510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K170413

B. Purpose for Submission:
The purpose of this submission is to show that the BioPlex 2200 Syphilis Total & RPR Kit (for use on the Bio-Rad BioPlex 2200 System) is substantially equivalent to the LIAISON Treponema Assay and BD Macro-Vue RPR Card Test, respectively and to obtain clearance for the BioPlex 2200 Syphilis Total & RPR Kit.

C. Measurand:
Serum antibodies (IgM and IgG) to *Treponema pallidum* (*T. pallidum*) and antibodies (cardiolipin and lecithin) to non-treponemal plasma reagin

D. Type of Test:
The BioPlex 2200 Syphilis Total & RPR Kit is a multiplex flow immunoassay. Both assays are performed using the same procedure, but with beads that will bind either IgG and IgM antibodies to *Treponema pallidum* or antibodies to plasma reagin.

E. Applicant:
Bio-Rad Laboratories

F. Proprietary and Established Names:
BioPlex 2200 Syphilis Total & RPR
BioPlex 2200 Syphilis Total & RPR Calibrator
BioPlex 2200 Syphilis Total & RPR Control

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.3830 – Treponema pallidum treponemal test reagents
   21 CFR §866.3820 – Treponema pallidum nontreponemal test reagents
   21 CFR §862.1150 – Calibrator
   21 CFR §862.1660 – Quality Control Material (assayed and unassayed)

2. Classification:
   Class II

3. Product code:
   LIP, Enzyme Linked Immunoabsorption Assay, *Treponema Pallidum*
   GMQ, Antigens, Nontreponemal, All

JIT, Calibrator, Secondary
JJX, Single (specified) Analyte Controls (Assayed and Unassayed)

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   The BioPlex Syphilis Total & RPR kit is a multiplex flow immunoassay intended for the qualitative detection of total (IgG/IgM) antibodies to *Treponema pallidum* and the qualitative detection and/or titer determination of non-treponemal reagin antibodies in human serum or plasma. The Syphilis Total or RPR assays may be used to supplement a previously determined reactive treponemal or non-treponemal test. The test system should be used in conjunction with other laboratory tests and clinical findings to aid in the diagnosis of syphilis infection.

   The BioPlex 2200 Syphilis Total assay is not intended for use in screening blood or plasma donors.

   The BioPlex 2200 Syphilis Total & RPR kit is intended for use with the Bio-Rad BioPlex 2200 System.

   The BioPlex 2200 Syphilis Total & RPR Calibrator Set is intended for the calibration of the BioPlex 2200 Syphilis Total & RPR Reagent Pack.

   The BioPlex 2200 Syphilis Total & RPR Control Set is intended for use as an assayed quality control to monitor the performance of the BioPlex 2200 Instrument and BioPlex 2200 Syphilis Total & RPR assay in the clinical laboratory. The performance of the BioPlex 2200 Syphilis Total & RPR Control Set has not been established with any other Syphilis Total & RPR assays.

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

3. Special conditions for use statement(s):
   For Prescription Use Only

4. Special instrument requirements:
   Bio-Rad BioPlex 2200 System

I. Device Description:
BioPlex 2200 Syphilis Total & RPR kit includes the following components:

- One (1) 10 mL vial, containing dyed beads coated with recombinant Syphilis rTP47/rTP17 fusion protein, a cardiolipin antigen, an Internal Standard Bead (ISB) and a Serum Verification Bead (SVB) in MOPS (3-[N-Morpholino] propanesulfonic acid) buffer containing bovine proteins with protein stabilizers. ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) are added as preservatives.
- One (1) 5 mL vial, 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%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) are added as preservatives.
- One (1) 10 mL vial, containing bovine and murine protein stabilizers in MOPS (3-[N-Morpholino] propanesulfonic acid) buffer. ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) are added as preservatives,

BioPlex 2200 Syphilis Total & RPR Calibrator Set: Four (4) 0.5 mL vials, containing T. pallidum and reagin antibodies in a human serum matrix made from defibrinated plasma, and one (1) 0.5 mL vial containing human serum matrix made from defibrinated plasma for a total of five (5) calibrator vials. All calibrators contain ProClin 300 (≤ 0.3%), sodium benzoate (< 0.1%) and sodium azide (< 0.1%) as preservatives,

BioPlex 2200 Syphilis Total & RPR Control Set: Two sets of three (3) control vials. Each set contains two (2) 1.5 mL Positive Control vials with antibodies to T. pallidum and reagin in a human serum matrix made from defibrinated plasma and one (1) 1.5 mL vial of Negative Control in a human serum matrix made from defibrinated plasma. ProClin 300 (≤ 0.3%) sodium benzoate (< 0.1%) and sodium azide (< 0.1%) are added as preservatives for all controls.

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

J. Substantial Equivalence Information:

1. Predicate device name(s):
   LIAISON Treponema Assay
   BD Macro-Vue RPR Card Tests

2. Predicate 510(k) number(s):
   K061247
   Pre-amendment prior to May 28, 1976

3. Comparison with predicate:

Treponemal Device Similarities		
Characteristics	New Device BioPlex 2200 Syphilis Total & RPR Kit	Predicate Device LIAISON Treponema Assay, K061247
Intended Use	Multiplex flow immunoassay intended for the qualitative detection of Total (IgG/IgM) antibodies to *Treponema pallidum* in human serum or plasma	Chemiluminescent immunoassay intended for the qualitative determination of total antibodies directed against *Treponema pallidum* in human serum
Indications for Use	Used in conjunction with other serological tests and clinical findings to aid in the diagnosis of syphilis infection.	Same
Measured Analyte	Total antibodies (IgG/IgM) to *T. pallidum*	Same
Assay Type	Qualitative	Same
Solid Phase	Antigen-coated paramagnetic microbeads	Antigen coated magnetic particles
Cut-off Index	1.0 Antibody Index (AI)	Index 1.0
Equivocal Zone	0.9 – 1.0	0.9 - 1.1
Standardization	The calibrator is referenced to an internal reference material.	The calibrator concentrations are referenced to an in-house antibody preparation
Controls	2 (Negative and Positive)	Same

Treponemal Device Differences		
Characteristics	New Device BioPlex 2200 Syphilis Total & RPR Kit	Predicate Device LIAISON Treponema Assay, K061247
Assay Technology	Automated multiplex flow immunoassay	Sandwich chemiluminescence immunoassay (CLIA)
Antigen	Recombinant fusion TP antigen: rTP17/rTP47	DNA-Tp17 Recombinant antigen
Conjugate	Phycoerythrin conjugated murine monoclonal anti-human IgG and murine monoclonal anti-human IgM	Conjugated to an Isoluminol derivative
Signal Detection	Fluorescence	Chemiluminescent
Sample Matrix	Serum or Plasma	Serum

Treponemal Device Differences		
Characteristics	New Device BioPlex 2200 Syphilis Total & RPR Kit	Predicate Device LIAISON Treponema Assay, k061247
Calibrator(s)	4 calibrator levels (sold separately)	Two positive calibrators
Open Pack Stability	60 days	4 weeks
Reagent Pack Calibration Frequency	Every 30 days	Every 14 days
Instrumentation	Bio-Rad BioPlex 2200 System	DiaSorin LIAISON Analyzer

Non-Treponemal Device Similarities		
Characteristics	New Device BioPlex 2200 Syphilis Total & RPR Kit	Predicate Device BD Macro-Vue RPR CARD TEST, Pre-amendment
Intended Use	Multiplex flow immunoassay intended for the qualitative detection of non- *Treponema pallidum* reagin antibodies in human serum or plasma	A non-treponemal testing procedure for the serological detection of syphilis in human serum or plasma
Measured Analyte	Non-*Treponema pallidum* reagin antibodies	Same
Antigen	Cardiolipin/lecithin/ cholesterol	Same
Sample matrix	Serum or plasma	Same

Non-Treponemal Device Differences		
Characteristics	New Device BioPlex 2200 Syphilis Total & RPR Kit	Predicate Device BD Macro-Vue RPR CARD TEST, Pre-amendment
Assay Technology	Automated multiplex flow immunoassay	Macroscopic flocculation
Solid phase	Antigen-coated paramagnetic microbeads	Antigen carbon particle suspension
Conjugate	Phycoerythrin conjugated murine monoclonal anti-human IgG and murine monoclonal anti-human IgM	None
Calibrator(s)	2 levels – negative and positive	None
Control(s)	2 (Negative and Positive)	3 (Negative and 2 Positive)

Non-Treponemal Device Differences		
Characteristics	New Device BioPlex 2200 Syphilis Total & RPR Kit	Predicate Device BD Macro-Vue RPR CARD TEST, Pre-amendment
Standardization	The calibrator is referenced to internal reference material	None
Cut- off Index	1.0 Antibody Index (AI)	None
Signal Detection	Fluorescence	Flocculation by naked eye
Reagent Pack Calibration Frequency	Every 30 days	None
Instrumentation	Bio-Rad BioPlex 2200 System	Card Test (Manual)

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

EP05-A3, Evaluation of Precision of Quantitative Measurement Methods; Approved Guideline, Third Edition

EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition

EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Third Edition (For Matrix Comparison only)

EP12-A2 – User Protocol for Evaluation of Qualitative Test Performance – Second Edition

EP15-A3, User Verification of Precision and Estimate of Bias, Approved Guideline, Third Edition

EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

L. Test Principle:

The BioPlex 2200 Syphilis Total & RPR kit employs *Treponema pallidum* fusion protein (rTP47/rTP17) and cardiolipin antigen-coated fluoromagnetic beads with unique fluorescent signatures to identify the presence of IgG and IgM antibodies to *Treponema pallidum* and reagin in a two-step assay format.

Dyed beads are coated with recombinant *T. pallidum* rTP47/rTP17 fusion protein or cardiolipin antigen.

The BioPlex 2200 System combines an aliquot of patient sample, sample diluent, and bead reagent into a reaction vessel. The mixture is incubated at 37ºC. 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 37ºC. The excess conjugate is removed in a 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

signature of the dyes, and the amount of antibody captured by the antigen is determined by the fluorescence intensity 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. Refer to the BioPlex 2200 System Operation Manual for more information.

The system is calibrated using a set of five (5) distinct calibrator vials, supplied separately by Bio-Rad Laboratories. One vial containing negative sample, and four vials containing human *Treponema pallidum* or human reagin antibodies, are used for qualitative calibration of assays. The results are expressed in antibody index (AI). The Syphilis Total treponemal assay results are reported as nonreactive (≤ 0.8 AI), equivocal (0.9, 1.0 AI) or reactive (≥ 1.1 AI); while the RPR assay results are reported as nonreactive (< 1.0 AI) or reactive (≥ 1.0 AI).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision
   Precision testing of the BioPlex 2200 Syphilis Total & RPR kit on the BioPlex 2200 instrument was performed in accordance with CLSI EP05-A3 guideline. A human serum panel consisting of 6 frozen samples spanning the measuring range was assayed in duplicate per run on two runs daily over 20 days (N=80) on one reagent lot. Two levels of the BioPlex Syphilis Total & RPR controls were also included. The analyte concentrations (target antibody index range (AI)) of the samples used are described in the table below.

Serum Panel	Syphilis Total, AI	RPR, AI
Low Negative	0.2 to 0.5	0.2 to 0.5
High Negative	0.6 to 0.8	0.6 to 0.8
Near Cut-Off	0.8 to1.2	0.8 to1.2
Low Positive	1.2 to1.5	1.2 to1.5
Medium Positive	1.6 to 2.9	1.6 to 2.9
High Positive	3.0 to 5.0	3.0 to 5.0
Assay Parameters		
Non-Reactive Range	0.2 to 0.8	0.2 to 0.8
Equivocal Zone	0.9 to1.0	N.A.
Clinical Cut-Off	1.0	1.0

The data from both the BioPlex Syphilis Total and BioPlex RPR tests were analyzed for within-run, between-run, between-day, and total precision and the mean (AI), standard deviation (AI) and percent coefficient of variation (%CV) are summarized below:

BioPlex 2200 SyphilisTotal – Precision

Serum Panel	N	Mean AI	Within Run		Between Run		Between Day		Total Precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Negative	80	0.3	0.03	10.4%	0.00	0.0%	0.03	8.4%	0.04	13.4%
High Negative	80	0.5	0.04	6.5%	0.02	3.5%	0.03	5.6%	0.05	9.3%
Near Cut-Off	80	0.9	0.03	3.3%	0.02	2.2%	0.02	2.7%	0.04	4.8%
Low Positive	80	1.5	0.06	3.7%	0.02	1.0%	0.12	7.8%	0.13	8.7%
Medium Positive	80	1.8	0.08	4.3%	0.00	0.0%	0.19	10.3%	0.20	11.2%
High Positive	80	3.2	0.08	2.5%	0.04	1.4%	0.09	2.9%	0.13	4.1%
Positive Control	80	2.6	0.13	4.7%	0.01	0.4%	0.05	1.8%	0.13	5.1%

BioPlex 2200 RPR – Precision

Serum Panel	N	Mean AI	Within Run		Between Run		Between Day		Total Precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Negative	80	0.2	0.03	11.2%	0.02	6.5%	0.04	16.2%	0.05	20.7%
High Negative	80	0.7	0.04	6.6%	0.06	9.0%	0.05	7.3%	0.09	13.4%
Near Cut-Off	80	0.9	0.04	4.2%	0.06	6.5%	0.05	5.1%	0.09	9.3%
Low Positive	80	1.0	0.05	4.7%	0.08	7.5%	0.08	7.8%	0.12	11.8%
Medium Positive	80	1.9	0.10	5.2%	0.10	5.3%	0.08	4.2%	0.16	8.6%
High Positive	80	3.4	0.09	2.6%	0.16	4.8%	0.12	3.5%	0.22	6.5%
Positive Control	80	2.0	0.05	2.4%	0.11	5.4%	0.08	4.0%	0.14	7.1%

Reproducibility

The reproducibility was also evaluated in accordance with CLSI EP15-A3 guideline "User Verification of Precision and Estimation of Bias, Third Edition".

Reproducibility testing was performed at each of three (3) US testing facilities using a single lot of the BioPlex 2200 Syphilis Total & RPR reagent. A serum panel consisting of 5 samples spanning the measuring range were assayed in 4 replicates per run, two runs per day over 5 days (4 reps x 2 runs x 5 days x 3 sites = 120 total data points per sample). The QC Controls were also included.

Serum Panel	Target AI Range
Negative	0.2 to 0.8
Near Cut-Off	0.9 to1.1
Low Positive	1.1 to1.5
Medium Positive	1.8 to 3.0
High Positive	3.0 to 5.0

The data for both the BioPlex Syphilis Total and BioPlex RPR tests were analyzed for within-run, between run, between day, between site/instrument and total precision and the mean AI, standard deviation and percent coefficient of variation (%CV) are summarized below:

BioPlex 2200 Syphilis Total - Reproducibility

Syphilis Total			Within Run		Between Run		Between Day		Between Site/Instrument		Total	
Sample	N	Mean (AI)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	120	0.5	0.02	5.1%	0.02	4.1%	0.00	0.9%	0.08	16.9%	0.08	18.2%
Sample 2	120	1.0	0.04	3.9%	0.02	1.6%	0.02	1.9%	0.08	7.5%	0.09	8.8%
Sample 3	120	1.5	0.05	3.5%	0.00	0.0%	0.02	1.5%	0.08	5.5%	0.10	6.7%
Sample 4	120	2.2	0.08	3.4%	0.03	1.5%	0.03	1.1%	0.01	0.5%	0.09	3.9%
Sample 5	120	6.8	0.22	3.2%	0.11	1.7%	0.17	2.6%	0.00	0.0%	0.30	4.4%
Positive Control	119	2.7	0.15	5.7%	0.07	2.7%	0.00	0.0%	0.08	3.1%	0.19	7.0%

BioPlex 2200 RPR - Reproducibility

RPR			Within Run		Between Run		Between Day		Between Site/Instrument		Total	
Sample	N	Mean (AI)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	120	0.8	0.05	5.9%	0.01	1.7%	0.04	5.4%	0.03	4.0%	0.07	9.1%
Sample 2	120	1.0	0.05	4.7%	0.01	0.9%	0.04	3.4%	0.04	3.5%	0.07	6.8%
Sample 3	120	2.0	0.08	4.0%	0.09	4.5%	0.09	4.7%	0.21	10.7%	0.26	13.2%
Sample 4	120	3.0	0.08	2.8%	0.10	3.2%	0.07	2.3%	0.07	2.4%	0.16	5.4%
Sample 5	120	7.4	0.26	3.6%	0.27	3.6%	0.24	3.3%	0.23	3.2%	0.51	6.9%
Positive Control	120	2.7	0.10	3.5%	0.04	1.5%	0.05	1.9%	0.05	1.9%	0.13	4.7%

RPR Titer On-board Dilution Reproducibility

The BioPlex 2200 System has a feature for the determination of an end point RPR titer result. All reactive RPR samples can be diluted on board at 1:4, 1:8, 1:16, 1:32, and 1:64. Four reactive RPR samples as well as negative and positive controls were selected to evaluate the titer precision were tested in two runs per day in duplicate per run for 5 days for a total of 20 data points. Samples with a titer within ±1 doubling dilution were considered acceptable. The results are summarized below.

Sample Reactivity	End Point Titer Results								% Agreement within ± 1 titer (95% CI)
	Non-Reactive	Neat	<1:4	1:4	1:8	1:16	1:32	>1:64	
Negative Control	20	0	0	0	0	0	0	0	100% (83.9 – 100%)
Positive Control	0	20	20	0	0	0	0	0	100% (83.9 – 100%)
Low Reactive (1:8)	0	0	0	1	19	0	0	0	100% (83.9 – 100%)
Moderately Reactive (1:16)	0	0	0	0	0	20	0	0	100% (83.9 – 100%)
High Reactive (>1:64)	0	0	0	0	0	0	0	20	100% (83.9 – 100%)
High Reactive (>1:64)	0	0	0	0	0	0	0	20	100% (83.9 – 100%)

The results demonstrate that the RPR titer dilution feature of the assay is reliably repeatable.

b. Linearity/assay reportable range:

Not applicable; this is a qualitative assay

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

Traceability:
Calibrator assignment is established for the matched lot of BioPlex 2200 Syphilis Total & RPR kit using the internal reference standards. For each calibrator level, three vials are tested in replicates of five on three BioPlex 2200 analyzers for a total of 45 data points. The mean values obtained for each kit calibrator level are verified and must fall within specified acceptable range as shown below.

Calibrator Set	Assay	Range (AI)
Vial 1	Syphilis Total and RPR	0.0 – 0.2
Vial 2	Syphilis Total	1.4 – 2.2
Vial 3	Syphilis Total	2.8 – 4.2
Vial 4	Syphilis Total	4.8 – 7.2
Vial 5	RPR	1.4 – 2.6

Calibrator
The BioPlex Syphilis Total & RPR Calibrators are traceable to an internal standard. The internal reference standard is manufactured by spiking high positive plasma into non-reactive immunodepleted human plasma. The internal reference calibrators are frozen at ≤ -80ºC.

The BioPlex Syphilis Total assay is calibrated using a set of four levels of distinct serum based calibrators whereas the BioPlex RPR assay is calibrated with a set of two levels of calibrators.

Controls

The BioPlex Syphilis & RPR Control Set contains one negative control, one positive control for Syphilis Total and one positive control for RPR. Positive controls are made from the human serum of infected individuals containing anti-*T. pallidum* and reagin antibodies. The control set is provided in a human serum matrix stabilized with ≤ 0.3% ProClin 300, ≤0.1% sodium benzoate and <0.1% sodium azide.

For each control level, three vials are tested in multiple replicates using multiple reagent lots on three BioPlex 2200 analyzers for a minimum of 45 replicates per reagent lot. The minimum number of replicates for each control level is 90 when two reagent lots are used and 135 when three reagent lots are used. For each control level, the mean values were derived from replicate analyses and should fall within the corresponding ranges as shown below.

The manufacturing target ranges of the Control Sets are listed below.

Control Set	Range (AI)
Negative Control	0.0 – 0.5
Syphilis Total Positive Control	1.8 – 3.8
RPR Positive Control	1.8 – 3.8

Stability:

Stability studies have been performed to support the following claims:

Sample Stability:

The stability of samples for Syphilis Total and RPR testing was evaluated for the following sample types: serum, dipotassium EDTA (K_2EDTA) plasma, and lithium heparin plasma. Four evaluation sets were generated by pooling at least 5 individual non-reactive samples for each sample type. The samples were then spiked to generate the following test samples.

Antibody Levels	Syphilis Total Antibody Index	RPR Antibody Index	Number of samples generated
High Non-Reactive	0.6 to 0.8	0.6 to 0.8	3
Near Cut-Off	0.8 to 1.2	0.8 to 1.2	5
Medium Reactive	1.6 to 2.9	1.6 to 2.9	3
High Reactive	3.0 to 8.0	3.0 to 10.0	3

For each condition listed below multiple aliquots of each sample was prepared and stored at the test condition. At the time of submission testing was only performed up to 6 months, testing will continue and the package insert will be modified to include additional time points that pass the acceptance criteria .

- Refrigerated stability (at 2-8°C) ≤ 15 days
  - Time points tested: Day 0, Day 4, Day 8, Day 12 and Day 15
- Room temperature stability (at 25° +/- 2°C) ≤ 3 days
  - Time points tested: Day 0, Day 1, Day 2 and Day 3
- Frozen stability (at ≤ -20° +/- 10°C) ≤ 12 months
  - Time points tested: Day 0, Month 2, Month 4, Month 6, Month 8, Month 10, Month 12
- Frozen stability (at ≤ -80° +/- 10°C) ≤ 12 months
  - Time points tested: Day 0, Month 12
- Freeze-thaw stability (at ≤ -20° +/- 10°C)
  - Conditions tested:1, 2, 3, 4 and 5 freeze thaw cycles
- Freeze-thaw stability (at ≤ -80° +/- 10°C)
  - Conditions tested: 1, 2, 3, 4 and 5 freeze thaw cycles

Each sample was tested with 5 replicates at each time point. The evaluation of results was performed separately for each analyte, concentration level, storage condition, and time point.

The acceptance criteria were defined as the antibody index for Syphilis Total ±15% from the Day 0 result and ±20% for the RPR result.

The results supported sample stability as shown:

Sample Type	Stability at 25°	Stability at -20°	Freeze/Thaw cycles at -20°	Freeze/Thaw cycles at -80°
Serum	3 days	6 months	5	5
K_2EDTA	3 days	6 months	5	5
Lithium Heparin plasma	3 days	6 months	5	5

Calibrator and Control:
The following are the stability claims for the calibrator and control sets for theBioPlex 2200 Syphilis Total & RPR:

- Calibrator set and Controls are stable for 60 days after opening the vial the first time when stored at 2 to 8°C.
- Onboard Calibration Curve Stability study showed that controls and samples can be measured accurately using a stored calibration curve for up to 30 days;
- Calibrators and Controls Real Time Stability study demonstrated stability for upt to 24 months at 2 to 8°C, the date of expiration may increase based on continuing stability studies;
- Calibrators and Controls Accelerated Stability study predicted 2 years stability at 25ºC.

- Calibrators and controls are stable for 5 freeze-thaw cycles when frozen at -20ºC and -80ºC.

Kit Stability:
The following are the stability claims for the BioPlex 2200 Syphilis Total & RPR Kit:

- Real Time (unopened) Kit Stability study demonstrated 24 month stability when stored unopened on the instrument or at 2 to 8°C, the date of expiration may increase based on continuing stability studie.
- Additionaly stability studies demonstrated stability of up to 60 days at room temperature or 2 to 8°C for an open kit.

Carryover:
This study was designed to look for potential carryover of sample when using the BioPlex 2200 instrument probe. High positive Syphilis Total and RPR samples were identified and chosen for this study. Testing was performed by having the probe sample 100uL (maximum probe dispensing volume) of high positive sample, immediately prior to running five replicates of low negative samples with 10uL aspiration volume. This study was conducted with one set of reagents on three separate instruments.

Carryover was defined as a change in antibody index of <0.1000. No significant carryover was observed for either the Syphilis Total or RPR assays.

High dose hook effect:
Not Applicable due to a two-step indirect immunoassay with a wash cycle.

IgM Detection:
An IgM depletion study was performed to demonstrate that the Syphilis Total RPR assay can detect IgM antibodies specifically. In this study three different detection reagent systems were used: (1) the original assay which contains anti-IgG and anti-IgM, (2) IgG only assay which contains only anti-IgG, and (3) a IgM only assay which contains only anti-IgM detection reagents. Fourteen known positive clinical samples were tested in this study. Each clinical sample was split and one aliquot was incubated with dithiothreitol to deplete IgM antibodies. Depletion was confirmed and the testing was performed.

The results demonstrated that there was a loss of RPR assay signal, when using the assay with only anti-IgM reagent after IgM depletion (by dithiothreitol). This test design demonstrates that the anti-IgM reagent in the BioPlex 2200 Syphilis Total RPR assay is specific for IgM antibodies.

A second study was performed testing a nine-member syphilis sero-convesion panel. It is well established that the IgM response to infection is detectable before the IgG response. Therefore testing of a serial bleed sero-conversion panel by the BioPlex 2200 Syphils Total assay should be positive at the same bleed as a IgM specific syphilis diagnostic assay. For this study each panel member was tested with the BioPlex 2200 Syphilis Total assay as well as syphilis IgM and IgG specific assays that are commercially available. The results below demonstrate that the BioPlex 2200 Syphilis Total assay was positive

when the sample was only positive in the IgM specific commercially available assay. This demonstrates that the BioPlex 2200 Syphilis Total assay is specific for IgM antibodies.

Sero-Conversion Panel Sample Test Results

Syphilis Sero-Conversion Panel	Syphilis Assay					
	Days Since 1st Bleed	BioPlex2200 Syphilis Total	Syph IgMonly Assay	Syph IgGonly Assay	Treponemal Assay	TPPA
PSS901-01	0	<0.2	0.42	0.15	Neg	Neg
PSS901-02	5	<0.2	0.45	0.12	Neg	Neg
PSS901-03	10	<0.2	0.41	0.12	Neg	Neg
PSS901-04	13	<0.2	0.40	0.13	Neg	Neg
PSS901-05	31	1.3	0.68	0.16	Neg	Neg
PSS901-06	45	6.6	1.17	0.50	POS	POS
PSS901-07	48	>8.0	1.26	0.80	POS	POS
PSS901-08	52	>8.0	1.51	0.98	POS	POS
PSS901-09	59	>8.0	1.87	1.32	POS	POS

d. Detection limit:

Not applicable

e. Analytical specificity:

Interfering Substances

An interfering substances study was conducted to evaluate the potential interference of specific endogenous and exogenous substances with the BioPlex 2200 Syphilis Total & RPR kit according to CLSI EP7-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:

Substance	Concentration
Hemoglobin	≤500 mg/dL
Bilirubin (unconjugated)	≤20 mg/dL
Bilirubin (conjugated)	≤30 mg/dL
Triglycerides	≤3300 mg/dL
Total Protein	≤12 g/dL
Cholesterol	≤450 mg/dL
Ascorbic Acid	≤3 mg/dL
Heparin Sodium	<8000 units/dL
EDTA	<800 mg/dL

Cross-Reactivity:

A study was conducted to determine if samples from various cross reactants interfered with test results when tested with the BioPlex 2200 Syphilis Total & RPR kit. A panel of at least ten (10) specimens positive for each potential cross reactant was evaluated for possible cross reactivity with the BioPlex 2200 Syphilis Total & RPR kit for Syphilis Total and RPR assays.

The majority of the samples evaluated were high positive (2x cut-off) for each cross reactant. The potential cross reactant samples were tested with commercially available predicate kits in order to confirm the negative status for the analytes intended to be measured. Cross reactivity, expressed as percent negative agreement is calculated by the ratio of the number of negative results to the total number of samples assayed for each cross reactant sample set. The results of each potential cross reactant are listed below:

Cross Reactant	Number Tested	BioPlex Syphilis Total % Negative Agreement	BioPlex RPR % Negative Agreement
Anti-HBs	12	100.0%	100.0%
Anti-Cardiolipin IgG	10	90.0% (9/10)	100.0%
Anti-Cardiolipin IgM	14	100.0%	100.0%
Anti-Cardiolipin IgA	10	100.0%	90.0% (9/10)
Anti-nuclear antibody (ANA)	10	100.0%	100.0%
B.burgdorferi IgG (US Strain)	10	100.0%	100.0%
B.burgdorferi IgG (EU Strain)	10	100.0%	100.0%
B.burgdorferi IgM (EU Strain)	12	100.0%	100.0%
Cytomegalovirus (anti-CMV IgG positive)	11	100.0%	100.0%
Cytomegalovirus (anti-CMV IgM positive)	12	100.0%	100.0%
E. coli	12	100.0%	100.0%
Epstein-Barr Virus (EBV IgG positive)	13	100.0%	100.0%
Epstein-Barr Virus (EBV IgM positive)	12	100.0%	100.0%
HBsAg	13	100.0%	100.0%
HCV	13	100.0%	100.0%
HIV	10	100.0%	100.0%
HSV (anti-HSV-2 IgG positive)	16	100.0%	100.0%
Hyper gamma-globulinemia IgG	10	100.0%	100.0%

Cross Reactant	Number Tested	BioPlex Syphilis Total % Negative Agreement	BioPlex RPR % Negative Agreement
Hyper gamma-globulinemia IgM	13	100.0%	100.0%
Leptospirosis	12	100.0%	100.0%
Pregnancy	17	100.0%	100.0%
Rheumatoid Factor (RF)	13	100.0%	100.0%
Rubella IgG	11	100.0%	100.0%
Rubella IgM	12	100.0%	100.0%
Systemic Lupus Erythematosus (SLE)	12	100.0%	91.7% (11/12)
Toxoplasma IgG positive	10	100.0%	100.0%
Toxoplasma IgM positive	10	100.0%	100.0%
Varicella Zoster Virus (anti-VZV IgG positive)	12	100.0%	100.0%
Total	332	99.7% (331/332)	99.4 %(330/332)

Testing demonstrated no cross reactivity except for Systemic Lupus Erythematosus (SLE) and Anti-Cardiolipin IgA in the RPR assay. A limitation will be added to the package insert to inform users of the potential for false positive results in Systemic Lupus Erythematosus (SLE) patients. No specific limitation regarding the Anti-Cardiolipin IgA cross-reactivity was added because this is detection of an antibody actually generated during syphilis infection and because there is no standard test for Anti-Cardiolipin IgA. It is not possible to know which patients would be cross-reactive if there were a specific Anti-Cardiolipin IgA limitation. The general limitation that restuls should be considered with other laboratory findings adequately covers the demonstrated cross-reactivity with Anti-Cardiolipin IgA.

f. Assay cut-off:

The cutoff values were established in the feasibility phase of BioPlex 2200 Syphilis Total & RPR assay development using native human samples from apparently healthy subjects, patients sent to the laboratory for syphilis testing and patients diagnosed with syphilis infection. Based on the Receiver Operating Characteristics (ROC) analysis using predicate results as standard, calibrator values were adjusted such that the cut-off value was equal to 1.0 AI.

To further confirm the cutoff value the BioPlex Syphilis Total and RPR results were compared to those from the commercially available assays for agreement. Subsequently, clinical studies conducted at three sites in the US provided the final validation of the cut-off value.

For the Syphilis Total assay an equivocal range of ±10% was then set around the cut-off value. Thus, results above or equal to 1.1 AI are considered reactive and results below 0.9

AI are considered nonreactive. The 10% range was established based on the precision of the assay.

For RPR assay, no equivocal range was set around the cut-off value. Thus results ≤0.9 are considered non-reactive and results ≥1.0 are considered reactive.

2. Comparison studies:

a. Method comparison with predicate device:
The results of the BioPlex 2200 Syphilis Total & RPR assay were compared to a composite comparator and an FDA cleared RPR assay, respectively. For additional details on the composite comparator please see the "Clinical Studies" section below.

b. Matrix comparison:
Five different sample types were evaluated for use with the BioPlex 2200 Syphilis Total & RPR assay:

- Serum
- K_2 EDTA Plasma
- K_3 EDTA Plasma
- Lithium heparain Plasma
- Sodium heparin Plasma

Matched serum and plasma sample sets were obtained from 113 doors and screeened for non-reactivity with both assays. Non-reactive samples were then spiked with high reactive Syphilis Total or RPR samples to generate samples with concentrations that span the assay range.

Serum Panel	**Syphilis Total, AI**	**RPR, AI**
Low Negative	0.2 to 0.5	0.2 to 0.5
High Negative	0.6 to 0.8	0.6 to 0.8
Near Cut-Off	0.8 to1.2	0.8 to1.2
Low Positive	1.2 to1.5	1.2 to1.5
Medium Positive	1.6 to 2.9	1.6 to 2.9
High Positive	3.0 to 5.0	3.0 to 10.0

Sample Comparison	# Samples for Syphilis Total Testing	Sample Comparison	# of Samples for RPR Testing
K_2 EDTA Plasma vs. Serum	83	K_2 EDTA Plasma vs. Serum	112
K_3 EDTA Plasma vs. Serum	82	K_3 EDTA Plasma vs. Serum	113
Lithium heparain	89	Lithium heparain	113

Plasma vs. Serum		Plasma vs. Serum	
Sodium heparin Plasma vs. Serum	89	Sodium heparin Plasma vs. Serum	113

A regression analysis was performed on the antibody index value of each test result to look for significant differences between the matrices compared to serum. No differences were found.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Clinical Studies

Clincical performance was evaluated at three sites from November to December 2016 using a total of 2008 prospective and retrospective samples. Samples were obtained from multiple commercial suppliers who collected samples in the following geographical areas (all prospective samples were collected in the US):

- US : 90.7%
  - 23.6% Northeast (Maryland, Massachusetts, New York, Pennsylvania)
  - 18.7% Southwest (California, Hawaii, New Mexico)
  - 28.4% Southeast (Florida, Georgia)
  - 9.2% Midwest
  - 10.8% Unknown

- Outside US: 9.3%
  - 3.5% Argentina
  - 3.2% France/Europe
  - 1.4% China
  - 1.2% Others

The samples tested included 1001 prospective samples, 546 retrospective samples, 160 clinically diagnosed syphilis patients and 301 apparently healthy subjects. Six samples generated no values due to repeat instrument error.

Performance with Prospectively Collected Samples

Prospective samples were purchased by Bio-Rad from multiple vendors. Each vendor prospectively collected samples per Bio-Rad's requisition order stating all comers arriving into the laboratory with a physician's request for Syphilis or RPR testing be collected and included in the clinical study regardless of testing results. Samples were collected in the following regions: Southern California, New Mexico, Florida, and Massachusetts. Testing was conducted at three study sites in California (one in house and two locations representative of intended use sites for the BioPlex 2200 System). Serum samples were collected, de-identified (only information on patient age, gender, and HIV or pregnancy status was made available), frozen, and sent to the laboratories for testing.

One thousand and one (1001) prospectively collected serum samples were tested, 401 samples were from subjects who had a physican order for Syphilis and/or RPR testing, 295 pregnant women, and 305 HIV positive patients. Patients were aged 7 years to 96 years with 586 females (58.5%) and 415 males (41.5%).

The serum samples were tested with the BioPlex 2200 Syphilis Total assay and compared to the result of a reference comparator algorithim. The comparator algorithm consists of three tests; a treponemal IgG/IgM assay, a non-treponemal assay and a second non-treponemal TP-PA assay. A final comparator result is determined from the three tests in the algorithm and used in the estimation of positive and negative percent agreement. The table below shows the comparator algorithm for treponemal syphilis assays used in this clinical study.

Comparator Algorithm for Treponemal Syphilis Assays

Treponemal IgG/IgM (Predicate)	Non-treponemal (Predicate)	2nd Treponemal (TP-PA) (Predicate)	Final Comparator Result
Negative	Non-reactive	Reactive	**Negative**
		Non-reactive	**Negative**
		Inconclusive	**Negative**
Negative	Reactive	Reactive	**Positive**
		Non-reactive	**Negative**
		Inconclusive	**Negative**
Positive	Reactive	Reactive	**Positive**
		Non-reactive	**Positive**
		Inconclusive	**Positive**
Positive	Non-reactive	Reactive	**Positive**
		Non-reactive	**Negative**
		Inconclusive	**Positive**
Equivocal	Non-reactive	Reactive	**Positive**
		Non-reactive	**Negative**
		Inconclusive	**Indeterminate**
Equivocal	Reactive	Reactive	**Positive**
		Non-reactive	**Negative**
		Inconclusive	**Indeterminate**

The BioPlex 2200 RPR kit performance was estimated compared to the performance of a previously FDA cleared RPR assay.

The tables below show the distribution of results obtained from all comparator assays performed on each sample for the BioPlex 2200 Syphiliis Total assay (treponemal assay) and BioPlex RPR kit, respectively.

BioPlex Syphilis Total Serological Profile – Prospective Sample Data

Trep Assay (predicate)	RPR (predicate)	TPPA (predicate)	Final Comparator Result	BioPlex Syphilis Total	Number of Subjects
N	NR	Inconclusive	N	NR	7
N	NR	NR	N	NR	793
N	NR	NR	N	R	4
N	NR	R	N	NR	5
N	NR	R	N	R	4
N	R	NR	N	NR	9
EQ	NR	NR	N	NR	1
EQ	NR	R	P	NR	2
EQ	NR	R	P	R	1
P	NR	Inconclusive	P	R	2
P	NR	NR	N	EQ	2
P	NR	NR	N	NR	9
P	NR	NR	N	R	8
P	NR	R	P	EQ	3
P	NR	R	P	NR	5
P	NR	R	P	R	63
P	R	Inconclusive	P	NR	1
P	R	NR	P	NR	1
P	R	NR	P	R	2
P	R	R	P	NR	1
P	R	R	P	R	78
Total					1001

P-Positive; N-Negative; EQ: Equivocal; NR-Non-reactive; R-Reactive

BioPlex RPR Serological Profile – Prospective Sample Data

Trep Assay (predicate)	RPR (predicate)	TPPA (predicate)	Final Comparator Result	BioPlex RPR	Number of Subjects
N	NR	Inconclusive	N	NR	7
N	NR	NR	N	NR	773
N	NR	NR	N	R	24
N	NR	R	N	NR	9
N	R	NR	N	NR	3
N	R	NR	N	R	6
EQ	NR	NR	N	NR	1
EQ	NR	R	P	NR	3
P	NR	Inconclusive	P	NR	2
P	NR	NR	N	NR	17
P	NR	NR	N	R	2
P	NR	R	P	NR	65
P	NR	R	P	R	6
P	R	Inconclusive	P	NR	1
P	R	NR	P	NR	1
P	R	NR	P	R	2
P	R	R	P	NR	24
P	R	R	P	R	55
Total					1001

P-Positive; N-Negative; EQ: Equivocal; NR-Non-reactive; R-Reactive

The positive percent agreement (PPA) and the negative percent agreement (NPA) of the BioPlex 2200 Syphilis Total assay and BioPles 2200 RPR kit, in prospective samples, when compared to the comparator algorithm or previously FDA approved RPR assay, respectively, along with the 95% confidence interval is shown in the two tables below.

BioPlex 2200 Syphilis Total vs. Final Comparator Results

Prospective Samples		Final Comparator Algorithm Result		
		Positive	Negative	Total
BioPlex 2200 Syphilis Total Assay (Treponemal Test)	Reactive	147	16	163
	Equivocal	3	2	5
	Non-Reactive	9	824	833
	Total	159	842	1001

PPA: 92.45% (95% CI: 87.27%-95.63%)
NPA: 97.86% (95%CI: 96.65%-98.64%)

BioPlex 2200 RPR vs. Predicate Results

Prospective Samples		Predicate RPR Result		
		Positive	Negative	Total
BioPlex 2200 RPR Assay (Non-Treponemal Test)	Reactive	75	32	107
	Non-Reactive	17	877	894
	Total	92	909	1001

PPA: 81.52% (95% CI: 72.39%-88.13%)
NPA: 96.48% (95%CI: 95.07%-97.50%)

To further investiage the serologic status of RPR non-reactive samples the non-treponemal result from the BioPlex 200 RPR kit and the RPR predicate assay were stratified by treponemal result (table below). The treponemal assay used in this analyses was the predicate treponmeal EIA,one of the three assays of the comparator algorithm.

Non-Treponemal (RPR) Assay Performance Stratified by Treponemal Assay Results

		Comparator NT Assay Reactive		Comparator NT Assay Non-Reactive	
		Treponemal Reactive	Treponemal Non-Reactive	Treponemal Reactive	Treponemal Non-Reactive
BioPlex 2200 RPR Assay Result	Reactive	69	6	8	24
	Non-Reactive	14	3	87	790

Performance with Retrospective/Pre-Selected Samples

Retrospective samples were purchased by Bio-Rad from multiple vendors. Testing was conducted at three study sites in California (one in house and two locations representative of intended use sites for the BioPlex 2200 System). Serum samples were frozen and de-identified (only information on patient age, gender and HIV or pregnancy status was made available), and sent to the laboratories for testing.

A total of 546 retrospective serum samples were tested, including 412 RPR or Treponemal test positive samples, 32 syphilis postive pregnant women, 45 pregnant women with a history of STD infection, and 57 known HIV/Syphilis dual positive patients. Patients were aged <1 year to 89 years with 207 females (38%), 338 males (62%), and one patient of unknown gender. Two samples generated no result due to repeated instrument error.

The serum samples were tested with the BioPlex 2200 Syphilis Total assay and compared

to the result of a reference comparator algorithim. The comparator algorithm consists of three tests; a treponemal IgG/IgM assay, a non-treponemal assay and a second non-treponemal TP-PA assay. A final comparator result is determined from the three tests in the algorithm and used in the estimation for positive and negative percent agreement. The BioPlex 2200 RPR kit performance was calculated compared to the performance of a previously FDA cleared RPR assay.

Retrospective Samples		Final Comparator Algorithm Result		
		Reactive	Non-Reactive	Total
BioPlex 2200 Syphilis Total Assay (Treponemal Test)	Reactive	486	0	486
	Equivocal	1	0	1
	Non-Reactive	1	56	57
	Total	488	56	544

PPA: 99.59% (95% CI: 98.52% - 99.89%)
NPA: 100% (95% CI: 93.58% - 100%)

Retrospective Samples		Predicate RPR Result		
		Positive	Negative	Total
BioPlex 2200 RPR Assay (Non-Treponemal Test)	Reactive	422	22	444
	Non-Reactive	8	92	100
	Total	430	114	544

PPA: 98.14% (95% CI: 96.37% - 99.05%)
NPA: 80.70% (95% CI: 72.51% - 86.90%)

Performance in Samples from Pregnant Women

A total of 372 pregnant women were tested in this study. The women were aged 15 – 42 years and covered all three trimesters of pregnancy. Two hundred ninety five samples were prospectively collected, 32 samples were preselected pregnant women positive for syphilis, and 45 samples were pre selected pregnant women with a history of sexually transmitted disease (high-risk). The performance in pregnant women of the BioPlex 2200 Syphilis Total and BioPlex 2200 RPR kit are shown below.

Pregnant Women		Final Comparator Algorithm Result		
		Positive	Negative	Total
BioPlex 2200 Syphilis Total Assay (Treponemal Test)	Reactive	32	2	34
	Equivocal	0	0	0
	Non-Reactive	0	338	338
	Total	32	340	372

PPA: 100% (95% CI: 89.28% - 100%)
NPA: 98.83% (95% CI: 97.03% - 99.54%)

Pregnant Women		Predicate RPR Result		
		Positive	Negative	Total
BioPlex 2200 RPR Assay (Non-Treponemal Test)	Reactive	25	6	31
	Non-Reactive	0	341	341
	Total	25	347	372

PPA: 100% (95% CI: 86.68% - 100%)
NPA: 98.27% (95% CI: 96.28% - 99.21%)

Performance in HIV Positive Population

A total of 362 samples from HIV positive individuals were tested in this study. The patients were aged 17 – 75 years, 121 female (33.5%) and 241 (66.5%) male. Three hundred and five samples were prospectively collected, 57 samples were pre selected from known HIV/Syphilis positive individuals. The performance of the BioPlex 2200 Syphilis Total and BioPlex 2200 RPR kit in HIV positive patients are shown below.

HIV Positive Patients		Final Comparator Algorithm Result		
		Positive	Negative	Total
BioPlex 2200 Syphilis Total Assay (Treponemal Test)	Reactive	140	11	151
	Equivocal	3	2	5
	Non-Reactive	7	199	206
	Total	150	212	362

PPA: 93.33% (95% CI: 88.16% - 96.34%)
NPA: 93.87% (95% CI: 89.79% - 96.38%)

HIV Positive Patients		Predicate RPR Result		
		Positive	Negative	Total
BioPlex 2200 RPR Assay (Non-Treponemal Test)	Reactive	36	30	66
	Non-Reactive	6	290	296
	Total	42	320	362

PPA: 85.71% (95% CI: 72.16% - 93.29%)
NPA: 90.63% (95% CI: 86.93% - 93.35%)

Clinical Performance in Apparently Healthy Subjects

Samples were prospectively collected from 301 apparently healthy subjects, aged 8-102 years undergoing a routine check-up including 178 females (59%) and 123 males (41%).

The results of the BioPlex 2200 Syphilis Total and BioPlex 2200 RPR kit is shown below.

Apparently Healthy Subjects		Final Comparator Algorithm Result		
		Reactive	Non-Reactive	Total
BioPlex 2200 Syphilis Total Assay (Treponemal Test)	Positive	3	1	4
	Equivocal	0	2	2
	Negative	1	294	295
	Total	4	297	301

PPA: 75% (95% CI: 30.06% - 95.45%)
NPA: 99.0% (95% CI 97.1 – 99.7%)

Apparently Healthy Subjects		Predicate RPR Result		
		Positive	Negative	Total
BioPlex 2200 RPR Assay (Non-Treponemal Test)	Reactive	0	6	6
	Non-Reactive	4	291	295
	Total	4	297	301

PPA: 0% (95% CI: 0% - 48.98%)
NPA: 97.98% (95% CI: 95.66% - 99.07%)

Performance in Medically Diagnosed Individuals

The performance of the BioPlex 2200 Syphilis Total and RPR kit was evaluated with samples from subject who were medically diagnosed with primary, secondary or latent syphilis. The diagnosis of syphilis and the stage of the disease were made by a licensed physican based on the patient's clinical sypmptoms, medial history, and laboratory test results at the time of diagnosis. Samples were collected from 160 individuals diagnosed with primary, secondary, or latent syphilis with treatment status including 43 females (27%) and 117 males (73%). Four samples with no results were excluded due to repeated instrument error flags observed. The comparison results between BioPlex Syphilis Total and RPR assays and predicate results are shown below.

Syphilis Phase	Treatment Status	N	Reactivity in Medically Diagnosed Syphilis Patients			
			BioPlex Syphilis Total	95% Confidence Interval	Final Comparator Result	95% Confidence Interval
Primary	Untreated	26	96.2% (25/26)	81.1% - 99.3%	100% (26/26)	87.1% - 100%
	Treated	29	86.2% (25/29)	69.4% - 94.5%	86.2% (25/29)	69.4% - 94.5%
Secondary	Untreated	25	100% (25/25)	86.7% - 100%	100% (25/25)	86.7% - 100%
	Treated	26	100% (26/26)	87.1% - 100%	100% (26/26)	87.1% -100%
Latent	Untreated	23	100% (23/23)	85.7% - 100%	100% (23/23)	85.7% - 100%
	Treated	27	100% (27/27)	85.1% -100%	100% (27/27)	85.1% -100%
All Phases	Untreated	74	98.6% (73/74)	92.7% - 99.8%	100% (74/74)	95.1% - 100%
	Treated	82	95.1% (78/82)	88.1% - 98.1%	95.1% (78/82)	88.1% - 98.1%
Total		156	96.8% (151/156)	92.7% - 98.6%	97.4% (152/156)	93.6% - 99.3%

Syphilis Phase	Treatment Status	N	Reactivity in Medically Diagnosed Syphilis Patients			
			BioPlex RPR	95% Confidence Interval	Commercially Available RPR	95% Confidence Interval
Primary	Untreated	26	92.3% (24/26)	75.9% - 97.9%	88.5% (23/26)	71.05 – 96.0%
	Treated	29	65.5% (19/29)	47.3% - 80.1%	75.9% (22/29)	57.9% - 87.8%
Secondary	Untreated	25	100% (25/25)	86.7% - 100%	100% (25/25)	86.7% - 100%
	Treated	26	88.5% (23/26)	71.05 – 96.0%	80.8% (21/26)	62.1% - 91.6%
Latent	Untreated	23	95.7% (22/23)	79.0% - 99.2%	95.7% (22/23)	79.0% - 99.2%
	Treated	27	66.7% (18/27)	47.8% - 81.4%	66.7% (18/27)	47.8% - 81.4%
All Phases	Untreated	74	95.9% (71/74)	88.7% - 98.6%	95.0% (70/74)	86.9% - 97.9%
	Treated	82	73.2% (60/82)	62.7% - 81.6%	74.4% (61/82)	64.0% - 82.6%
Total		156	84.0% (131/156)	77.4% - 88.9%	84.0% (131/156)	77.4% - 88.9%

Of the total number of 2002 samples tested in the clinical study, 6 invalid results were generated on the initial testing and had to be retested, none gave valid results upon retest. The calculated rate of invalid results in this study was 0.3%.

4. Clinical cut-off**:**
   Not Applicable.

5. Expected values/Reference range:
   A total of 1001 specimens collected from the intended use population were tested with the BioPlex Syphilis Total and RPR kit. The three cohorts consisted of 401 specimens sent for routine syphilis or RPR testing (194 females, 207 males, 7 - 96 years old), 295 pregnant women (15 -42 years old), 45 pregnant women with STD (18 -36 years old), and 305 HIV positive individuals (97 females, 208 males, 17 -75 years old). The syphilis prevalence in each cohort is presented below.

Prevalence of Syphilis Among Subjects Sent for Syphilis Testing

Age (years)	Gender	N	BioPlex Syphilis Total N (%)			BioPlex RPR N (%)	
			R(+)	EQ	NR(-)	R(+)	NR(-)
<21	Female	30	0 (0.0%)	0 (0.0%)	30 (100.0%)	1 (3.3%)	29 (96.7%)
	Male	22	2 (9.1%)	0 (0.0%)	20 (90.9%)	1 (4.5%)	21 (95.5%)
21-30	Female	56	1 (1.8%)	0 (0.0%)	55 (98.2%)	3 (5.4%)	53 (94.6%)
	Male	59	7 (11.9%)	1 (1.7%)	51 (86.4%)	5 (8.5%)	54 (91.5%)
31-40	Female	42	3 (7.1%)	0 (0.0%)	39 (92.9%)	6 (14.3%)	36 (85.7%)
	Male	50	10 (20.0%)	0 (0.0%)	40 (80.0%)	9 (18.0%)	41 (82.0%)
41-50	Female	33	4 (12.2%)	0 (0.0%)	29 (87.9%)	3 (9.1%)	30 (90.9%)
	Male	24	9 (37.5%)	0 (0.0%)	15 (62.5%)	6 (25.0%)	18 (75.0%)
51-60	Female	13	5 (38.5%)	0 (0.0%)	8 (61.5%)	4 (30.8%)	9 (69.2%)
	Male	27	10 (37.0%)	0 (0.0%)	17 (63.0%)	7 (25.9%)	20 (74.1%)
>61	Female	20	2 (10%)	0 (0.0%)	18 (90.0%)	3 (15.0%)	17 (85.0%)
	Male	25	7 (28.0%)	0 (0.0%)	18 (72.0%)	5 (20.0%)	20 (80.0%)
Overall 7 - 96	Female	194	15 (7.7%)	0 (0.0%)	179 (92.3%)	20 (10.3%)	174 (89.7%)
	Male	207	45 (21.7%)	1 (0.5%)	161 (77.8%)	33 (15.9%)	174 (84.1%)
Combined		401	60 (15.0%)	1 (0.2%)	340 (84.8%)	53 (13.2%)	348 (86.8%)

Prevalence of Syphilis among Pregnant Women with STDs and for whom Syphilis Testing is Ordered

Age (years)	N	BioPlex Syphilis Total N (%)			BioPlex RPR N (%)	
		R(+)	EQ	NR(-)	R(+)	NR(-)
<21	47	0 (0.0%)	0 (0.0%)	47 (100%)	1 (2.1%)	46 (97.9%)
21-30	199	2 (1.0%)	0 (0.0%)	197 (99.0%)	1 (0.5%)	198 (99.5%)
31-40	92	1(1.1%)	0 (0.0%)	91 (98.9%)	3 (3.3%)	89 (96.7%)
>41	2	0 (0.0%)	0 (0.0%)	2 (100%)	0 (0.0%)	2 (1005)
Overall 15 - 42	340	3 (0.9%)	0 (0.0%)	337 (99.1%)	5 (1.5%)	335 (98.5%)

Prevalence of Syphilis among HIV Positive Individuals

Age (years)	Gender	N	BioPlex Syphilis Total N (%)			BioPlex RPR N (%)	
			R(+)	EQ	NR(-)	R(+)	NR(-)
<21	Female	2	0 (0.0%)	0 (0.0%)	2 (100%)	0 (0.0%)	2 (100%)
	Male	5	1 (20%)	0 (0.0%)	4 (80.0%)	2 (40.0%)	3 (60.0%)
21-30	Female	6	0 (0.0%)	0 (0.0%)	6 (100%)	0 (0.0%)	6 (100%)
	Male	25	4 (16.0%)	0 (0.0%)	21 (84.0%)	4 (16.0%)	21 (84.0%)
31-40	Female	20	5 (25%)	0 (0.0%)	15 (75.0%)	4 (20.0%)	16 (80.0%)
	Male	26	8 (30.8%)	1 (3.8%)	17 (65.4%)	6 (23.1%)	20 (76.9%)
41-50	Female	42	12 (28.6%)	0 (0.0%)	30 (71.4%)	6 (14.3%)	36 (85.7%)
	Male	71	26 (36.6%)	1 (1.4%)	44 (62.0%)	12 (16.9%)	59 (83.1%)
51-60	Female	21	8 (38.1%)	0 (0.0%)	13 (61.9%)	3 (14.3%)	18 (85.7%)
	Male	61	30 (49.2%)	1 (1.6%)	30 (49.2%)	10 (16.4%)	51 (83.6%)
61 -70	Female	6	2 (33.3%)	0 (0.0%)	4 (66.7%)	0 (0.0%)	6 (100%)
	Male	16	5 (31.3%)	1 (6.3%)	10 (62.5%)	2 (12.5%)	14 (87.5%)
>71	Female	0	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Male	2	0 (0.0%)	0 (0.0%)	2 (100%)	0 (0.0%)	2 (100%)
Unknown	Male	2	0 (0.0%)	0 (0.0%)	2 (100%)	0 (0.0%)	2 (100%)
All 17-75	Female	97	27 (27.8%)	0 (0.0%)	70 (72.2%)	13 (13.4%)	84 (86.6%)
	Male	208	74 (35.6%)	4 (1.8%)	130 (62.5%)	37 (17.8%)	171 (82.2%)
Combined		305	101 (33.1%)	4 (1.3%)	200 (65.6%)	50 (16.4%)	255 (83.6%)

N. Instrument Name:

The BioPlex 2200 System, software version 4.3

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes __X__ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No __X__

2. Software:

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

   Yes __X__ or No _____

3. Specimen Identification, Sampling and Handling:

   The BioPlex 2200 software is designed to control and process data for the BioPlex 2200 instrument system. The instrument system operates in a continuous processing mode with an on-demand STAT mode as well as when connected to a track (TLA) system. Orders, either imported from an LIS or entered manually by the user, are processed by the software from a worklist. Calculated results are both reported to the user and exported to the LIS. Some instrument functions controlled by the software include order scheduling, sample verification, sample aspiration/dispense, bead reagent aspiration/dispense, conjugate reagent aspiration/dispense, in process agitation, magnetic separation and wash, detector aspiration, multiplexed flow cytometric detection, results processing, detector calibration, assay calibration, assay quality control, internal quality control, reagent kit and consumables inventory, and waste management.

   The device also contains service software that allows only authorized personnel to set and check parameters and service the system. No diagnostic patient results are generated or accessed through the service software.

   Significant software features can be categorized under Instrument Control, Data Analysis, Data Management, and LIS functionality:

   - Instrument Control
     - Manage a random-access work list using barcodes to process the assays for each sample, entered manually or from the LIS.
     - Use a workflow that is compatible with existing laboratory instruments and practice.
     - Control all hardware modules automatically.

    - Optimize scheduling of processing steps in order to complete the assay of each sample as soon as possible.
    - Provide for STAT sample processing.
    - Provide for track (TLA) sample processing.
    - Acquire and process detector data.
    - Perform setup operations that are required at installation or for new reagent packs.
    - Monitor and display instrument supplies and reagents.
    - Monitor and report system parameters.
    - Track the status of samples on the instrument.
- Data Analysis
    - For quantitative assays, determine analyte concentration. Qualitative tests may report "positive" or "negative".
    - Multiple-level calibration of concentration versus intensity as required the analyte.
    - Chemistry calibration.
    - Run control samples and display the data.
- Data Management
    - Sample data storage and retrieval.
    - Storage and management of test parameter sets.
    - Tracking of consumables used with the assay.
    - QC data management to facilitate compliance.
- Connection to a Laboratory Information System (LIS) using ASTM protocols.
    - Work lists may be imported from an LIS.
    - Patient results may be exported to an LIS.

The BioPlex 2200 software runs on a PC running the Windows operating system. The software serves as the user interface, data processing, and instrument control center. The user interacts with the software via touch screen, and keyboard/mouse inputs. The software communicates with the instrument via USB connections. The software interacts with the firmware and modules through dynamic link libraries (DLL's). The user can modify or control software functions—Barcode symbologies and parameters, reportable analytes, report settings (lab name, lab details), define test groups, and LIS configuration.

4. Calibration:
Syphilis Total and Negative calibrators are prepared in a serum base that has been stripped of immunoglobulins while RPR calibrators are derived from disease free human plasma that are pre-screened for immune-reactivity to Syphilis and RPR antigens. Positive plasma are tested with FDA cleared methods, pooled, processed, and diluted to target concentrations in the appropriate base matrix. Calibrators and controls may use common high positive plasma units. Both calibrators and controls are supplemented with protein preservatives. Control materials use a serum base containing a full contingency of immunoglobulins to simulate actual human serum specimens.

The serum units used to prepare these materials are tested with FDA-cleared products to ensure that they are non-reactive for hepatitis B and C viruses as well as for HIV-1 and HIV-2. The antibody indices for each antigen are determined for each raw serum unit,

and appropriate dilutions are determined to obtain the target calibrator and control values.

5. Quality Control:
   At the beginning of each day that the Syphilis Total & RPR kit is to be used, load and process the Syphilis Total & RPR Control Set as indicated in the BioPlex 2200 System Operation Manual. The Syphilis Total & RPR Control Set should be run at least once per day, and with each new Reagent Pack lot.

   The Syphilis Total & RPR Control Set includes a negative control and a positive control in a human serum matrix made from defibrinated plasma. Each positive control contains antibodies for analytes within the Syphilis Total & RPR kit. The positive control is manufactured to give reactive results, with values above the cut-off for each specific bead. The negative control is manufactured to give nonreactive results with values below the cut-off for each specific bead. The negative control must have a nonreactive result, and the positive control must have a reactive result.

   Note: The Negative and Positive Controls of the Syphilis Total & RPR Control Set are intended to monitor for substantial reagent failure.

   Lot specific values for the positive control are loaded into the BioPlex 2200 System database via the provided media or by manual input. After identifying the control via the barcoded vial, the BioPlex 2200 System compares the control results to the expected lot specific control values stored in the BioPlex 2200 System database.

   Failure to obtain the appropriate values for controls will invalidate the assay and indicates procedural error, improper sample handling or deterioration of reagents. Additional controls may be tested in accordance with a laboratory's quality control policy.

   If a control result is out of its specified range, any test results generated since the last acceptable control results must be evaluated to determine if test results may have been adversely affected. Adversely affected results are invalid, and these samples must be re-tested.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

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

R. Conclusion:

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