DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

May 11, 2017

BIO-RAD Laboratories
Juang Wang
Regulatory Affairs Representative, Clinical Immunology Division
5500 E. 2nd Street
Benicia, CA 94510

Re: K170413
Trade/Device Name: Bioplex 2200 Syphilis Total & RPR Kit
Bioplex 2200 Syphilis Total & RPR Calibrator Set
Bioplex 2200 Syphilis Total & RPR Control Set
Regulation Number: 21 CFR 866.3830
Regulation Name: *Treponema pallidum* treponemal test reagents
Regulatory Class: II
Product Code: LIP, GMQ, JIT, JJX
Dated: February 9, 2017
Received: February 10, 2017

Dear Mr. Wang:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S

for Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

# Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
*See PRA Statement below.*

510(k) Number *(if known)*
K170413

Device Name
BioPlex 2200 Syphilis Total & RPR
BioPlex 2200 Syphilis Total & RPR Control Set
BioPlex 2200 Syphilis Total & RPR Calibrator Set

Indications for Use *(Describe)*

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 & RPR kit 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 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.

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

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

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# BioPlex 2200 Syphilis Total & RPR
# 510(k) Summary

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

## I. Applicant/Sponsor

Bio-Rad Laboratories, INC
Clinical Immunology Division
5500 E. Second Street
Benicia, CA 94510

Contact Person:
Juang Wang, PhD, RAC
Tel: 510-741-4609
FAX: 510-741-3941
Juang_wang@bio-rad.com

Date Prepared: May 09, 2017

## II. Device Name

Proprietary Name:
BioPlex 2200 Syphilis Total & RPR
BioPlex 2200 Syphilis Total & RPR Calibrator
BioPlex 2200 Syphilis Total & RPR Control

Classification Name:
- Enzyme Linked Immunoabsorption Assay, *Treponema pallidum*
- Antigens, Nontreponemal, All
- Calibrator, Secondary
- Single (specified) Analyte Controls (Assayed and Unassayed)

## III. Regulatory Information:

| Product Code | Classification | Regulation Section | Panel |
|---|---|---|---|
| Enzyme linked immunoabsorption assay, *Treponema pallidum* (LIP) | Class II | 21 CFR §866.3830 – *Treponema pallidum* treponemal test reagents | Microbiology (83) |
| Antigens, Nontreponemal, All (GMQ) | Class II | 21 CFR §866.3820 – *Treponema pallidum* nontreponemal test reagents | Microbiology (83) |

| Product Code | Classification | Regulation Section | Panel |
|---|---|---|---|
| Calibrator, Secondary(JIT) | Class II | 21 CFR § 862.1150 – Calibrator | Clinical Chemistry |
| Single (specified) analyte controls (assayed and unassayed) (JJX) | Class I | 21 CFR § 862.1660 –Quality control Material (Assayed and Unassayed) | Clinical Chemistry |

**IV. Predicate Devices**

LIAISON Treponema Assay, k061247
BD Macro-Vue RPR Card Tests, Pre-amendment prior to May 28, 1976

**V. 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.

**VI. Intended Use:**

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 kit 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.

## VII. Test Principle and Procedure

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 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 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 presence of serum or plasma in 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 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).

## VIII. Technological Characteristics and Substantial Equivalence

The following tables summarize the similarities and differences between the BioPlex 2200 Syphilis Total & RPR kit and the predicate devices used in comparative studies with the BioPlex 2200 Syphilis Total & RPR kits.

| **Device Similarities** | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 Syphilis Total & RPR Kit | Predicate Device<br>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 |

| **Device Differences** | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 Syphilis Total & RPR Kit | Predicate Device<br>LIAISON Treponema Assay, k061247 |

| Device Differences | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 Syphilis Total & RPR Kit | Predicate Device<br>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 |
| 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 |

| Device Similarities | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 Syphilis Total & RPR Kit | Predicate Device<br>BD Macro-Vue RPR CARD TEST, Pre-amendment |
| Intended Use | Multiplex flow immunoassay intended for the qualitative detection and/or titer determination 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 |

| Device Differences | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 Syphilis Total & RPR Kit | Predicate Device<br>BD Macro-Vue RPR CARD TEST, Pre-amendment |
| Assay Technology | Automated multiplex flow immunoassay | Macroscopic flocculation |
| Solid phase | Antigen-coated paramagnetic | Antigen carbon particle |

| Device Differences | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 Syphilis Total & RPR Kit | Predicate Device<br>BD Macro-Vue RPR CARD TEST, Pre-amendment |
| | microbeads | 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) |
| Standardization | The calibrator is referenced to an 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) |

## IX. Performance Characteristics

1. Analytical Performance:

   a. *Precision/Reproducibility:*

Internal Precision (CLSI EP05-A3)

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 data were analyzed for within-run, between-run, between-day. The total precision and the mean (AI), standard deviation (AI) and percent coefficient of variation (%CV) are summarized below:

**BioPlex 2200 Syphilis Total –Precision (CLSI EP5-A3)**

| Serum Panel | N | Mean AI | Within Run | | Between Run | | Between Day | | Total Precision | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Sample 1 | 80 | 0.3 | 0.03 | 10.4% | 0.00 | 0.0% | 0.03 | 8.4% | 0.04 | 13.4% |
| Sample 2 | 80 | 0.5 | 0.04 | 6.5% | 0.02 | 3.5% | 0.03 | 5.6% | 0.05 | 9.3% |
| Sample 3 | 80 | 0.9 | 0.03 | 3.3% | 0.02 | 2.2% | 0.02 | 2.7% | 0.04 | 4.8% |
| Sample 4 | 80 | 1.5 | 0.06 | 3.7% | 0.02 | 1.0% | 0.12 | 7.8% | 0.13 | 8.7% |
| Sample 5 | 80 | 1.8 | 0.08 | 4.3% | 0.00 | 0.0% | 0.19 | 10.3% | 0.20 | 11.2% |
| Sample 6 | 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 (CLSI EP05-A3)**

| Serum Panel | N | Mean AI | Within Run | | Between Run | | Between Day | | Total Precision | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Sample 1 | 80 | 0.2 | 0.03 | 11.2% | 0.02 | 6.5% | 0.04 | 16.2% | 0.05 | 20.7% |
| Sample 2 | 80 | 0.7 | 0.04 | 6.6% | 0.06 | 9.0% | 0.05 | 7.3% | 0.09 | 13.4% |
| Sample 3 | 80 | 0.9 | 0.04 | 4.2% | 0.06 | 6.5% | 0.05 | 5.1% | 0.09 | 9.3% |
| Sample 4 | 80 | 1.0 | 0.05 | 4.7% | 0.08 | 7.5% | 0.08 | 7.8% | 0.12 | 11.8% |
| Sample 5 | 80 | 1.9 | 0.10 | 5.2% | 0.10 | 5.3% | 0.08 | 4.2% | 0.16 | 8.6% |
| Sample 6 | 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 (CLSI EP15-A3)

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 lot. 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). The QC Controls were also included. The data 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 (CLSI EP15-A3)**

| Syphilis Total | | | Within Run | | Between Run | | Between Day | | Between Site/Instrument | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sample* | *N* | *Grand 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 (CLSI EP15-A3)**

| RPR | | | Within Run | | Between Run | | Between Day | | Between Site/Instrument | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Sample*** | ***N*** | ***Grand 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, negative and positive controls 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. 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%) |

b. Traceability:

Value Assignment:

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 |

| Calibrator Set | Assay | Range (AI) |
|---|---|---|
| Vial 5 | RPR | 1.4 – 2.6 |

Calibrator

There is no international or certified reference material available. The BioPlex Syphilis Total & RPR Calibrators are traceable to 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 disease state serum 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 |

c. Stability:

Stability studies have been performed to support the following claims:

Calibrator and Control:
BioPlex 2200 Syphilis Total & RPR Calibrator set is stable for 60 days after opening the vial the first time; Control Open Vial Stability (2 to 8°C), 60 days from first opening; Onboard Calibration Curve Stability, 30

days; Calibrators and Controls Real Time Stability (2 to 8°C), 24 month claim or until the date of expiration; Calibrators and Controls Accelerated Stability at 25ºC, 2 years predicted. Calibrators and controls freeze-thaw (-20ºC and -80ºC), 5 freeze-thaw cycles.

Kit Stability:
BioPlex 2200 Syphilis Total & RPR Kit: Real Time (unopened) Kit Stability, 24 month claim or until the date of expiration when stored unopened on the instrument or at 2 to 8°C. The open kit claim is 60 days.

Sample Stability:

Sample stability studies were also performed: Sample stability fresh (2 to 8°C), 7 days; Sample stability frozen (-20 or -80°C), 24 months claimed; Sample Freeze-thaw (-20 or -80°C), up to 5-freeze thaw cycles at -20ºC and 5-freeze thaw cycles at -80ºC acceptable.

d. *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 containing various cross reactants interfere 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.

Most 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. The potentially cross reactive samples were assayed in a single point. 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% |
| 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) |

*e. 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. Further confirmation of the cutoff values were compared the BioPlex Syphilis Total and RPR results from patient samples to those from the commercially available assays. 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 <1.0AI are considered non-reactive and results ≥1.0 AI are considered reactive.

2. Comparison studies:

*a. Method comparison with predicate device:*
The results of the BioPlex 2200 Syphilis Total and RPR assays were compared to a composite comparator and RPR card test for Syphilis Total and RPR assays, respectively. The composite comparator is based on an algorithm of results obtained from three commercially available syphilis assays: (a) Treponemal chemiluminescent immunoassay (predicate), (b) a non-treponemal assay (RPR Card Test), and (c) a second treponemal assay (Treponema Pallidum Particle Agglutination (TP-PA). Additional details of how the final comparator result was determined are provided below in the "Clinical Studies" section.

*b. Matrix comparison:*
The effect of anticoagulants in the samples on the performance of the BioPlex 2200 Syphilis Total and RPR assays was determined by comparing values obtained from the native serum samples spiked with anti-*T. pallidum* and reagin antibodies to the values of the matched donor's plasma containing the following anti-coagulants:

- Dipotassium EDTA
- Tripotassium EDTA
- Lithium heparin
- Sodium heparin

A total of 113 serum/plasma pairs, screened to be non-reactive using the BioPlex 2200 Syphilis Total & RPR kit, were spiked with an equal amount of a highly reactive syphilis and RPR sample to obtain concentrations that span the assay measuring range.

Each sample was tested in duplicate on one BioPlex 2200 instrument and the mean of the results for each matrix type was compared to the mean of the respective serum control sample by using Passing-Bablok analysis method to generate regression parameters (slope, intercept and correlation) along with 95% Confidence Interval. Sample results that exceeded the measuring range were excluded from the data analysis. Acceptance criteria for the regression analysis were: slope 0.8 to 1.2, intercept -0.2 to 0.2 and correlation ≥0.95.

| Matrix | BioPlex Assay | Slope (95% CI) | Intercept (95% CI) | Correlation (r) | N |
|---|---|---|---|---|---|
| Lithium heparin | Syphilis Total | 0.8421 (0.8148 to 0.8694) | 0.0316 (-0.0312 to 0.0944) | 0.9899 | 89 |
| | RPR | 0.8684 (0.8333 to 0.9035) | 0.0908 (0.0625 to 0.1191) | 0.9893 | 113 |
| Sodium heparin | Syphilis Total | 0.8333 (0.8148 to 0.8519) | 0.0500 (0.0050 to 0.0950) | 0.9924 | 89 |
| | RPR | 0.8353 (0.8095 to 0.8611) | 0.1147 (0.0833 to 0.1461) | 0.9849 | 113 |
| K2EDTA | Syphilis Total | 1.1923 (1.1538 to 1.230) | -0.0923 (-0.1538 to -0.0308) | 0.9943 | 83 |
| | RPR | 1.0930 (1.0709 to 1.1151) | -0.0314 (-0.0663 to 0.0035) | 0.9864 | 112 |
| K3EDTA | Syphilis Total | 1.1667 (1.1290 to 1.2043) | -0.0625 (-0.1073 to -0.0177) | 0.9915 | 82 |
| | RPR | 1.0776 (1.0500 to 1.1052) | -0.0233 (-0.0484 to 0.0018) | 0.9810 | 113 |

The resulting data support the package insert claim that serum, lithium heparin, sodium heparin, K2 EDTA, and K3EDTA specimens are acceptable for use with the BioPlex Syphilis Total and RPR assays.

3. Clinical studies:

The clinical Performance of the BioPlex 2200 Syphilis Total & RPR kit was evaluated in three testing sites from November to December 2016 using a total of 2008 samples prospectively and retrospectively collected from multiple commercial suppliers (approximately 91% in the US and 9% from outside of the US). There were 6 samples that generated no values due to repeat instrument error flags. The tested specimens consisted of 1347 prospective samples (301 apparently healthy subjects, 201 syphilis and 200 RPR test ordered for a total of 401 test ordered samples, 295 pregnant women and 45 pregnant women with STD, 305 HIV positive individuals) and 661 retrospective samples (412 RPR/VDRL positive, 160 clinically diagnosed syphilis, 32 pregnant syphilis positive, 57 HIV/Syphilis dual positive).

The 2008 specimens enrolled in the multiple site study were collected in the following areas:

- 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

Performance of the BioPlex Syphilis Total assay was evaluated by calculating positive percent agreement and negative percent agreement of the assay with the final comparator results based on an algorithm of results from three commercially available assays: treponemal chemiluminescence immunoassay (Predicate), RPR Card Test, and a second treponemal assay (TP-PA). The final comparator results were determined using a "2 out of 3" rule (TP-CLIA, RPR Card and TP-PA) as shown below. Results with indeterminate final comparator results would be excluded from the data analysis.

Performance of the BioPlex RPR assay was also evaluated by calculating positive percent agreement and negative percent agreement against a commercially available RPR Card Test (Predicate).

| Treponemal IgG/IgM (Predicate) | Non-treponemal (Predicate) | 2nd Treponemal (TP-PA) (Predicate) | Final Comparator Algorithm 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** |

a. Clinical Performance in Prospectively Collected Specimens in the Intended Use Population

The 1001 prospectively collected specimens in the intended use population evaluated with the BioPlex 2200 Syphilis Total & RPR kit 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), and 305 HIV positive individuals (97 females, 208 males, 17 -75 years old).

The comparison between the BioPlex Syphilis Total assay result and the final comparator result for the prospectively-collected specimens in the intended use population is shown below.

| Prospective Samples | | | Final Comparator Algorithm Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR(-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 Syphilis Total Assay (Treponemal Test) | Syphilis Test Ordered | Pos (+) | 26 | 3 | 29 | 89.7% (26/29) 73.6%-96.4% | 98.3% (169/172) 95.0%-99.4% |
| | | EQ | 1 | 0 | 1 | | |
| | | Neg (-) | 2 | 169 | 171 | | |
| | | Total | 29 | 172 | 201 | | |
| | RPR Test Ordered | Pos (+) | 31 | 0 | 31 | 96.9% (31/32) 84.3%-99.4% | 100% (168/168) 97.8%-100% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 1 | 168 | 169 | | |
| | | Total | 32 | 168 | 200 | | |
| | HIV Positive | Pos (+) | 90 | 11 | 101 | 91.8% (90/98) 84.7% - 95.8% | 93.7%(194/207) 89.6% - 96.3% |
| | | EQ | 2 | 2 | 4 | | |
| | | Neg (-) | 6 | 194 | 200 | | |
| | | Total | 98 | 207 | 305 | | |
| | Pregnant Women | Pos (+) | 0 | 2 | 2 | N/A | 99.3% (293/295) 97.6% - 99.8% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 293 | 293 | | |
| | | Total | 0 | 295 | 295 | | |
| | Overall | Pos (+) | 147 | 16 | 163 | 92.5% (147/159) 87.3% – 95.6% | 97.9% (824/842) 96.6% - 98.6% |
| | | EQ | 3 | 2 | 5 | | |
| | | Neg (-) | 9 | 824 | 833 | | |
| | | Total | 159 | 842 | 1001 | | |

R- Reactive; EQ-Equivocal; NR-Non-reactive; Pos – Positive; Neg- Negative

The comparison between the BioPlex RPR assay result and the predicate RPR result is shown below.

| Prospective Samples | | | Predicate RPR Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR (-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 RPR Assay (Non-Treponemal Test) | Syphilis Test Ordered | R (+) | 19 | 5 | 24 | 79.2% (19/24) 59.5%-90.8% | 97.2% (172/177) 93.6%-98.8% |
| | | NR (-) | 5 | 172 | 177 | | |
| | | Total | 24 | 177 | 201 | | |
| | RPR Test Ordered | R (+) | 27 | 2 | 29 | 77.1% (27/35) 61.0%-87.9% | 98.8% (163/165) 95.7%-99.7% |
| | | NR (-) | 8 | 163 | 171 | | |
| | | Total | 35 | 165 | 200 | | |
| | HIV Positive | R (+) | 29 | 21 | 50 | 87.9% (29/33) 72.7%-95.2% | 92.3%(251/272) 88.5%-94.9% |
| | | NR (-) | 4 | 251 | 255 | | |
| | | Total | 33 | 272 | 305 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pregnant Women | R (+) | 0 | 4 | 4 | N/A | 98.6% (291/295)<br>96.6%-99.5% |
| | | NR (-) | 0 | 291 | 291 | | |
| | | Total | 0 | 295 | 295 | | |
| | Overall | R(+) | 75 | 32 | 107 | 81.5% (75/92)<br>72.4% -88.1% | 96.5%(877/909)<br>95.1% - 97.5% |
| | | NR (-) | 17 | 877 | 894 | | |
| | | Total | 92 | 909 | 1001 | | |

b. Clinical Performance in Retrospective known Syphilis Positive Samples

Clinical performance in the retrospective known syphilis positive samples was evaluated by testing a total of 544 samples including 412 known RRP/VDRL positive (106 females, 305 males, 1 unknown gender, <1 – 89 years old), 32 pregnant positive women (18- 36 years old), 45 pregnant women with a history of STD (20 - 39 years old), and 57 known HIV/Syphilis dual positive (24 females, 33 males, 20 – 67 years old). Two (2) samples from RPR/VDRL positive cohort obtained no values due to repeated instrument errors were excluded from analysis. The comparison between the BioPlex Syphilis Total results and the final comparator results is shown below.

| Retrospective Samples | | | Final Comparator Algorithm Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR(-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 Syphilis Total Assay (Treponemal Test) | RPR/VDRL Positive Pregnant Positive Women | Pos (+) | 404 | 0 | 404 | 100% (404/404)<br>99.1%-100% | 100% (6/6)<br>61.0%-100% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 6 | 6 | | |
| | | Total | 404 | 6 | 410 | | |
| | Pregnant Women with STD | Pos (+) | 31 | 0 | 31 | 100% (31/31)<br>89.0%-100% | 100% (1/1)<br>20.7%-100% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 1 | 1 | | |
| | | Total | 31 | 1 | 32 | | |
| | HIV/ Syphilis Dual Positive | Pos (+) | 1 | 0 | 1 | 100% (1/1)<br>20.7% - 100% | 100% (44/44)<br>92.0% - 100% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 44 | 44 | | |
| | | Total | 1 | 44 | 45 | | |
| | All known positive RPR/VDRL | Pos (+) | 50 | 0 | 50 | 96.2%(50/52)<br>87.0% - 98.9% | 100% (5/5)<br>56.6% - 100% |
| | | EQ | 1 | 0 | 1 | | |
| | | Neg (-) | 1 | 5 | 6 | | |
| | | Total | 52 | 5 | 57 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | All known positive | Pos (+) | 486 | 0 | 486 | 99.6% (486/488)<br>98.5% – 99.9% | 100% (56/56)<br>93.6% - 100% |
| | | EQ | 1 | 0 | 1 | | |
| | | Neg (-) | 1 | 56 | 57 | | |
| | | Total | 488 | 56 | 544 | | |

The comparison between the BioPlex RPR results and the predicate RPR results is shown below.

| Retrospective Samples | | | Predicate RPR Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR (-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 RPR Assay (Non-Treponemal Test) | RPR/VDRL Positive | R (+) | 390 | 11 | 401 | 98.5%(390/396)<br>96.7%-99.3% | 21.4% (3/14)<br>7.6%-47.6% |
| | | NR (-) | 6 | 3 | 9 | | |
| | | Total | 396 | 14 | 410 | | |
| | Pregnant Positive Women | R (+) | 25 | 1 | 26 | 100% (25/25)<br>86.7%-100% | 85.7% (6/7)<br>48.7%-97.4% |
| | | NR (-) | 0 | 6 | 6 | | |
| | | Total | 25 | 7 | 32 | | |
| | Pregnant Women with STD | R (+) | 0 | 1 | 1 | N/A | 97.8% (44/45)<br>88.4% - 99.6% |
| | | NR (-) | 0 | 44 | 44 | | |
| | | Total | 0 | 45 | 45 | | |
| | HIV/ Syphilis Dual Positive | R (+) | 7 | 9 | 16 | 77.8% (7/9)<br>45.3% - 93.7% | 81.3% (39/48)<br>68.1%- 89.8% |
| | | NR (-) | 2 | 39 | 41 | | |
| | | Total | 9 | 48 | 57 | | |
| | All known positive | R (+) | 422 | 22 | 444 | 98.1%(422/430)<br>96.4% -99.1% | 80.7% (92/114)<br>72.5% - 86.9% |
| | | NR (-) | 8 | 92 | 100 | | |
| | | Total | 430 | 114 | 544 | | |

c. Clinical Performance in Clinically Diagnosed Individuals

Samples were collected from 160 individuals diagnosed with primary, secondary or latent syphilis with treatment status including 43 females (18 – 78 years old) and 117 males (19 – 64 years old). 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.

Sensitivity of the BioPlex Syphilis Total and RPR assays and predicate assays in individuals clinically diagnosed with syphilis along with the treatment status is shown below.

| Syphilis Phase | Treatment Status | N | Reactivity in Medically Diagnosed Syphilis Patients (95% CI) | | | |
|---|---|---|---|---|---|---|
| | | | BioPlex Syphilis Total | BioPlex RPR | Final Comparator | Predicate RPR |
| Primary | Untreated | 26 | 96.2% | 92.3% | 100% | 88.5% |

| Syphilis Phase | Treatment Status | N | Reactivity in Medically Diagnosed Syphilis Patients (95% CI) | | | |
|---|---|---|---|---|---|---|
| | | | BioPlex Syphilis Total | BioPlex RPR | Final Comparator | Predicate RPR |
| | | | (25/26)<br>81.1% - 99.3% | (24/26)<br>75.9% - 97.9% | (26/26)<br>87.1% - 100% | (23/26)<br>71.05 – 96.0% |
| | Treated | 29 | 86.2%<br>(25/29)<br>69.4% - 94.5% | 65.5%<br>(19/29)<br>47.3% - 80.1% | 86.2%<br>(25/29)<br>69.4% - 94.5% | 75.9%<br>(22/29)<br>57.9% - 87.8% |
| Secondary | Untreated | 25 | 100%<br>(25/25)<br>86.7% - 100% | 100%<br>(25/25)<br>86.7% - 100% | 100%<br>(25/25)<br>86.7% - 100% | 100%<br>(25/25)<br>86.7% - 100% |
| | Treated | 26 | 100%<br>(26/26)<br>87.1% - 100% | 88.5%<br>(23/26)<br>71.05 – 96.0% | 100%<br>(26/26)<br>87.1% -100% | 80.8%<br>(21/26)<br>62.1% - 91.6% |
| Latent | Untreated | 23 | 100%<br>(23/23)<br>85.7% - 100% | 95.7%<br>(22/23)<br>79.0% - 99.2% | 100%<br>(23/23)<br>85.7% - 100% | 95.7%<br>(22/23)<br>79.0% - 99.2% |
| | Treated | 27 | 100%<br>(27/27)<br>85.1% -100% | 66.7%<br>(18/27)<br>47.8% - 81.4% | 100%<br>(27/27)<br>85.1% -100% | 66.7%<br>(18/27)<br>47.8% - 81.4% |
| All Phases | Untreated | 74 | 98.6%<br>(73/74)<br>92.7% - 99.8% | 95.9%<br>(71/74)<br>88.7% - 98.6% | 100%<br>(74/74)<br>95.1% - 100% | 95.0%<br>(70/74)<br>86.9% - 97.9% |
| | Treated | 82 | 95.1%<br>(78/82)<br>88.1% - 98.1% | 73.2%<br>(60/82)<br>62.7% - 81.6% | 95.1%<br>(78/82)<br>88.1% - 98.1% | 74.4%<br>(61/82)<br>64.0% - 82.6% |
| Total | | 156 | 96.8% (151/156)<br>92.7% - 98.6% | 84.0% (131/156)<br>77.4% - 88.9% | 97.4% (152/156)<br>93.6% - 99.3% | 84.0% (131/156)<br>77.4% - 88.9% |

d. Clinical Performance in HIV Positive Individuals

Clinical performance in the HIV positive samples was evaluated by testing a total of 362 samples from 305 HIV positive individuals (97 females, 208 males, 17 -75 years old).and 57 known HIV/Syphilis dual positive individuals (24 females, 33 males, 20 – 67 years old). The comparison between the BioPlex Syphilis Total results and the final comparator results is shown below.

| HIV Positive | | | Final Comparator Algorithm Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR(-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 Syphilis Total Assay (Treponemal Test) | HIV Positive | Pos (+) | 90 | 11 | 101 | 91.8% (90/98)<br>84.7% - 95.8% | 93.7%(194/207)<br>89.6% - 96.3% |
| | | EQ | 2 | 2 | 4 | | |
| | | Neg (-) | 6 | 194 | 200 | | |
| | | Total | 98 | 207 | 305 | | |
| | HIV /Syphilis Dual Positive | Pos (+) | 50 | 0 | 50 | 96.2%(50/52)<br>87.0% - 98.9% | 100% (5/5)<br>56.6% - 100% |
| | | EQ | 1 | 0 | 1 | | |
| | | Neg (-) | 1 | 5 | 6 | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Total | 52 | 5 | 57 | | |
| | Overall | Pos (+) | 140 | 11 | 151 | 93.3%(140/150)<br>88.2% – 96.3% | 93.9%(199/212)<br>89.8% - 96.4% |
| | | EQ | 3 | 2 | 5 | | |
| | | Neg (-) | 7 | 199 | 206 | | |
| | | Total | 150 | 212 | 362 | | |

| HIV Positive | | | Predicate RPR Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR (-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 RPR Assay (Non-Treponemal Test) | HIV Positive | R (+) | 29 | 21 | 50 | 87.9% (29/33)<br>72.7%-95.2% | 92.3%(251/272)<br>88.5%-94.9% |
| | | NR (-) | 4 | 251 | 255 | | |
| | | Total | 33 | 272 | 305 | | |
| | HIV /Syphilis Dual Positive | R (+) | 7 | 9 | 16 | 77.8% (7/9)<br>45.3% - 93.7% | 81.3% (39/48)<br>68.1%-89.8% |
| | | NR (-) | 2 | 39 | 41 | | |
| | | Total | 9 | 48 | 57 | | |
| | Overall | R(+) | 36 | 30 | 66 | 85.7% (36/42)<br>72.2% -93.3% | 90.6%(290/320)<br>86.9% - 93.4% |
| | | NR (-) | 6 | 290 | 296 | | |
| | | Total | 42 | 320 | 362 | | |

e. Clinical Performance in Pregnant Women

A total of 372 specimens were from 295 pregnant women (15 – 42 years old) including 295 samples sent for Syphilis testing ,32 preselected pregnant women (18 – 36 years old) with positive syphilis and 45 pregnant women with a history of STD (20 - 39 years old). The comparison results for pregnant women on the BioPlex Syphilis Total and RPR assay are shown below.

| Pregnant Women | | | Final Comparator Algorithm Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR(-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 Syphilis Total Assay (Treponemal Test) | Test Ordered | Pos (+) | 0 | 2 | 2 | N/A | 99.3%(293/295) 97.6%-99.8% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 293 | 293 | | |
| | | Total | 0 | 295 | 295 | | |
| | Syphilis Positive | Pos (+) | 31 | 0 | 31 | 100% (31/31) 89.0%-100.0% | 100% (1/1) 20.7%-100% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 1 | 1 | | |
| | | Total | 31 | 1 | 32 | | |
| | SDT | Pos (+) | 1 | 0 | 1 | 100% (1/1) 20.7% - 100% | 100% (44/44) 92.0% - 100% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 44 | 44 | | |
| | | Total | 1 | 44 | 45 | | |
| | All Combined | Pos (+) | 32 | 2 | 34 | 100% (32/32) 89.3%-100.0% | 99.4%(338/340) 97.9%-99.8% |
| | | EQ | 0 | 0 | 0 | | |
| | | Neg (-) | 0 | 338 | 338 | | |
| | | Total | 32 | 340 | 372 | | |

| Pregnant Women | | | Predicate RPR Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | R (+) | NR(-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 RPR Assay (Non-Treponemal Test) | Test Ordered | R (+) | 0 | 4 | 4 | N/A | 98.6% (291/295) 98.6%-99.5% |
| | | NR (-) | 0 | 291 | 291 | | |
| | | Total | 0 | 295 | 295 | | |
| | Syphilis Positive | R(+) | 25 | 1 | 26 | 100% (25/25) 86.7%-100% | 85.7% (6/7) 48.7%-97.4% |
| | | NR (-) | 0 | 6 | 6 | | |
| | | Total | 25 | 7 | 32 | | |
| | STD | R (+) | 0 | 1 | 1 | N/A | 97.8% (44/45) 88.4% - 99.6% |
| | | NR (-) | 0 | 44 | 44 | | |
| | | Total | 0 | 45 | 45 | | |
| | All Combined | R(+) | 25 | 6 | 31 | 100% (25/25) 86.7%-100% | 98.3% (341/347) 96.3%-99.2% |
| | | NR (-) | 0 | 341 | 341 | | |
| | | Total | 25 | 347 | 372 | | |

f. Clinical Performance in Apparently Healthy Subjects

A total of 301 samples prospectively collected from apparently healthy subjects undergoing a routine check-up including 178 females (13 – 102 years old) and 123 males (8 – 93 years old) were evaluated with the BioPlex Syphilis Total & RPR. The comparison results of the BioPlex Syphilis Total and RPR assays are shown below.

| Apparently Healthy Subjects | | Final Comparator Algorithm Result | | | | |
|---|---|---|---|---|---|---|
| | | R (+) | NR(-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 Syphilis Total Assay (Treponemal Test) | Pos (+) | 3 | 1 | 4 | 75,0% (3/4) 30.1% - 95.5% | 99.0% (294/297) 97.1% - 99.7% |
| | EQ | 0 | 2 | 2 | | |
| | Neg (-) | 1 | 294 | 295 | | |
| | Total | 4 | 297 | 301 | | |

| Apparently Healthy Subjects | | Predicate RPR result | | | | |
|---|---|---|---|---|---|---|
| | | R (+) | NR (-) | Total | % Pos Agreement (95% CI) | % Neg Agreement (95% CI) |
| BioPlex 2200 RPR Assay (Non-Treponemal Test) | R(+) | 0 | 6 | 6 | 0% (0/4) 0.0%– 49.0% | 98.0% (291/297) 95.7% - 99.1% |
| | NR (-) | 4 | 291 | 295 | | |
| | Total | 4 | 297 | 301 | | |

g. Summary of the Serological Test Profile

The serological test profile for all prospective samples tested in the clinical study is summarized below for both BioPlex Syphilis Total and RPR assays.

BioPlex Syphilis Total serological test profile

| 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 | R | 2 |
| P | R | NR | P | NR | 1 |
| P | R | R | P | NR | 1 |
| P | R | R | P | R | 78 |

| Trep Assay (predicate) | RPR (Predicate) | TPPA (Predicate) | Final Comparator Result | BioPlex Syphilis Total | Number of Subjects |
|---|---|---|---|---|---|
| Total | | | | | 1001 |

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

BioPlex RPR serological test profile

| 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; R- Reactive; NR – Non-reactive; EQ – Equivocal

4. Expected values/Reference range:

There are a total of 1001 samples prospectively collected specimens for the intended use population that 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), and 305 HIV positive individuals (97 females, 208 males, 17 -75 years old). The prevalence of each cohort is presented below.

Prevalence of 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%) |

| Age (years) | Gender | N | BioPlex Syphilis Total N (%) | | | BioPlex RPR N (%) | |
|---|---|---|---|---|---|---|---|
| | | | R(+) | EQ | NR(-) | R(+) | NR(-) |
| | 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 Pregnant Women for whom syphilis testing is ordered

| Age (years) | N | BioPlex Syphilis Total N (%) | | | BioPlex RPR N (%) | |
|---|---|---|---|---|---|---|
| | | R(+) | EQ | NR(-) | R(+) | NR(-) |
| <21 | 45 | 0 (0.0%) | 0 (0.0%) | 45 (100%) | 1 (2.2%) | 44 (97.8%) |
| 21-30 | 175 | 1 (0.6%) | 0 (0.0%) | 174 (99.4%) | 1 (0.6%) | 174 (99.4%) |
| 31-40 | 73 | 1 (1.4%) | 0 (0.0%) | 72 (98.6%) | 2 (2.7%) | 71 (97.3%) |
| >41 | 2 | 0 (0.0%) | 0 (0.0%) | 2 (100%) | 0 (0.0%) | 2 (100%) |
| Overall 15 – 42 | 295 | 2 (0.7%) | 0 (0.0%) | 293 (99.3%) | 4 (1.4%) | 291 (98.6%) |

Prevalence of 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%) |

| Age (years) | Gender | N | BioPlex Syphilis Total N (%) | | | BioPlex RPR N (%) | |
|---|---|---|---|---|---|---|---|
| | | | R(+) | EQ | NR(-) | R(+) | NR(-) |
| >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%) |

**X. Instrument Name:**

The BioPlex 2200 System, software version 4.3

**XI. Conclusion:**

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