11	3.3%	0.26	8.2%	0.08	2.4%	0.30	9.1%
High Positive 2	80	2.9	0.07	2.5%	0.20	7.1%	0.09	3.1%	0.23	8.2%

N/A = No precision specification established for samples below cut-off.

Table Y. Precision Results; BioPlex 2200 Syphilis IgG 47kD

Syphilis 47kD Panel Members	Sample N	Mean	Within-Run		Between-Day		Between-Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	80	0.0	0.00	N/A	0.00	N/A	0.00	N/A	0.00	N/A
High Negative 1	80	0.6	0.01	1.9%	0.03	5.4%	0.00	0.0%	0.04	5.9%
High Negative 2	80	0.6	0.03	5.2%	0.00	0.0%	0.03	5.1%	0.05	7.4%
Near Cut-off 1	80	1.1	0.03	2.4%	0.04	4.3%	0.03	3.0%	0.06	5.7%
Near Cut-off 2	80	1.1	0.04	3.1%	0.05	4.8%	0.03	2.8%	0.07	6.4%
Low Positive 1	80	1.2	0.05	4.1%	0.08	6.8%	0.03	2.6%	0.10	8.5%
Low Positive 2	80	1.4	0.05	3.4%	0.09	6.5%	0.07	5.2%	0.12	8.8%
High Positive 1	80	3.4	0.06	1.8%	0.21	6.2%	0.08	2.5%	0.23	7.0%
High Positive 2	80	3.8	0.06	1.7%	0.18	4.7%	0.14	3.9%	0.24	6.3%

N/A = No precision specification established for samples below cut-off.

b. Linearity/assay reportable range:

High dose effect was not evaluated as it is not relevant to two step indirect heterogenous assay formats.

However, an internal dilution linearity study was conducted using 5 high analyte positive patient samples. These high samples were initially diluted in negative human serum to bring within the assay range. Each dilution was tested a minimum of four times. The results of this study demonstrated that all dilutions recovered were within the acceptance criteria. The data demonstrated that the BioPlex™ 2200 Syphilis IgG has an acceptable linear range between 0.2 to 8.0 AI. Under and over-range results would be reported as <0.2 AI, and >8.0 AI.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

QC was performed each day of testing with the following results.

BioPlex Syphilis Assay	Control	Contol Lot AI Range			Total AI Range	Site 1		Site 2		Site 3		Grand Total	
		SGN11	SGN20	SGN30		Mean	Total CV%	Mean	Total CV%	Mean	Total CV%	Mean	Total CV%
r15 IgG	Negative	<0.6	<0.6	<0.6	<0.6	0.1	N/A	0.1	N/A	0.1	N/A	0.1	N/A
	Positive	1.4 - 2.6	1.7 - 3.3	1.3 - 2.5	1.3 - 3.3	2.2	17.2%	2.5	21.3%	2.3	14.3%	2.4	18.7%
r17 IgG	Negative	<0.6	<0.6	<0.6	<0.6	0.1	N/A	0.2	N/A	0.1	N/A	0.1	N/A
	Positive	1.5 - 2.7	1.7 - 3.1	1.5 - 2.9	1.5 - 3.1	2.2	12.1%	2.4	11.4%	2.5	8.3%	2.4	11.4%
r47 IgG	Negative	<0.6	<0.6	<0.6	<0.6	0.0	N/C	0.0	N/C	0.0	N/C	0.0	N/C
	Positive	1.4 - 2.6	1.7 - 3.1	1.5 - 2.7	1.4 - 3.1	2.2	15.5%	2.5	16.3%	2.4	13.8%	2.4	15.5%

N/A: Not applicable
N/C: Not calculable

On-Board Calibration stability study was performed using one lot. After initial calibration of newly opened reagent lot, calibrators and controls were tested as samples up to 35 days. A 30-day calibration stability is claimed in the insert.

Stability Studies

An **Open Vial Stability** study was conducted where control and calibrator vials were stored at 2-8°C and tested for 72 days. The study was carried out to mimic customer handling of open controls and calibrators. The results of the study support the 60-day open vial claim.

Accelerated Stability Testing: These studies were performed on pilot lots at elevated temperatures in order to observe changes in product performance more rapidly than would be seen under normal storage conditions.

To support the product's 18-month shelf life at 2-8°C, accelerated stability studies were conducted for the calibrators and controls by incubating each vial for multiple time points at 25°C, 35°C, and 41°C. Unopened vials were incubated at each temperature for the times indicated in the stability procedure to predict the shelf life stability. The data predicts stability for all analytes to be greater than 18 months when stored at 2-8°C.

d. Detection limit:

This is a qualitative assay (Reactive, Non-Reactive, or Equivocal Results) so there is no limit of detection.

e. Analytical specificity:

Separate interfering substances and cross reactivity studies were conducted internally to evaluate the potential interference of specific endogenous and exogenous substances and to determine whether or not cross-reactivity occurs in the presence of non-Syphilis IgG antibodies with the BioPlex™ 2200 Syphilis IgG kit.

Interfering Substances

For the interfering substances study, human serum samples that were previously characterized as positive for the various Syphilis IgG markers at concentrations near the middle of the assay range were spiked with various interfering substances. A control was made for each interfering substance by spiking the pooled sera with the solvent or solution used to make the interfering substance spiking solution. The test sample and control were run in replicates of 10 using one reagent lot. No significant interference was observed in any of the endogenous or exogenous substances tested.

Results are summarized in the table below.

Endogenous Substances	Exogenous Substances
Hemoglobin ≤ 500mg/dL	Ascorbic Acid ≤ 3mg/dL
Bilirubin (unconjugated) ≤ 20mg/dL	Sodium Citrate ≤ 1000 mg/dL
Bilirubin (Conjugated) ≤ 20mg/dL	EDTA ≤ 800 mg/dL
Triglycerides ≤ 3000mg/dL	Lithium Heparin ≤ 8000 mg/dL
Protein (total) ≤ 12g/dL	Sodium Heparin ≤ 8000mg/dL
Beta-Carotene ≤ 0.6mg/dL	
Cholesterol ≤ 500mg/dL	
Red Blood Cells ≤ 0.4% Concentration	
Gamma-globulin ≤ 5g/dL	

Cross Reactivity

A cross-reactivity study was performed to determine if samples from various disease states and other potentially interfering factors interfere with test results when tested with the BioPlex™ 2200 Syphilis IgG kit. A panel of ten (10) specimens positive for each cross reactant were evaluated for possible cross reactivity with the BioPlex™ 2200 Syphilis IgG kit. The test specimens were also evaluated by TPPA, and where BioPlex™ 2200 Syphilis IgG results did not agree with TPPA, samples were further tested by RPR and a commercially available EIA. The results demonstrated that the various disease state samples evaluated do not cross react with the BioPlex™ 2200 Syphilis IgG kit. Most of the samples evaluated were high positive for each disease state. The majority of all samples that elicited a positive result were also confirmed positive by another treponemal antibody test (TPPA), indicating reactivity to Syphilis (*T. pallidum*) IgG antibodies rather than cross reactivity. Results are summarized in the table below.

Table Z. Cross-Reactivity

Cross Reactives	N	Method	Syphilis IgG
ANA	10	BioPlex 2200	3
		TPPA	2
		Discrepants	1
dsDNA	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
HCV	10	BioPlex 2200	3
		TPPA	3
		Discrepants	0
E. Coli	4*,†††	BioPlex 2200	0
		TPPA	N/A
		Discrepants	0
HSV-1 IgG	10	BioPlex 2200	1
		TPPA	0
		Discrepants	1
Lyme IgG Borrelia burgdorferi	10	BioPlex 2200	1
		TPPA	1
		Discrepants	0
VZV IgG	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
Hyper-gamma-globulinemia	10	BioPlex 2200	2
		TPPA	1
		Discrepants	1
Leptospirosis	7	BioPlex 2200	2
		Predicate	2
		Discrepants	0

Cross Reactives	N	Method	Syphilis IgG
Rheumatoid Factor	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
CMV IgG	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
HIV	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
HTLV	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
Pregnant women	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
HSV-2 IgG	10	BioPlex 2200	1
		TPPA	1
		Discrepants	0
Lyme IgG Afzelli / Garni	49**,†	BioPlex 2200	1
		TPPA	1††
		Discrepants	0
EBV IgG	10	BioPlex 2200	0
		TPPA	0
		Discrepants	0
Small Pox	10††	BioPlex 2200	0
		TPPA	N/A
		Discrepants	0

* *Due to limited availability of samples, only four E. coli specimens were evaluated.*
** *Additional samples were tested to evaluate the various lyme strains.*
† *Only positive samples were tested by the predicate.*
†† *Sample was negative by TPPA but positive by both RPR and EIA*
††† *Testing was only performed on the BioPlex 2200 Syphilis IgG assay.*

Sample Probe and Reagent Probe Carryover Studies were performed with no observable effect.

f. Assay cut-off:

The BioPlex™ 2200 Syphilis IgG assay displays results as an antibody index (AI) and reports the highest value based on the three assay beads.

A final cut-off of 1.2 was established for all Syphilis IgG assays based on an evaluation of 735 serum samples. The population included 521 Syphilis IgG negative samples of which 312 were from pregnant women. The remaining samples were positive by one or more Syphilis analytes. All samples were characterized by commercially available methods. ROC analysis was performed for each analyte using this population of samples. For the purpose of establishing a cut-off, samples that were equivocal on the commercially available methods were not used

during ROC analysis or in evaluating the concordance statistics. The BioPlex™ 2200 Syphilis cut-off was adjusted slightly to reduce the number of false positive samples. Using this cut-off, Syphilis IgG exhibited negative and positive agreement of 98.5% and 100%. Calibrators will be re-assigned prior to product introduction so that the final cut-off is 1.0.

2. Comparison studies:

a. Method comparison with predicate device:

Clinical study consisted of 1,750 serum samples evaluated at 3 US sites using the BioPlex™ 2200. Three lots of BioPlex™ 2200 Syphilis IgG Reagent Packs, three lots of calibrator sets and three lots of control sets were used in the evaluation as stated in the submission. Approximately one-third of each sample population was tested on one of the three BioPlex™ 2200 Syphilis IgG lots. Each lot was calibrated using the appropriately matched calibrator.

All serum samples evaluated for concordance were tested with reference nontreponemal (RPR) and treponemal (TPPA) assays. Samples that were positive by both RPR and TPPA were reference assay positive. Samples that were negative by both RPR and TPPA were reference assay negative. Samples with discordant RPR and TPPA results were further tested with a treponemal IgG EIA to determine the final reference assays result. However, samples with BioPlex™ results that differ from RPR/TPPA results were not further tested unless a suspected error has occurred.

The following populations were tested.

1. Five hundred (500) purchased banked serum samples from pregnant women:

These frozen serum samples were purchased from a New York vendor. One sample was excluded because it did not meet the eligibility criteria for pregnancy. Two samples with a BioPlex™ 2200 analysis error message which did not resolve on repeat testing were excluded from the data analysis. Therefore, only four hundred ninety seven samples are represented.

Results are summarized below.

Table G. Unselected Serum Samples From Pregnant Women (N=497)

		BioPlex 2200 Syphilis IgG							
		Reactive	Equivocal	Nonreactive	Total	Positive (+) % Agreement	95% Confidence Interval	Negative (-) % Agreement	95% Confidence Interval
Reference Assays Result	Positive	5	0	0	5	100% (5/5)	56.5 - 100%	99.8% (491/492)	98.9 - 100.%
	Equivocal	0	0	0	0				
	Negative	1*	0	491	492				
	Total	6	0	491	497				

* *One (1) sample with a BioPlex 2200 reactive result was RPR and TPPA nonreactive.*

2. Five hundred (500) banked serum samples from patients with a syphilis test ordered:

These frozen serum samples were collected at a hospital in CA. Two hundred twenty seven (227) samples were from females, and 273 were from males. The age range for this population was from 3 – 86 years. Ethnicity information was also included in submission.

Table F. Serum Samples From Patients Who Had a Syphilis Test Ordered (N=500)

		BioPlex 2200 Syphilis IgG							
		Reactive	Equivocal	Nonreactive	Total	Positive (+) % Agreement	95% Confidence Interval	Negative (-) % Agreement	95% Confidence Interval
Reference Assays Result	Positive	46	0	0	46	100% (46/46)	92.0 - 100%	93.8% (426/454)	91.2 - 95.7%
	Equivocal	3*	0	0	3				
	Negative	22**	3	426	451				
	Total	71	3	426	500				

* *Three (3) BioPlex 2200 Syphilis IgG reactive samples were RPR nonreactive, TPPA reactive and treponemal EIA equivocal.*

** *Of 22 samples with reactive results on the BioPlex 2200 Syphilis IgG assay, 16 were RPR and TPPA nonreactive, and 6 samples were RPR reactive, TPPA nonreactive and treponemal EIA negative.*

3. Two hundred-fifty (250) banked purchased serum samples requested to be RPR/TPPA reactive:

These frozen serum samples were purchased from a New York vendor.

The results from testing with the reference assays and BioPlex™ 2200 are presented below.

Table H. Serum Samples Requested to be RPR and TPPA Reactive (N=250)

		BioPlex 2200 Syphilis IgG					
		Reactive	Equivocal	Nonreactive	Total	Positive (+) % Agreement	95% Confidence Interval
Reference Assays Result	Positive	249	0	0	249	100% (249/249)	98.5 - 100%
	Equivocal	0	0	0	0		
	Negative	0	0	1	1		
	Total	249	0	1	250		

4. Four hundred thirty nine (439) purchased banked samples from pregnant women; 189 requested to be treponemal assay positive and 250 requested to be RPR/TPPA non-reactive:

These frozen serum samples were purchased from a vendor in New York. Four of the 189 samples requested to be treponemal assay positive were excluded because they did not meet eligibility criteria; three did not meet the eligibility criteria for pregnancy, and one did not meet eligibility criteria for positive treponemal assay result. Additionally, two of the 189 samples requested to be treponemal assay positive were excluded because they had BioPlex™ 2200 analysis error message. Therefore a total of 183 samples requested to be treponemal assay positive, and 250 samples requested to be RPR/TPPA non-reactive were evaluated. Results are summarized below.

Table I. Serum Samples From Pregnant Women Requested to be Treponemal Assay Positive (N=183)

		BioPlex 2200 Syphilis IgG					
		Reactive	Equivocal	Nonreactive	Total	Positive (+) % Agreement	95% Confidence Interval
Reference Assays Result	Positive	166	0	0	166	100% (166/166)	97.7 -100%
	Equivocal	4*	0	0	4		
	Negative	8**	0	5	13		
	Total	178	0	5	183		

* *Four (4) BioPlex 2200 Syphilis IgG reactive samples were RPR nonreactive, TPPA reactive and treponemal EIA equivocal.*

** *Of the eight (8) BioPlex 2200 Syphilis IgG reactive samples, 1 sample was RPR and TPPA nonreactive and 7 were RPR reactive, TPPA nonreactive and treponemal EIA negative.*

Table J. Serum Samples From Pregnant Women Requested to be RPR/TPPA Nonreactive (N=250)

		BioPlex 2200 Syphilis IgG					
		Reactive	Equivocal	Nonreactive	Total	Negative (+) % Agreement	95% Confidence Interval
Reference Assays Result	Positive	0	0	0	0	98.8% (247/250)	96.5 -99.6%
	Equivocal	0	0	0	0		
	Negative	3*	0	247	250		
	Total	3	0	247	250		

* *Three (3) BioPlex 2200 Syphilis IgG reactive samples were RPR and TPPA nonreactive.*

5. <u>One hundred (100) purchased propectively collected banked serum samples from treated or untreated patients with primary or secondary syphilis infections:</u>

These serum samples were collected with informed consent at 2 different sites in FL, and one site in TX and were purchased by the device manufacturer. Four samples were excluded due to informed consent or enrollment issues. Thirteen samples were excluded because they did not meet eligibility criteria after source data verification. Three samples were excluded because eligibility could not be located. Seventy (70) of the samples could be verified with a medical diagnosis but primary or secondary syphilis could not be verified, and/or collection date of the samples did not coincide with the syphilis diagnosis date. Therefore, these samples were analyzed for concordance with the reference assays. Of the 100 samples collected; ten (10) samples were verified as treated or untreated primary or secondary syphilis.

Table L. Serum Samples From Patients Medically Diagnosed With Syphilis (N=70)

		BioPlex 2200 Syphilis IgG					
		Reactive	Equivocal	Nonreactive	Total	Positive (+) % Agreement	95% Confidence Interval
Reference Assays Result	Positive	63	0	0	63	100.0% (63/63)	94.2 - 100%
	Equivocal	1*	0	0	1		
	Negative	3**	0	3	6		
	Total	67	0	3	70		

* *One (1) sample with BioPlex 2200 Syphilis IgG reactive results was RPR nonreactive, TPPA reactive and treponemal EIA equivocal.*
** *Three (3) samples with BioPlex 2200 Syphilis IgG reactive results were RPR and TPPA nonreactive.*

6. <u>Two hundred twenty (220) Banked Known HIV-1 Positive Serum samples</u>

Performance of the BioPlex™ 2200 Syphilis IgG was evaluated in two hundred (220) banked known HIV-1. Results were compared to reference assay results for RPR/TPPA and if applicable EIA. Using reference assays

samples that were positive by both RPR and TPPA were reference assay positive. Samples that were negative by both RPR and TPPA were reference assay negative. Samples with discordant RPR and TPPA results were further tested with a treponemal IgG EIA to determine the final reference assay results. Results are summarized in the table below.

Table 4
BioPlex 2200 Syphilis IgG vs. Reference Assay Results
Banked Known HIV- I Positive Serum Samples
N=220

Reference Assays Result	BioPlex 2200 Syphilis IgG							
	Reactive	Equivocal	Non-reactive	Total	% Pos(+) Agreement	95% Confidence Interval	% Neg(-) Agreement	95% Confidence Interval
Positive	39	0	0	39	100% (39/39)	91.0 -100%	68.0% (123/181)	60.8 – 74.3%
Equivocal	4[a]	0	0	4				
Negative	35[b]	19[c]	123	177				
Total	78	19	123	220				

[a] Four (4) BioPlex 2200 Syphilis IgG reactive samples were RPR non-reactive, TPPA reactive, and treponemal EIA equivocal .

[b] Of 35 BioPlex 2200 Syphilis IgG reactive samples, ten (10) were RPR non-reactive, TPPA reactive and treponemal EIA negative; and 25 samples were RPR and TPPA non-reactive.

[c] Nineteen (19) BioPlex 2200 Syphilis IgG equivocal samples were RPR and TPPA non-reactive.

b. Matrix comparison:

Human serum is the only sample type utilized by the BioPlex™ 2200 Syphilis IgG kit assays.

3. Clinical studies:

CDC panel of one hundred forty-three (143) well-characterized serum samples from treated and untreated patients with primary, secondary, and latent syphilis infections:

The performance of the BioPlex™ 2200 Syphilis IgG kit was evaluated with a well characterized CDC panel of one hundred forty-three samples from treated or untreated patients with primary, secondary, or latent syphilis infection. Three (3) samples from this population had a BioPlex™ 2200 analysis error message. These samples were excluded from the data analysis. Results for the 140 samples by known clinical status are presented below.

The CDC samples were also tested with reference assay tests for RPR and TPPA and if applicable EIA. Samples that are positive by both RPR and TPPA were reference assay positive. Samples that were negative by both RPR and TPPA were reference assay positive. Samples that were negative by both RPR and TPPA were reference assay negative. Samples with discordant RPR and TPPA results further tested with a treponemal IgG EIA to determine the reference assay result. Results are summarized below.

Table 34
Reference Assay Results
CDC Panel of Characterized Serum Samples
N = 140

Known Clinical Status		RPR	TPPA	EIA	N	Reference Assays Result	Total N	% Clinical Sensitivity	95% Confidence Interval
Untreated	Primary	Reactive(+)	Reactive(+)	Not Tested	10	Positive	10	83.3%(10/12)	55.2 - 100%
		Reactive(+)	Non-reactive (-)	Neg (-)	1	Negative	2		
		Non-reactive (-)	Non-reactive (-)	Not Tested	1				
	Secondary	Reactive(+)	Reactive(+)	Not Tested	10	Positive	10	100% (10/10)	72.2 - 100%
	Latent	Reactive(+)	Reactive(+)	Not Tested	8	Positive	8	61.5%(8/13)	35.5 - 82.3%
		Reactive(+)	Non-reactive (-)	Neg (-)	5	Negative	5		
Treated	Primary	Reactive(+)	Reactive(+)	Not Tested	15	Positive	15	93.8%(15/16)	71.6 - 98.9%
		Reactive(+)	Non-reactive (-)	Neg (-)	1	Negative	1		
	Secondary	Reactive(+)	Reactive(+)	Not Tested	36	Positive	36	100% (36/36)	90.3 - 100%
	Latent	Reactive(+)	Reactive(+)	Not Tested	48	Positive	49	92.5%(49/53)	82.1 - 97.0%
		Non-reactive (-)	Reactive(+)	Pos (+)	1				
		Reactive(+)	Non-reactive (-)	Neg (-)	4	Negative	4		
						Total	140	91.4%(128/140)	85.6 - 95.0%

The performance of the Syphilis IgG kit was further evaluated at one clinical testing site using banked prospectively collected serum samples from treated and untreated patients with primary and secondary syphilis infections (N=10). Performance of the Syphilis IgG kit in the 10 samples was compared to a corresponding composite result using commercially available (RPR, TPPA and if required treponemal EIA) kits by known clinical status. Results are summarized in the table below.

Table Q. Bio-Rad Syphilis IgG vs. Prospectively Collected Serum Samples With Known Clinical Status (N=10)

Known Clinical Status		N	BioPlex 2200 Syphilis IgG					Reference Assays Result				
			Reactive	Equivocal	Nonreactive	Clinical Sensitivity %	95% Confidence Interval	Positive	Equivocal	Negative	Clinical Sensitivity %	95% Confidence Interval
Untreated	Primary	1	1	0	0	100% (1/1)	20.6 - 100%	0	0	1	0.0% (0/1)	N/A*
	Secondary	0	0	0	0	N/A*	N/A*	0	0	0	N/A*	N/A*
Treated	Primary	2	2	0	0	100% (2/2)	34.2 - 100%	2	0	0	100% (2/2)	34.2 - 100%
	Secondary	7	7	0	0	100% (7/7)	64.5 - 100%	7	0	0	100% (7/7)	64.5 - 100%
Total		10	10	0	0	100% (10/10)	72.2 - 100%	9	0	1	90.0% (9/10)	59.5 - 98.2%

* *In cases where agreement resulted in a numerator of zero (0), 95% confidence interval could not be calculated; in cases where agreement resulted in (0/0) samples, percent agreement and 95% confidence interval could not be calculated.*

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

c. *Other clinical supportive data (when a. and b. are not applicable):*

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Results are calculated for each of the three (3) antibodies, compared against its respective cut-off, and are expressed as an antibody index (AI). A single result is reported after completing a composite analysis of all the antibodies (the highest AI value is reported). Results of ≤0.8 AI are nonreactive, 0.9 and 1.0 AI are equivocal, and ≥1.1 AI are reported as reactive.

Antibody Index	Result
≤ 0.8 AI	Nonreactive
0.9 AI, 1.0 AI	Equivocal
≥ 1.1 AI	Reactive

For specimens that are equivocal, the patient sample can be re-collected for additional testing. In conjunction with these results, the immune status of patients should be evaluated based on their clinical status, related risk factors, and other diagnostic test results. Initially, reactive results should be supplemented with a non-treponemal test to distinguish between present and past infection. An additional sample should be obtained (one drawn at a later date) if the results of non-treponemal and treponemal tests do not establish a diagnosis.

The following table can be used for additional interpretation of results:

Table A. BioPlex 2200 Syphilis IgG and non-treponemal Interpretation

BioPlex 2200 Syphilis IgG Result	Non-treponemal Result (RPR)	Interpretation
Nonreactive	Nonreactive	No serological evidence of infection with *T. Pallidum* (incubating or early syphilis cannot be excluded).
Nonreactive	Reactive	Current infection unlikely, most likely a biological false positive to other secondary medical conditions.[1] Recommend repeating both samples by other test method (treponemal and non-treponemal).
Reactive	Nonreactive	Probable past infection. Possible cross-reactivity with other spirochetes/related antigens. Recommend additional testing consistent with clinical history/findings.[2]
Reactive	Reactive	Presumptive evidence of current infection. Possible inadequately treated infection, persistent infection, re-infection, or biological false positive if prior infection. Recommend additional testing consistent with clinical history/findings.[2]
Reactive	Not performed	Current infection unlikely. Cannot exclude incubating or early syphilis.

1) *Febrile diseases, Immunizations, IVDU, Autoimmune diseases, etc.*
2) *Quantitative non-treponemal testing; repeat (sequential) serological test for changes in titer; immunocompromised individuals may have delayed sero-reactivity or negative serology.*

N. Instrument Name:

BioPlex™ 2200 Multi-Analyte Detection System

O. System Descriptions:

1. Modes of Operation:

   The BioPlex™ 2200 is a fully-automated, random access, multiplex testing platform.

2. Software:

   FDA has reviewed applicants Hazard Analysis and software development processes for this line of product types:

   Yes __X_____ or No _____

   The BioPlex™ 2200 instrument incorporates a dedicated software package for instrument control, data collection, results analysis, calibration, quality control, as well as service software. BioPlex™ 2200 System Software version 1.0 was previously reviewed by FDA under the original submission (k041658). The Device Hazard Analysis was reviewed by FDA under that submission. As indicated in the Product Classification section, BioPlex™ 2200 System Software is now at version 2.0.

3. Specimen Identification:

   Samples are identified on the BioPlex™ 2200 instrument by barcodes on each sample

tube, corresponding to the sample's accession identification number.

4. Specimen Sampling and Handling:

   The Instructions For Use for the BioPlex™ 2200 Syphilis IgG kit instructs the user to thoroughly mix thawed specimens before testing and also recommends that users centrifuge specimens prior to loading specimens on the instrument. As described in BioPlex™ 2200 Operation Manual, the instrument samples specimens directly from open sample tubes.

5. Calibration:

BioPlex™ 2200 Syphilis IgG Calibrator Set:

4 vials – 0.5 mL each	Human Serum made from defibrinated plasma containing antibodies to *Treponema pallidum* (15kD, 17kD, and 47kD) Preservatives: 0.3% ProClin® 300

The BioPlex™ 2200 Syphilis IgG Calibrator Set is intended for the calibration of the BioPlex™ 2200 Syphilis IgG Reagent Pack.

The Syphilis IgG Calibrator Set should be loaded and assayed at minimum in duplicate every 30 days or with each new lot of BioPlex™ 2200 Syphilis IgG Reagent Pack. They are loaded before control or patient samples are processed. A BioPlex™ 2200 Syphilis IgG Calibrator Lot Data CD-ROM is required to load the necessary value assignment data into the instrument. The Calibrator Lot Data CD-ROM has an XML file that contains calibrator value assignment data and describes all the properties for the calibrator lot that are needed to perform calibrations. All the vials have a pre-affixed barcode, identifying them as calibrators. The barcode contains the level and lot number of the calibrator.

A calibration event occurs when a calibrator set is processed for an active reagent lot. All reagent lots must have successful calibration events to be used for sample analysis. When calibration is performed, all reportable analytes for a given reagent lot are calibrated simultaneously. Therefore, all analytes in a kit become available for analysis upon calibration.

6. Quality Control:

   Reagent Bead Quality Control

   There are three quality control beads in every BioPlex™ 2200 assay. These beads serve to evaluate samples as they are processed and report any control issues as they occur and are described below:

   Reagent Blank Bead (RBB): The Reagent Blank Bead is a non-antigen coated bead that serves to identify sample problems arising from non-specific binding.

Internal Standard Bead (ISB): The Internal Standard Bead performs fluorescence intensity data-correction. The inherent fluorescent signal of the Internal Standard Bead is used to detect and compensate for Detector Module fluctuations that may occur during sample analysis.

Serum Verification Bead (SVB): The Serum Verification Bead ensures that serum or plasma is present.

In-Kit Quality Control

BioPlex™ 2200 Syphilis IgG Control Set

NEGATIVE CONTROL 2 vials – 1.5 mL each	Human Serum made from defibrinated plasma negative for antibodies to *Treponema pallidum* Preservatives: 0.3% ProClin® 300
POSITIVE CONTROL 4 vials – 1.5 mL each	Human Serum made from defibrinated plasma containing antibodies to *T. pallidum* 17kD, and containing antibodies to *T. pallidum* 15kD and 47kD Preservatives: 0.3% ProClin® 300

The BioPlex™ 2200 Syphilis IgG Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex™ 2200 Instrument and BioPlex™ 2200 Syphilis IgG Reagent Pack in the clinical laboratory. The performance of the BioPlex™ 2200 Syphilis IgG Control Set has not been established with any other Syphilis assays.

The recommended minimum frequency for performing quality control is once every 24-hour testing period. The vials can be directly loaded into the instrument sample racks and processed with the Syphilis IgG Reagent Pack in the same manner as patient samples. All the vials have a pre-affixed barcode, a unique lot number and a QC prefix identifying it as a quality control sample.

Performing quality control is also necessary after each new assay calibration and certain service procedures. Quality control procedures are performed by first informing the BioPlex™ 2200 system software of the provided lot specific control values, either manually or by CD-ROM, and then loading and processing the quality control samples in the same manner as patient samples.

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

Q. Conclusion:

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