**DEPARTMENT OF HEALTH & HUMAN SERVICES** Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

July 25, 2017

BIO-RAD LABORATORIES
ARLENE CARILLO
REGULATORY AFFAIRS REPRESENTATIVE
5500 EAST SECOND STREET
BENICIA, CA 94510

Re: K170509
Trade/Device Name: Bioplex 2200 ToRC IgM, Bioplex 2200 ToRC IgM Calibrator Set, Bioplex 2200 ToRC IgM Control Set
Regulation Number: 21 CFR 866.3510
Regulation Name: Rubella virus serological reagents
Regulatory Class: II
Product Code: PUQ, JIX, JJX, LKQ, LFZ, LGD
Dated: February 17, 2017
Received: February 21, 2017

Dear Ms. Carillo:

This letter corrects our substantially equivalent letter of May 19, 2017.

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

510(k) Number *(if known)*
K170509

Device Name
BioPlex 2200 ToRC IgM Kit
BioPlex 2200 ToRC IgM Calibrator Set
BioPlex 2200 ToRC IgM Control Set

Indications for Use *(Describe)*

BioPlex 2200 ToRC IgM Kit
The BioPlex 2200 ToRC IgM kit is a multiplex flow immunoassay intended for the qualitative detection of IgM antibodies to *Toxoplasma gondii* (*T. gondii*), Rubella, and Cytomegalovirus (CMV) in human serum and plasma (K3 EDTA, lithium heparin, or sodium heparin).

The BioPlex 2200 ToRC IgM kit is intended for use with the Bio-Rad BioPlex 2200 System.

This kit is intended as an aid in the diagnosis of a current or recent *T. gondii*, Rubella and/or CMV infection, in individuals suspected of having one of the respective disease states, including women of child bearing age.

This assay is not FDA cleared or approved for use in testing (screening) blood or plasma donors.

Performance characteristics for the ToRC IgM assay have not been evaluated in immunosuppressed or organ transplant individuals. Performance characteristics of this kit have not been established for use in neonatal screening or for use at point of care facilities.

BioPlex 2200 ToRC IgM Calibrator Set
The BioPlex 2200 ToRC IgM Calibrator Set is intended for the calibration of the BioPlex 2200 ToRC IgM Reagent Pack.

BioPlex 2200 ToRC IgM Control Set
The BioPlex 2200 ToRC IgM Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and BioPlex 2200 ToRC IgM Reagent Pack in the clinical laboratory.

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 ToRC IgM
# 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 provides the information to support a determination of substantial equivalence for the BioPlex 2200 ToRC IgM kit.

**510(k) Number:**
K170509

**Summary Preparation Date:**
February 17, 2017

**Applicant:**
Bio-Rad Laboratories

**Contact:**
Arlene Carillo
Regulatory Affairs Representative
5500 East Second Street
Benicia, CA 94510

**Purpose for Submission:**
New Device

**Measurand:**
IgM antibodies to *Toxoplasma gondii* (*T. gondii*), Rubella and Cytomegalovirus (CMV)

**Type of Test:**
Multiplexed Microparticle Immunoassay based on Luminex Technology

**Proprietary and Established Names:**
BioPlex 2200 ToRC IgM
BioPlex 2200 ToRC IgM Calibrator Set
BioPlex 2200 ToRC IgM Control Set

**Regulatory Information:**

| Product code | Classification | Regulation section | Panel |
|---|---|---|---|
| PUQ: Multiplex flow immunoassay, *T. gondii*, Rubella, CMV IgM | Class II | 21 CFR § 866.3510 – Rubella virus Serological Agents | Microbiology |
| JIT, Calibrator, Secondary | Class II | 21 CFR §862.1150 – Calibrator | Clinical Chemistry |
| JJY, Multi-Analyte Controls, All Kinds (Assayed) | Class I | 21 CFR §862.1660 – Quality Control Material (assayed | Clinical Chemistry |

| Product code | Classification | Regulation section | Panel |
|---|---|---|---|
| | | and unassayed) | |

The following is a list of regulation sections and product codes that are applicable to the individual analytes detected by the device subject of this submission.

1. 21 CFR § 866.3780 – *Toxoplasma gondii* Serological Agents (Microbiology Panel: Class II), Product Code LGD, Enzyme Linked Immunoabsorbent Assay, *Toxoplasma gondii*
2. 21 CFR § 866.3510 – Rubella virus Serological Agents (Microbiology Panel: Class II), Product Code LFX, Enzyme Linked Immunoabsorbent Assay, Rubella
3. 21 CFR § 866.3175– Cytomegalovirus Serological Agents (Microbiology Panel: Class II), Product Code LFZ, Enzyme Linked Immunoabsorbent Assay, Cytomegalovirus and LKQ, Antibody IgM, if, Cytomegalovirus Virus

**Intended Use:**

1. Intended use(s):

   The BioPlex 2200 ToRC IgM kit is a multiplex flow immunoassay intended for the qualitative detection of IgM antibodies to *Toxoplasma gondii* (*T. gondii*), Rubella and Cytomegalovirus (CMV) in human serum and plasma (K3 EDTA, lithium heparin, or sodium heparin).

   The BioPlex 2200 ToRC IgM kit is intended for use with the Bio-Rad BioPlex 2200 System.

   This kit is intended as an aid in the diagnosis of a current or recent *T. gondii*, Rubella and/or CMV infection, in individuals suspected of having one of the respective disease states including women of child bearing age.

   This assay is not FDA cleared or approved for use in testing (screening) blood or plasma donors.

   Performance characteristics for the ToRC IgM assay have not been evaluated in immunosuppressed or organ transplant individuals. Performance characteristics of this kit have not been established for use in neonatal screening or for use at point of care facilities.

   The BioPlex 2200 ToRC IgM Calibrator Set is intended for the calibration of the BioPlex 2200 ToRC IgM Reagent Pack.

   The BioPlex 2200 ToRC IgM Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and BioPlex 2200 ToRC IgM Reagent Pack in the clinical laboratory.

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

**Device Description:**

BioPlex ToRC IgM Reagent Pack includes the following components:

- One (1) 10 mL vial, containing dyed beads coated with lysates of *T. gondii*, Rubella and CMV plus an Internal Standard bead (ISB) and a Serum Verification bead (SVB) in buffer with Glycerol and protein stabilizers (bovine and caprine). ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) as preservatives.
- One (1) 5 mL vial, containing phycoerythrin-conjugated murine monoclonal anti-human IgM antibody and phycoerythrin-conjugated murine monoclonal anti-human FXIII antibody, in buffer with protein stabilizers (bovine and murine). ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) as preservatives.
- One (1) 10 mL vial, containing goat anti-human IgG antibody and protein stabilizers (bovine and murine) in buffer. ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) as preservatives.

BioPlex 2200 ToRC IgM Calibrator set contains two (2) 0.5 mL vials. The calibrators are provided in a human serum matrix made from defibrinated plasma with added known analyte concentrations consisting of HuCAL® recombinant IgM antibodies for rubella and human disease state plasma derived antibodies for T. gondii and CMV. All calibrators contain ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%) as preservatives.

BioPlex 2200 ToRC IgM Control set contains two (2) 1.5 mL Positive Control serum vials, containing human disease state plasma derived IgM antibodies to T. gondii and CMV, and HuCAL® recombinant IgM antibodies to Rubella in a human serum matrix made from defibrinated plasma; and two (2) 1.5 mL Negative Control serum vials, in a human serum matrix made from defibrinated plasma. All controls contain Amikacin (0.003%), Cycloheximide (C15H23NO4) (0.009%), Amphotericin B (0.002%), Cefotaxime Sodium (0.002%), Ciprofloxacin (0.005%), ProClin 300 (≤ 0.3%), sodium benzoate (≤ 0.1%) and sodium azide (< 0.1%).

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.

**Substantial Equivalence Information:**

1. Predicate device name(s):
   BioPlex 2200 Rubella and CMV IgM Kit, K092587
   bioMeriéux, Inc. VIDAS® TOXO IgM, K923166

2. Comparison with predicate:
   *Toxoplasma gondii* IgM Predicate Device: VIDAS® TOXO IgM

| **Device Similarities** | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 ToRC IgM | Predicate Device<br>bioMeriéux, Inc.,<br>VIDAS® TOXO IgM<br>K923166 |
| Intended Use | Qualitative detection of IgM antibodies to ***Toxoplasma gondii* (*T. gondii*)**, Rubella and Cytomegalovirus (CMV) | Qualitative detection of anti-*Toxoplasma gondii* antibodies |
| Indications for Use | To be used as an aid in the diagnosis of a current or recent *T. gondii*, Rubella and/or CMV infection | To be used as an aid in the diagnosis of acute, recent, or reactivated *Toxoplasma gondii* infection |
| Measured Analyte | IgM antibodies to ***Toxoplasma gondii* (*T. gondii*)**, Rubella and Cytomegalovirus (CMV) | Same |
| Signal Detection | Fluorescence | Same |

| **Device Differences** | | |
|---|---|---|
| Characteristics | New Device<br>BioPlex 2200 ToRC IgM | Predicate Device<br>bioMeriéux, Inc.,<br>VIDAS® TOXO IgM<br>K923166 |
| Sample Handling Process | Automated | Manual |
| Assay Technology | Automated multiplex flow immunoassay | Automated enzyme linked fluorescent immunoassay (ELFA) |
| Solid Phase | Antigen-coated paramagnetic | Antibody Coated Solid Phase Receptacle |

| Device Differences | | |
|---|---|---|
| Characteristics | New Device BioPlex 2200 ToRC IgM | Predicate Device bioMeriéux, Inc., VIDAS® TOXO IgM K923166 |
| | microbeads | (SPR®) |
| Reagent Integral Storage | On-board or in refrigerator at 2-8ºC | No reagent storage |
| Enzyme Conjugate | Phycoerythrin conjugated | Alkaline phosphatase conjugated |
| Unit of Measure | AI | RFV |
| Calibrator(s) | 2 calibrator levels (sold separately) | Single calibrator included with the kit |
| Reagent Pack Calibration Frequency | Every 30 days | Every 14 days |
| Control Intended Use | Use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and BioPlex 2200 ToRC IgM Reagent Pack in the clinical laboratory | Intended for use as assayed quality control samples to monitor the accuracy and precision of the assay |
| Instrumentation | Bio-Rad BioPlex 2200 System | Vitek® ImmunoDiagnostic Assay System,VIDAS® System |
| Sample Matrix | Serum and Plasma (EDTA and Heparin) | Serum |
| Sample Size | 3 µL | 100 µL |

Rubella and CMV IgM Predicate Device: BioPlex 2200 **Rubella** and CMV IgM Kit

| **Device Similarities** | | |
|---|---|---|
| Characteristics | New Device BioPlex 2200 ToRC IgM | Predicate Device BioPlex 2200 **Rubella** and CMV IgM Kit K092587 |
| Intended Use | Qualitative detection of IgM antibodies to *Toxoplasma gondii* (*T. gondii*), **Rubella** and **Cytomegalovirus** (CMV) | Same |
| Indications for Use | To be used as an aid in the diagnosis of a current or recent *T. gondii*, **Rubella** and/or **CMV** infection, in individuals suspected of having one of the respective disease states including women of child bearing age. | Same |
| Measured Analyte | IgM antibodies to *Toxoplasma gondii* (*T. gondii*), **Rubella** and **Cytomegalovirus** (CMV) | Same |
| Assay Technology | Automated multiplex flow immunoassay | Same |
| Test Principle | Multiplexed flow immunoassay | Same |
| Solid Phase | Antigen-coated paramagnetic microbeads | Same |
| Sample Handling/Process | Automated | Same |
| Reagent Integral Storage | On-board or in refrigerator at 2-8ºC | Same |
| Signal Detection | Fluorescence | Same |

| Device Similarities | | |
|---|---|---|
| Characteristics | New Device BioPlex 2200 ToRC IgM | Predicate Device BioPlex 2200 **Rubella** and CMV IgM Kit K092587 |
| Unit of Measure | AI | Same |
| Reagent Pack Calibration Frequency | Every 30 days | Same |
| Control Intended Use | Use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and BioPlex 2200 ToRC IgM Reagent Pack in the clinical laboratory | Same |
| Instrumentation | Bio-Rad BioPlex 2200 System | Same |

| Device Differences | | |
|---|---|---|
| Characteristics | New Device BioPlex 2200 ToRC IgM | Predicate Device BioPlex 2200 **Rubella** and CMV IgM Kit K092587 |
| Conjugate | Phycoerythrin-conjugated murine monoclonal anti-human IgM antibody and phycoerythrin-conjugated murine monoclonal anti-human FXIII antibody | Phycoerythrin-conjugated donkey polyclonal anti-human IgM antibody and phycoerythrin-conjugated murine monoclonal anti-human FXIII antibody |
| Sample Matrix | Serum and Plasma | Serum, potassium EDTA or Sodium |

| Device Differences | | |
|---|---|---|
| Characteristics | New Device BioPlex 2200 ToRC IgM | Predicate Device BioPlex 2200 **Rubella** and CMV IgM Kit K092587 |
| | (EDTA and Heparin) | Heparin plasma |
| Calibrator(s) | 2 calibrator levels (sold separately) | 3 calibrator levels (sold separately) |

**Standard/Guidance Document Referenced (if applicable):**

EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, Third Edition (Vol. 34 No.13)
EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition (Vol. 25 No.27)
EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples, Approved Guideline- Third Edition (Vol. 33, No. 11)
EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline- Second Edition (Vol. 28, No. 3)
EP15-A3, User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition (Vol. 34, No. 12)
EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline (Vol. 29, No. 20)

**Test Principle:**

The BioPlex 2200 ToRC IgM kit employs a panel of three antigen-coated fluoromagnetic beads with unique fluorescent signatures to identify the presence of IgM class antibodies to *T. gondii*, Rubella, and CMV antigens in a two-step assay format. In the first step, the system combines an aliquot of patient sample with sample diluent and bead reagent then agitates the mixture at 37°C. In the second step, immobilized IgM is identified indirectly using a fluorescent anti-human IgM reporter conjugate in a manner similar to antibody detection using an enzyme-linked reporter in an EIA. The assay is calibrated using a set of two distinct calibrator vials, supplied separately by Bio-Rad Laboratories. One vial containing negative sample, one vial containing *T. gondii* IgM, Rubella IgM, and CMV IgM are used for qualitative calibration of the assays.

Two control beads are used. One is used to normalize assay output for fluctuations in detector function (internal standard bead, ISB) and the other control bead is used to verify that the sample is serum or plasma (serum verification bead, SVB). Refer to the BioPlex 2200 System Operation Manual for more information about control beads. The fluorescent properties of the beads allow multi-analyte data to be acquired simultaneously from a single sample and segregated based upon the fluorescent codes embedded in the antigen-coated and control beads. The magnetic properties of the beads allow rapid washing to remove unbound molecules in between assay steps. Bead classification and reporter data are acquired in flow using a dual laser detector employing the same principles utilized by fluorescence activated cell sorters. Raw data are reported as relative fluorescent intensity (RFI).

**Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*

Precision Studies:

Precision testing of the BioPlex 2200 ToRC IgM kit on the BioPlex 2200 instrument was performed in accordance with CLSI EP5-A3 guideline. Samples were tested in duplicate, two (2) runs per day, over 20 days (2 replicates per run x 2 runs per day x 20 days (N= 80 data points per panel member) using one reagent lot, calibrator set and control set.

The data were analyzed for within-run (repeatability), between-run, between-day, and total reproducibility and the mean (AI), standard deviation (AI) and percent coefficient of variation (%CV) are summarized below. SD was evaluated for samples with AI values <0.8 and %CV was used for samples with AI values >0.8.

**BioPlex 2200 Toxo IgM – CLSI EP5-A3 Precision**

| | | | | Within Run | | Between Run | | Between Day | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample Type** | ***T. gondii* IgM Panel Members** | **N** | **Mean (AI)** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Serum | Negative | 80 | 0.5 | 0.039 | 7.9% | 0.000 | 0.0% | 0.007 | 1.3% | 0.039 | 8.0% |
| | High Negative | 80 | 0.7 | 0.034 | 4.8% | 0.011 | 1.6% | 0.012 | 1.7% | 0.037 | 5.3% |
| | Cut-Off | 80 | 0.9 | 0.050 | 5.5% | 0.000 | 0.0% | 0.031 | 3.4% | 0.059 | 6.5% |
| | Cut-Off | 80 | 1.0 | 0.064 | 6.3% | 0.000 | 0.0% | 0.030 | 2.9% | 0.071 | 6.9% |
| | Low Positive | 80 | 1.3 | 0.057 | 4.3% | 0.042 | 3.2% | 0.039 | 2.9% | 0.081 | 6.1% |
| | Positive | 80 | 2.3 | 0.113 | 5.0% | 0.000 | 0.0% | 0.034 | 1.5% | 0.118 | 5.2% |
| | Positive | 80 | 3.3 | 0.164 | 4.9% | 0.034 | 1.0% | 0.053 | 1.6% | 0.175 | 5.3% |
| Potassium EDTA | Negative | 80 | 0.5 | 0.019 | 3.9% | 0.000 | 0.0% | 0.002 | 0.4% | 0.019 | 3.9% |
| | High Negative | 80 | 0.7 | 0.046 | 6.2% | 0.000 | 0.0% | 0.027 | 3.7% | 0.053 | 7.2% |
| | Cut-Off | 80 | 0.9 | 0.056 | 6.3% | 0.000 | 0.0% | 0.017 | 1.9% | 0.059 | 6.6% |
| | Low Positive | 80 | 1.2 | 0.073 | 6.0% | 0.000 | 0.0% | 0.049 | 4.0% | 0.088 | 7.3% |
| | Positive | 80 | 2.0 | 0.089 | 4.4% | 0.037 | 1.8% | 0.062 | 3.1% | 0.114 | 5.7% |
| | Positive | 80 | 3.2 | 0.167 | 5.3% | 0.059 | 1.9% | 0.098 | 3.1% | 0.203 | 6.4% |
| Sodium Heparin | Negative | 80 | 0.4 | 0.045 | 10.3% | 0.000 | 0.0% | 0.018 | 4.1% | 0.048 | 11.1% |
| | High Negative | 80 | 0.7 | 0.032 | 4.3% | 0.030 | 4.0% | 0.025 | 3.4% | 0.050 | 6.8% |
| | Cut-Off | 80 | 0.9 | 0.058 | 6.2% | 0.025 | 2.7% | 0.022 | 2.4% | 0.067 | 7.2% |
| | Low Positive | 80 | 1.3 | 0.068 | 5.3% | 0.000 | 0.0% | 0.035 | 2.7% | 0.076 | 5.9% |
| | Positive | 80 | 2.1 | 0.109 | 5.3% | 0.000 | 0.0% | 0.060 | 2.9% | 0.124 | 6.0% |
| | Positive | 80 | 3.1 | 0.117 | 3.8% | 0.000 | 0.0% | 0.049 | 1.6% | 0.127 | 4.1% |
| Lithium Heparin | Negative | 80 | 0.5 | 0.050 | 10.9% | 0.011 | 2.4% | 0.010 | 2.3% | 0.052 | 11.4% |
| | High Negative | 80 | 0.7 | 0.042 | 6.0% | 0.000 | 0.0% | 0.017 | 2.4% | 0.045 | 6.4% |
| | Cut-Off | 80 | 0.9 | 0.061 | 6.7% | 0.000 | 0.0% | 0.018 | 2.0% | 0.064 | 7.0% |
| | Low Positive | 80 | 1.3 | 0.063 | 5.0% | 0.050 | 4.0% | 0.019 | 1.5% | 0.083 | 6.6% |
| | Positive | 80 | 2.0 | 0.108 | 5.4% | 0.045 | 2.2% | 0.031 | 1.6% | 0.121 | 6.1% |
| | Positive | 80 | 3.1 | 0.145 | 4.7% | 0.000 | 0.0% | 0.064 | 2.1% | 0.159 | 5.1% |

## BioPlex 2200 Rubella IgM – CLSI EP5-A3 Precision

| | | | | Within Run | | Between Run | | Between-Day | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample Type** | **Rubella IgM Panel Members** | **N** | **Mean (AI)** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Serum | Negative | 80 | 0.3 | 0.011 | 3.7% | 0.000 | 0.0% | 0.000 | 0.0% | 0.011 | 3.7% |
| | High Negative | 80 | 0.6 | 0.040 | 6.6% | 0.000 | 0.0% | 0.016 | 2.6% | 0.043 | 7.1% |
| | Cut-Off | 80 | 0.8 | 0.049 | 5.9% | 0.000 | 0.0% | 0.014 | 1.6% | 0.051 | 6.1% |
| | Cut-Off | 80 | 1.1 | 0.066 | 5.8% | 0.000 | 0.0% | 0.031 | 2.7% | 0.073 | 6.4% |
| | Low Positive | 80 | 1.2 | 0.063 | 5.4% | 0.027 | 2.3% | 0.027 | 2.2% | 0.074 | 6.3% |
| | Positive | 80 | 1.5 | 0.085 | 5.7% | 0.039 | 2.6% | 0.000 | 0.0% | 0.094 | 6.2% |
| | Positive | 80 | 2.7 | 0.146 | 5.5% | 0.000 | 0.0% | 0.000 | 0.0% | 0.146 | 5.5% |
| | Positive | 80 | 3.0 | 0.157 | 5.3% | 0.035 | 1.2% | 0.091 | 3.0% | 0.185 | 6.2% |
| Potassium EDTA | Negative | 80 | 0.3 | 0.011 | 3.7% | 0.000 | 0.0% | 0.000 | 0.0% | 0.011 | 3.7% |
| | High Negative | 80 | 0.6 | 0.045 | 7.0% | 0.000 | 0.0% | 0.029 | 4.6% | 0.053 | 8.4% |
| | Cut-Off | 80 | 1.0 | 0.055 | 5.6% | 0.016 | 1.6% | 0.026 | 2.7% | 0.063 | 6.5% |
| | Low Positive | 80 | 1.1 | 0.064 | 5.7% | 0.016 | 1.4% | 0.016 | 1.4% | 0.068 | 6.1% |
| | Positive | 80 | 1.6 | 0.084 | 5.4% | 0.000 | 0.0% | 0.057 | 3.7% | 0.101 | 6.5% |
| | Positive | 80 | 2.4 | 0.118 | 4.9% | 0.000 | 0.0% | 0.084 | 3.5% | 0.145 | 6.0% |
| | Positive | 80 | 3.0 | 0.168 | 5.6% | 0.000 | 0.0% | 0.103 | 3.4% | 0.198 | 6.5% |
| Sodium Heparin | Negative | 80 | 0.3 | 0.045 | 13.3% | 0.011 | 3.3% | 0.014 | 4.1% | 0.048 | 14.4% |
| | High Negative | 80 | 0.6 | 0.042 | 6.8% | 0.011 | 1.8% | 0.023 | 3.7% | 0.049 | 8.0% |
| | Cut-Off | 80 | 1.0 | 0.045 | 4.6% | 0.034 | 3.4% | 0.029 | 2.9% | 0.063 | 6.4% |
| | Low Positive | 80 | 1.1 | 0.066 | 5.8% | 0.016 | 1.4% | 0.018 | 1.6% | 0.070 | 6.2% |
| | Positive | 80 | 1.6 | 0.092 | 5.7% | 0.000 | 0.0% | 0.044 | 2.7% | 0.102 | 6.3% |
| | Positive | 80 | 2.5 | 0.123 | 4.9% | 0.039 | 1.5% | 0.036 | 1.4% | 0.134 | 5.3% |
| | Positive | 80 | 3.2 | 0.155 | 4.9% | 0.019 | 0.6% | 0.029 | 0.9% | 0.159 | 5.0% |
| Lithium Heparin | Negative | 80 | 0.3 | 0.019 | 6.5% | 0.000 | 0.0% | 0.000 | 0.0% | 0.019 | 6.5% |
| | High Negative | 80 | 0.6 | 0.047 | 8.3% | 0.000 | 0.0% | 0.023 | 4.1% | 0.053 | 9.2% |
| | Cut-Off | 80 | 1.0 | 0.063 | 6.6% | 0.000 | 0.0% | 0.032 | 3.3% | 0.071 | 7.4% |
| | Low Positive | 80 | 1.2 | 0.079 | 6.6% | 0.000 | 0.0% | 0.027 | 2.2% | 0.083 | 7.0% |
| | Positive | 80 | 1.5 | 0.089 | 5.9% | 0.047 | 3.1% | 0.026 | 1.7% | 0.105 | 6.8% |
| | Positive | 80 | 2.6 | 0.158 | 6.1% | 0.000 | 0.0% | 0.000 | 0.0% | 0.158 | 6.1% |
| | Positive | 80 | 3.1 | 0.150 | 4.8% | 0.070 | 2.2% | 0.039 | 1.3% | 0.170 | 5.5% |

## BioPlex 2200 CMV IgM – CLSI EP5-A3 Precision

| | | | | Within Run | | Between Run | | Between-Day | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample Type** | **CMV IgM Panel Members** | **N** | **Mean (AI)** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Serum | Negative | 80 | 0.4 | 0.039 | 10.3% | 0.019 | 5.2% | 0.005 | 1.5% | 0.044 | 11.6% |
| | High Negative | 80 | 0.8 | 0.060 | 7.5% | 0.000 | 0.0% | 0.017 | 2.2% | 0.063 | 7.8% |
| | Cut-Off | 80 | 1.1 | 0.065 | 6.1% | 0.030 | 2.8% | 0.032 | 3.0% | 0.078 | 7.4% |
| | Cut-Off | 80 | 1.2 | 0.152 | 12.2% | 0.000 | 0.0% | 0.000 | 0.0% | 0.152 | 12.2% |
| | Low Positive | 80 | 1.2 | 0.089 | 7.2% | 0.000 | 0.0% | 0.000 | 0.0% | 0.089 | 7.2% |
| | Positive | 80 | 1.7 | 0.122 | 7.0% | 0.000 | 0.0% | 0.000 | 0.0% | 0.122 | 7.0% |
| | Positive | 80 | 3.0 | 0.196 | 6.6% | 0.000 | 0.0% | 0.030 | 1.0% | 0.198 | 6.7% |
| | Positive | 80 | 3.1 | 0.214 | 7.0% | 0.000 | 0.0% | 0.077 | 2.5% | 0.227 | 7.4% |
| Potassium EDTA | Negative | 80 | 0.3 | 0.047 | 13.8% | 0.000 | 0.0% | 0.022 | 6.5% | 0.052 | 15.3% |
| | High Negative | 80 | 0.8 | 0.047 | 6.2% | 0.030 | 3.9% | 0.015 | 1.9% | 0.058 | 7.5% |
| | Cut-Off | 80 | 1.1 | 0.070 | 6.5% | 0.000 | 0.0% | 0.028 | 2.6% | 0.075 | 7.0% |
| | Low Positive | 80 | 1.3 | 0.089 | 6.7% | 0.000 | 0.0% | 0.000 | 0.0% | 0.089 | 6.7% |
| | Positive | 80 | 1.9 | 0.128 | 6.8% | 0.000 | 0.0% | 0.078 | 4.1% | 0.150 | 8.0% |
| | Positive | 80 | 3.1 | 0.185 | 6.0% | 0.000 | 0.0% | 0.088 | 2.9% | 0.205 | 6.7% |
| | Positive | 80 | 2.9 | 0.196 | 6.7% | 0.000 | 0.0% | 0.122 | 4.2% | 0.231 | 7.9% |
| Sodium Heparin | Negative | 80 | 0.3 | 0.022 | 7.5% | 0.000 | 0.0% | 0.000 | 0.0% | 0.022 | 7.5% |
| | High Negative | 80 | 0.8 | 0.065 | 7.8% | 0.000 | 0.0% | 0.000 | 0.0% | 0.065 | 7.8% |
| | Cut-Off | 80 | 0.8 | 0.047 | 6.3% | 0.022 | 3.0% | 0.017 | 2.3% | 0.055 | 7.3% |
| | Low Positive | 80 | 1.3 | 0.083 | 6.2% | 0.019 | 1.4% | 0.000 | 0.0% | 0.085 | 6.3% |
| | Positive | 80 | 2.0 | 0.121 | 6.0% | 0.000 | 0.0% | 0.048 | 2.4% | 0.130 | 6.5% |
| | Positive | 80 | 3.1 | 0.193 | 6.2% | 0.000 | 0.0% | 0.064 | 2.1% | 0.203 | 6.5% |
| | Positive | 80 | 3.1 | 0.167 | 5.4% | 0.000 | 0.0% | 0.076 | 2.5% | 0.184 | 6.0% |
| Lithium Heparin | Negative | 80 | 0.3 | 0.040 | 14.2% | 0.000 | 0.0% | 0.000 | 0.0% | 0.040 | 14.2% |
| | High Negative | 80 | 0.7 | 0.060 | 8.8% | 0.000 | 0.0% | 0.022 | 3.3% | 0.064 | 9.4% |
| | Cut-Off | 80 | 1.0 | 0.085 | 8.7% | 0.000 | 0.0% | 0.037 | 3.7% | 0.093 | 9.5% |
| | Low Positive | 80 | 1.4 | 0.104 | 7.5% | 0.000 | 0.0% | 0.055 | 4.0% | 0.118 | 8.5% |
| | Positive | 80 | 1.9 | 0.133 | 7.1% | 0.019 | 1.0% | 0.042 | 2.2% | 0.140 | 7.6% |
| | Positive | 80 | 3.0 | 0.222 | 7.5% | 0.096 | 3.2% | 0.000 | 0.0% | 0.242 | 8.1% |
| | Positive | 80 | 2.7 | 0.153 | 5.7% | 0.030 | 1.1% | 0.000 | 0.0% | 0.156 | 5.8% |

Reproducibility Studies:

A reproducibility panel, consisting of six (6) panel members made using serum matrix and BioPlex ToRC IgM QC controls was prepared by Bio-Rad Laboratories. The serum sample panel includes one (1) low negative, one (1) high negative, one (1) low positive near cut-off, one (1) medium positive, and one (1) high positive sample, and one (1) positive control for all three (3) analytes. Reproducibility testing was performed at three (3) US testing facilities using one (1) lot of the BioPlex 2200 ToRC IgM Pack, one (1) lot of BioPlex 2200 ToRC IgM Calibrator Set and one (1) lot of BioPlex 2200 ToRC IgM Control Set. The panels were provided to each of the testing sites. Each of the panel members and control sets were tested in replicates of four (4) on two runs per day over five (5) days at three (3) sites (4 replicates x 2 run x 5 days x 3 Sites = 120 replicates per panel member). The data were analyzed for within-run (repeatability), between-run, between-day, between-site and total precision according to the principles described in Clinical Laboratory Standards Institute (CLSI) EP15-A3. The grand mean, standard deviation (SD) and percent coefficient of variation (% CV) were calculated. SD was evaluated for samples with AI values <0.8 and %CV was used for samples with AI values >0.8.

**BioPlex 2200 Toxo IgM – CLSI EP15-A3 Reproducibility**

| ***T. gondii* IgM Panel Member** | **Mean (AI)** | **Within Run** | | **Between Run** | | **Between Day** | | **Between Site** | | **Total** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Positive Control | 2.0 | 0.092 | 4.7% | 0.103 | 5.2% | 0.000 | 0.0% | 0.178 | 9.0% | 0.225 | 11.4% |
| Low Negative | 0.2 | 0.029 | 13.8% | 0.016 | 7.6% | 0.000 | 0.0% | 0.010 | 4.7% | 0.035 | 16.5% |
| High Negative | 0.8 | 0.040 | 4.8% | 0.012 | 1.5% | 0.028 | 3.3% | 0.100 | 12.0% | 0.112 | 13.4% |
| Low Positive | 1.3 | 0.051 | 4.0% | 0.032 | 2.5% | 0.023 | 1.8% | 0.148 | 11.6% | 0.162 | 12.7% |
| Mid Positive | 2.2 | 0.081 | 3.7% | 0.058 | 2.6% | 0.032 | 1.5% | 0.257 | 11.7% | 0.278 | 12.7% |
| High Positive | 3.2 | 0.172 | 5.3% | 0.093 | 2.9% | 0.043 | 1.3% | 0.280 | 8.7% | 0.344 | 10.7% |

**BioPlex 2200 Rubella IgM – CLSI EP15-A3 Reproducibility**

| **Rubella IgM Panel Member** | **Mean (AI)** | **Within Run** | | **Between Run** | | **Between Day** | | **Between Site** | | **Total** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Positive Control | 1.9 | 0.086 | 4.5% | 0.053 | 2.8% | 0.043 | 2.3% | 0.198 | 10.5% | 0.227 | 12.0% |
| Low Negative | 0.3 | 0.026 | 9.3% | 0.013 | 4.7% | 0.000 | 0.0% | 0.042 | 15.3% | 0.051 | 18.5% |
| High Negative | 0.8 | 0.048 | 6.2% | 0.000 | 0.0% | 0.026 | 3.3% | 0.097 | 12.4% | 0.111 | 14.2% |
| Low Positive | 1.2 | 0.056 | 4.8% | 0.019 | 1.7% | 0.000 | 0.0% | 0.143 | 12.3% | 0.155 | 13.3% |
| Mid Positive | 1.5 | 0.051 | 3.5% | 0.014 | 1.0% | 0.027 | 1.8% | 0.197 | 13.6% | 0.206 | 14.2% |
| High Positive | 2.8 | 0.139 | 4.9% | 0.015 | 0.5% | 0.030 | 1.1% | 0.328 | 11.6% | 0.358 | 12.6% |

**BioPlex 2200 CMV IgM – CLSI EP15-A3 Reproducibility**

| **CMV IgM Panel Member** | **Mean (AI)** | **Within Run** | | **Between Run** | | **Between Day** | | **Between Site** | | **Total** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Positive Control | 2.3 | 0.098 | 4.3% | 0.053 | 2.4% | 0.058 | 2.6% | 0.145 | 6.4% | 0.192 | 8.5% |
| Low Negative | 0.3 | 0.032 | 9.1% | 0.000 | 0.0% | 0.013 | 3.8% | 0.052 | 15.1% | 0.063 | 18.1% |
| High Negative | 0.7 | 0.048 | 7.0% | 0.011 | 1.6% | 0.009 | 1.3% | 0.062 | 9.0% | 0.080 | 11.6% |
| Low Positive | 1.2 | 0.071 | 6.2% | 0.029 | 2.5% | 0.000 | 0.0% | 0.096 | 8.3% | 0.123 | 10.6% |
| Mid Positive | 1.7 | 0.081 | 4.7% | 0.021 | 1.3% | 0.038 | 2.3% | 0.208 | 12.2% | 0.227 | 13.4% |
| High Positive | 2.9 | 0.146 | 5.1% | 0.026 | 0.9% | 0.065 | 2.3% | 0.257 | 9.0% | 0.304 | 10.6% |

*b. Linearity/assay reportable range:*

Not Applicable.

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

Calibrator Traceability

The BioPlex ToRC IgM calibrators are traceable against frozen internal standards which are anchored to a quantitation panel. The quantitation panel consists of patient samples whose analyte values span the assay range.

The BioPlex 2200 ToRC IgM Reagent Kit is calibrated using a set of two distinct serum based calibrators. A negative and multianalyte *T. gondii*, Rubella CMV IgM calibrator is used to calibrate the *T. gondii*, Rubella and CMV IgM assays. The cut-off value and assignment of the calibrators are determined by determining the 98-99th percentile on normal samples and performing concordance and Receiver Operator Characteristic (ROC) analysis during feasibility and development.

The manufacturing target ranges of the Calibrator Set are listed below.

| Calibrator Set | Range (AI) |
|---|---|
| Calibrator Level 1 | 0.0 – 0.2 |
| Calibrator Level 2 | 1.1 – 2.0 |

Controls

The control set includes two vials each of negative control, multianalyte *T. gondi*i, Rubella and CMV IgM. The positive controls contain known concentrations of human *T. gondii*, Rubella and CMV IgM, and are prepared by blending human disease state serum (*T. gondii* and CMV) and human recombinant Rubella IgM (huCal) with ToRC IgM negative serum matrix.

The assignment for the BioPlex 2200 ToRC IgM Control Set is performed using a minimum of two reagent lots along with matched calibrators with a target of three reagent lots with matched calibrators. For each control lot, three vials per control level are tested in replicates of five on all reagent lots for a total of fifteen replicates per reagent lot. This testing is performed on three analyzers yielding a total of forty-five replicates per reagent lot. The total number of replicates for each control level is 90 when two reagent lots are used and 135 when three reagent lots are used.

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

| Control Set | Range (AI) |
|---|---|
| Negative Control | 0.0 – 0.5 |
| Positive Control | 2.0 – 2.6 |

Kit Stability

BioPlex 2200 ToRC IgM Kit: Real Time (unopened) Kit Stability, 12 months 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.

Calibrator and Control Stability

BioPlex 2200 ToRC IgM Control and Calibrator Sets:
Calibrator Open Vial Stability (2 to 8°C), 60 days from first opening; Control Open Vial Stability (2 to 8°C), 60 days from first opening; Onboard Calibration Curve Stability, 30 days; Real Time Stability of calibrators and controls (2 to 8°C), 12 months; labeled as until expiration date; Calibrators and Controls Accelerated Stability (2 to 8°C), 18 months predicted. Calibrators and Controls Freeze-thaw (-20ºC or -70ºC), 5 freeze thaw cycles.

Sample Stability

Serum or plasma (K3 EDTA, lithium heparin, or sodium heparin) samples may be stored at room temperature (18 – 30°C) for up to 3 days and under refrigeration (2 – 8°C) for up to 7 days. For longer storage of samples, keep at -20°C or colder. Up to 5-freeze thaw cycles at -20ºC and -70ºC is acceptable.

*d. Detection limit:*
Not Applicable.

*e. Analytical specificity:*

Cross Reactivity:

The study was conducted to determine if samples from various disease states interfere with test results when tested with the BioPlex 2200 ToRC IgM kit. A panel of at least ten (10) specimens that are positive for each cross reactant were evaluated for possible cross reactivity with the BioPlex 2200 ToRC IgM kit for each of the three antibody assays. The cross reactive samples were tested in commercially available predicate kits in order to confirm their negative status for the target analyte.

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 set of samples The results of each potential cross reactant are listed below.

**BioPlex 2200 ToRC IgM Cross Reactivity**

| Potential Cross Reactant | *T. gondii* IgM | | | Rubella IgM | | | CMV IgM | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | Neg | Pos | N | Neg | Pos | N | Neg | Pos |
| ANA Screen | 19 | 19 | 0 | 19 | 19 | 0 | 19 | 19 | 0 |
| CMV IgM | 10 | 10 | 0 | 10 | 10 | 0 | N/A | N/A | 0 |
| EBV IgM | 11 | 11 | 0 | 11 | 11 | 0 | 11 | 11 | 0 |
| HAMA | 15 | 15 | 0 | 15 | 15 | 0 | 15 | 15 | 0 |
| hCG | 12 | 12 | 0 | 12 | 12 | 0 | 12 | 12 | 0 |
| HIV | 10 | 10 | 0 | 10 | 10 | 0 | 10 | 10 | 0 |
| HSV-1/2 IgM | 13 | 13 | 0 | 13 | 13 | 0 | 13 | 13 | 0 |
| Hypergamma-globulinemia IgM | 21 | 21 | 0 | 21 | 20 | 1 | 21 | 21 | 0 |
| Influenza | 18 | 17 | 1 | 18 | 18 | 0 | 18 | 18 | 0 |
| Measles IgM | 16 | 16 | 0 | 16 | 16 | 0 | 16 | 16 | 0 |
| Mumps IgM | 13 | 12 | 1 | 13 | 13 | 0 | 13 | 13 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Multiple Myeloma | 17 | 17 | 0 | 17 | 17 | 0 | 17 | 17 | 0 |
| Parvovirus B 19 IgM | 14 | 14 | 0 | 14 | 13 | 1 | 14 | 14 | 0 |
| Rheumatoid Factor | 11 | 11 | 0 | 11 | 11 | 0 | 11 | 11 | 0 |
| Rubella IgM | 16 | 16 | 0 | N/A | N/A | 0 | 16 | 16 | 0 |
| Toxo IgM | N/A | N/A | 0 | 10 | 10 | 0 | 10 | 10 | 0 |
| VZV IgM | 13 | 12 | 1 | 13 | 13 | 0 | 13 | 12 | 1 |

Interfering Substances:

An interfering substances study was conducted to evaluate the potential interference of specific endogenous and exogenous substances with the BioPlex 2200 ToRC IgM kit according to CLSI EP7-A2 guideline.

No significant interference was observed in 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 |
| Cholesterol | ≤ 500 mg/dL |
| Red Blood Cells | ≤ 0.4% (v/v) |
| Gamma Globulin | ≤ 6 g/dL |
| Triglycerides | ≤ 3300 mg/dL |
| Beta Carotene | ≤ 0.6 mg/dL |
| Protein (total) | ≤ 12 g/dL |
| Ascorbic Acid | ≤ 6 mg/dL |
| Sodium Heparin | ≤ 8000 units/dL |
| Lithium Heparin | ≤ 8000 units/dL |
| EDTA (K2 and K3) | ≤ 800 mg/dL |

High dose hook effect:
Not Applicable

*f. Assay cut-off:*

A final cut-off of 1.0 AI was established for the BioPlex 2200 ToRC IgM assay based on an evaluation of 401, 454 and 511 test ordered and retrospective positive samples for *T. gondii*, Rubella and CMV IgM, respectively, of which the serological status was determined from the predicate *T. gondii*, Rubella and CMV IgM assays. The assay will employ an equivocal zone that brackets the cut-off, which results in samples >1.1 AI being positive and <0.9 AI being negative. This analysis was used to optimize sensitivity and specificity for the BioPlex 2200 ToRC IgM assay.

2. Comparison studies:

*Matrix comparison:*

Matched serum and plasma (EDTA and heparin sodium) samples drawn from the same donor were acquired from an outside reference lab. For each assay in the panel, a minimum of 40 sets of paired serum and plasma samples were prepared and values within the measurement range of the assay were analyzed in accordance with CLSI EP09-A3. Samples were assayed in replicates of two (2) with the second replicate run in reverse order. Mean plasma AI values were compared to matched mean serum AI values. Linear regression analysis was used to determine the presence of a matrix effect when compared to serum. The regression correlation parameters for slope, intercept and correlation coefficient (r) are shown below.

| Matrix Comparison | N | BioPlex ToRC IgM Assay | Slope (95% CI) | Intercept (95% CI) | Correlation (r) |
|---|---|---|---|---|---|
| K3 EDTA vs. Serum | 54 | *T. gondii* IgM | 1.02 (0.99 to 1.06) | -0.05 (-0.12 to 0.02) | 0.994 |
| | 53 | Rubella IgM | 1.03 (1.00 to 1.06) | -0.02 (-0.08 to 0.05) | 0.994 |
| | 60 | CMV IgM | 1.01 (0.97 to 1.05) | -0.02 (-0.10 to 0.05) | 0.989 |
| Lithium Heparin vs. Serum | 54 | *T. gondii* IgM | 1.02 (0.98 to 1.06) | -0.04 (-0.12 to 0.04) | 0.992 |
| | 51 | Rubella IgM | 1.03 (1.00 to 1.06) | -0.02 (-0.08 to 0.04) | 0.995 |
| | 60 | CMV IgM | 0.98 (0.93 to 1.02) | 0.00 (-0.09 to 0.09) | 0.985 |
| Sodium Heparin vs. Serum | 54 | *T. gondii* IgM | 1.03 (0.99 to 1.06) | -0.05 (-0.12 to 0.02) | 0.994 |
| | 51 | Rubella IgM | 1.01 (0.97 to 1.05) | -0.01 (-0.09 to 0.06) | 0.993 |
| | 59 | CMV IgM | 0.99 (0.96 to 1.03) | -0.04 (-0.11 to 0.04) | 0.991 |

3. Clinical studies:

*a. Clinical Sensitivity:*

Not Applicable

*b. Clinical Specificity:*
Not Applicable

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

4. Clinical cut-off:
Not Applicable

5. Expected values/Reference range:

Expected values for the BioPlex 2200 ToRC IgM kit are presented by age and gender in the following tables for samples from pregnant women and patients sent to the lab for *T. gondii*, Rubella or CMV IgM testing. A total of two hundred (200) each of pregnant women samples sent to the lab for *T. gondii*, Rubella or CMV IgM testing and approximately five hundred (500) each of samples sent to the lab for *T. gondii*, Rubella or CMV IgM testing were tested.

**BioPlex 2200 ToRC IgM Prevalence Results by Age and Gender: Samples from pregnant women who were sent for *T.gondii*, Rubella, or CMV IgM testing by Age Group**

| Age | *T. gondii* IgM | | Rubella IgM | | CMV IgM | |
|---|---|---|---|---|---|---|
| | Pos/Total | % Prevalence | Pos/Total | % Prevalence | Pos/Total | % Prevalence |
| 15-25 | 1/81 | 1.2% | 0/86 | 0.0% | 4/85 | 4.7% |
| 26-35 | 2/98 | 2.0% | 0/93 | 0.0% | 4/100 | 4.0% |
| 36-43 | 0/21 | 0.0% | 0/21 | 0.0% | 0/15 | 0.0% |
| Total | 3/200 | 1.5% | 0/200 | 0.0% | 8/200 | 4.0% |

**BioPlex 2200 ToRC IgM Prevalence Results by Age and Gender: Samples from patients who were sent for *T.gondii*, Rubella, or CMV IgM testing**

| Age | Gender | T. gondii IgM | | Rubella IgM | | CMV IgM | |
|---|---|---|---|---|---|---|---|
| | | Pos/Total | % Prevalence | Pos/Total | % Prevalence | Pos/Total | % Prevalence |
| <1-90 | F | 5/357 | 1.4% | 3/357 | 0.8% | 14/325 | 4.3% |
| | M | 5/137 | 3.6% | 2/155 | 1.3% | 9/198 | 4.5% |
| Total | | 10/494 | 2.0% | 5/512 | 1.0%* | 23/523 | 4.4% |

*Since 2001 the incidence of Rubella in the US has been less than 10/1,000,000 population

6. Other Clinical Supportive Data:

*a. Method comparison with predicate device:*
Method comparison studies were performed following CLSI EP12-A2 guideline.

Performance of the BioPlex 2200 ToRC IgM kit was tested against corresponding commercially available predicate *T. gondii*, Rubella and CMV IgM assays. A total of 2129 prospective samples (~700 samples per analyte) submitted for *T. gondii*, Rubella, or CMV testing were tested at 3 U.S. clinical testing sites. Of the approximate 700 samples per analyte, 200 were from pregnant women for each analyte. Results from all sites are shown and summarized below.

Comparative Testing: Prospective

**BioPlex 2200 ToRC IgM vs commercially available immunoassay for samples sent for *T. gondii*, Rubella or CMV IgM testing**

| Test Ordered | | | | BioPlex 2200 ToRC IgM Agreement | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Pos (+) | Eqv | Neg (-) | Total | Pos (+) % Agreement 95% CI | Neg (-) % Agreement 95% CI |
| Commercially Available Immunoassay | *T. gondii* IgM | Pregnant Women | Pos (+) | 0 | 0 | 0 | 0 | N/A | 98.0% (196/200) 95.0 to 99.2% |
| | | | Eqv | 0 | 0 | 0 | 0 | | |
| | | | Neg (-) | 3 | 1 | 196 | 200 | | |
| | | | Total | 3 | 1 | 196 | 200 | | |
| | | Test Ordered | Pos (+) | 0 | 0 | 0 | 0 | N/A | 97.4% (481/494) 95.6 to 98.5% |
| | | | Eqv | 1 | 0 | 0 | 1 | | |
| | | | Neg (-) | 9 | 3 | 481 | 493 | | |
| | | | Total | 10 | 3 | 481 | 494 | | |
| | Rubella IgM | Pregnant Women | Pos (+) | 0 | 0 | 0 | 0 | N/A | 100.0% (198/198) 98.1 to 100.0% |
| | | | Eqv | 0 | 0 | 2 | 2 | | |
| | | | Neg (-) | 0 | 0 | 198 | 198 | | |
| | | | Total | 0 | 0 | 200 | 200 | | |
| | | Test Ordered | Pos (+) | 4 | 1 | 2 | 7 | 40.0% (4/10)* 16.8 to 68.7% | 99.6% (498/500) 98.6 to 99.9% |
| | | | Eqv | 0 | 2 | 3 | 5 | | |
| | | | Neg (-) | 1 | 1 | 498 | 500 | | |
| | | | Total | 5 | 4 | 503 | 512 | | |
| | CMV IgM | Pregnant Women | Pos (+) | 8 | 2 | 4 | 14 | 50.0% (8/16)** 28.0 to 72.0% | 100.0% (183/183) 97.9 to 100.0% |
| | | | Eqv | 0 | 1 | 2 | 3 | | |
| | | | Neg (-) | 0 | 0 | 183 | 183 | | |
| | | | Total | 8 | 3 | 189 | 200 | | |
| | | Test Ordered | Pos (+) | 20 | 1 | 11 | 32 | 55.6% (20/36)*** 39.6 to 70.5% | 98.6% (480/487) 97.1 to 99.3% |
| | | | Eqv | 0 | 0 | 4 | 4 | | |
| | | | Neg (-) | 3 | 4 | 480 | 487 | | |
| | | | Total | 23 | 5 | 495 | 523 | | |

* 10 samples were considered predicate positive (7 positives and 3 equivocal). Of these 10 samples BioPlex Rubella IgM was negative for 5 of 10 and equivocal on 1 of 10.
** 2 out of the 16 CMV IgM pregnant women samples were equivocal by predicate and negative by BioPlex CMV IgM. 14 out of 16 were positive by predicate and BioPlex results in these were as follows: 2 were equivocal, 4 were negative and 8 were positive by Bioplex CMV IgM. Of the 8 discrepant samples, 7 were negative and one equivocal by another FDA cleared device.
*** 4 out of 36 CMV IgM test ordered samples were equivocal by predicate device and negative by BioPlex CMV IgM. 32 out of 36 were positive by predicate and BioPlex results in these were as follows: 1 was equivocal, 11 were negative and 20 were positive by Bioplex CMV IgM. 13 of the 16 discrepant samples were confirmed negative by another FDA cleared device

Performance of the BioPlex 2200 ToRC IgM kit was also evaluated against corresponding commercially available *T. gondii*, Rubella, and CMV IgM immunoassays using presumptive positive samples. Three clinical sites tested 210 *T. gondii* (134 female, 76 male), 101 Rubella (44 female, 57 male) and 213 CMV (119 female, 94 male) IgM presumptive positive samples. Presumed positive banked samples for ToRC IgM were further selected by the respective predicate
device used for the comparative analysis. The characteristics of samples with presumptive positive status are shown below.

Comparative Testing: Retrospective

**BioPlex 2200 ToRC IgM vs commercially available immunoassay for *T. gondii*, Rubella or CMV IgM presumptive positive samples**

| Presumptive Positive for *T. gondii*, Rubella or CMV IgM | | | BioPlex 2200 ToRC IgM Agreement | | | | |
|---|---|---|---|---|---|---|---|
| | | | Pos (+) | Eqv | Neg (-) | Total | Pos (+) % Agreement 95% CI |
| Commercially Available Immunoassay | *T.gondii* IgM | Pos (+) | 203 | 3 | 3 | 209 | 97.1% (203/209) 93.9 to 98.7% |
| | | Eqv | 1 | 0 | 0 | 1 | |
| | | Neg (-) | 0 | 0 | 0 | 0 | |
| | | Total | 204 | 3 | 3 | 210 | |
| | Rubella IgM | Pos (+) | 96 | 1 | 1 | 98 | 98.0% (96/98) 92.9 to 99.4% |
| | | Eqv | 1 | 0 | 0 | 1 | |
| | | Neg (-) | 1 | 0 | 1 | 2 | |
| | | Total | 98 | 1 | 2 | 101 | |
| | CMV IgM | Pos (+) | 198 | 2 | 1 | 201 | 98.5% (198/201) 95.7 to 99.5% |
| | | Eqv | 4 | 1 | 0 | 5 | |
| | | Neg (-) | 4 | 0 | 3 | 7 | |
| | | Total | 206 | 3 | 4 | 213 | |

*b. Correlation with CDC Evaluation Panels:*

A correlation study was performed to evaluate the characteristics of the BioPlex 2200 ToRC IgM kit with serum panels provided by the Centers for Disease Control and Prevention (CDC) for T. gondii IgM. The results are presented as a means to convey further information on the performance. This does not imply an endorsement of the BioPlex 2200 ToRC IgM kit by the CDC. Results are presented in the table below.

**Characteristics of CDC *T. gondii* Reference Sera (N=97)**

| CDC Panel *T. gondii* IgM | BioPlex *T. gondii* IgM | | | | | |
|---|---|---|---|---|---|---|
| | Pos(+) | Eq | Neg(-) | Total | Positive Agreement | Negative Agreement |
| Pos (+) | 32 | 0 | 0 | 32 | 100.0%<br>89.3 to 100.0 | 100.0%<br>94.4 to 100.0 |
| Neg (-) | 0 | 0 | 65 | 65 | | |
| Total | 32 | 0 | 65 | 97 | | |

*c. Seroconversion Testing:*

Commercially available seroconversion panels for *T. gondii*, Rubella and CMV IgM were tested with the BioPlex 2200 ToRC IgM kit and compared with the predicate commercial method.

**BioPlex 2200 ToRC IgM Kit, Comparison to Commercial Method – *T. gondii* IgM**

| Panel | Day | *T. gondii* IgM | |
|---|---|---|---|
| | | VIDAS Toxo IgM | BioPlex ToRC IgM |
| | | Index | Antibody Index (AI) |
| Antibody Systems | 0 | 0.24(Neg) | <0.2(Neg) |
| | 7 | 1.62(Pos) | 2.1(Pos) |
| | 9 | 3.24(Pos) | 3.3(Pos) |
| | 14 | 5.78(Pos) | >4.0(Pos) |
| | 16 | 5.80(Pos) | >4.0(Pos) |
| | 23 | 5.38(Pos) | >4.0(Pos) |
| | 27 | 5.24(Pos) | >4.0(Pos) |
| | 30 | 5.04(Pos) | >4.0(Pos) |
| | 49 | 4.59(Pos) | >4.0(Pos) |
| | 53 | 4.35(Pos) | >4.0(Pos) |
| | 86 | 3.30(Pos) | >4.0(Pos) |
| | 88 | 2.94(Pos) | 3.6(Pos) |
| | 93 | 2.67(Pos) | 3.1(Pos) |
| | 95 | 2.62(Pos) | 2.7(Pos) |
| | 100 | 2.58(Pos) | 2.8(Pos) |
| | 104 | 2.34(Pos) | 2.6(Pos) |
| | 107 | 2.36(Pos) | 2.2(Pos) |
| | 112 | 2.28(Pos) | 2.4(Pos) |
| | 114 | 2.21(Pos) | 2.3(Pos) |
| | 119 | 2.29(Pos) | 2.1(Pos) |

**BioPlex 2200 ToRC IgM Kit, Comparison to Commercial Method – Rubella IgM**

| Panel | Day | Rubella IgM | |
|---|---|---|---|
| | | BioPlex RC IgM | BioPlex ToRC IgM |
| | | Antibody Index (AI) | Antibody Index (AI) |
| Liquicheck - RP011 | 0 | <0.2 (Neg) | <0.2 (Neg) |
| | 3 | <0.2 (Neg) | <0.2 (Neg) |
| | 9 | <0.2 (Neg) | <0.2 (Neg) |
| | 12 | <0.2 (Neg) | <0.2 (Neg) |
| | 16 | 1.3 (Pos) | 1.0 (Eq) |
| | 19 | >4.0 (Pos) | >4.0 (Pos) |
| | 24 | >4.0 (Pos) | >4.0 (Pos) |
| | 27 | >4.0 (Pos) | >4.0 (Pos) |
| | 31 | 3.6 (Pos) | 3.4 (Pos) |
| | 36 | 2.1 (Pos) | 2.0 (Pos) |
| | 39 | 1.5 (Pos) | 1.4 (Pos) |
| | 43 | 1.0 (Eq) | 1.0 (Eq) |
| | 46 | 0.7 (Neg) | 0.6 (Neg) |
| | 50 | 0.6 (Neg) | 0.5 (Neg) |
| | 53 | 0.3 (Neg) | 0.4 (Neg) |
| | 57 | 0.3 (Neg) | 0.4 (Neg) |
| | 60 | 0.3 (Neg) | 0.3 (Neg) |
| | 64 | 0.2 (Neg) | 0.2 (Neg) |
| | 67 | 0.2 (Neg) | 0.2 (Neg) |
| | 71 | 0.2 (Neg) | 0.2 (Neg) |

**BioPlex 2200 ToRC IgM Kit, Comparison to Commercial Method – CMV IgM**

| Panel | Day | CMV IgM | |
|---|---|---|---|
| | | BioPlex RC IgM | BioPlex ToRC IgM |
| | | Antibody Index (AI) | Antibody Index (AI) |
| Liquicheck - RP003 | 1 | >4.0 (Pos) | 2.8 (Pos) |
| | 4 | >4.0 (Pos) | >4.0 (Pos) |
| | 8 | >4.0 (Pos) | >4.0 (Pos) |
| | 51 | 1.9 (Pos) | 2.3 (Pos) |
| | 55 | 1.8 (Pos) | 2.1 (Pos) |
| | 59 | 1.2 (Pos) | 1.8 (Pos) |
| | 65 | 1.3 (Pos) | 1.5 (Pos) |
| | 67 | 1.4 (Pos) | 1.5 (Pos) |
| | 72 | 1.3 (Pos) | 1.1 (Pos) |
| | 74 | 1.1 (Pos) | 1.0 (Eq) |
| | 79 | 1.6 (Pos) | 1.2 (Pos) |
| | 84 | 1.4 (Pos) | 1.3 (Pos) |
| | 88 | 1.3 (Pos) | 1.4 (Pos) |
| | 95 | 1.3 (Pos) | 1.1 (Pos) |
| | 99 | 1.4 (Pos) | 1.0 (Eq) |

*d. IgM Specificity:*

Samples positive for *T. gondii*, Rubella and CMV IgM and IgG were treated with dithiothreitol (DTT) to inactivate IgM activity. The samples were assayed neat and diluted into assay range in replicates of two. IgM was measured using the BioPlex 2200 ToRC IgM kit.

**BioPlex ToRC IgM - *T. gondii* IgM Specificity**

| Sample ID | *T. gondii* IgM (AI) Before DTT | DTT Treatment AI (% recovery) |
|---|---|---|
| Sample 1 | 9.5 | 0.1 (1.05%) |
| Sample 2 | 9.5 | 0.1 (1.05%) |
| Sample 3 | 18.0 | 0.3 (1.67%) |
| Sample 4 | 15.5 | 0.5 (3.23%) |
| Sample 5 | 9.0 | 0.1 (1.11%) |
| Sample 6 | 14.0 | 0.2 (1.43%) |
| Sample 7 | 14.0 | 0.1 (0.71%) |
| Sample 8 | 15.0 | 0.1 (0.67%) |
| Sample 9 | 15.0 | 0.1 (0.67%) |
| Sample 10 | 16.5 | 0.1 (0.61%) |

**BioPlex ToRC IgM – Rubella IgM Specificity**

| Sample ID | Rubella IgM (AI) Before DTT | DTT Treatment AI (% recovery) |
|---|---|---|
| Sample 1 | 8.0 | 0.1 (1.25%) |
| Sample 2 | 9.0 | 0.0 (0.00%) |
| Sample 3 | 8.5 | 0.0 (0.00%) |
| Sample 4 | 5.0 | 0.1 (2.00%) |
| Sample 5 | 9.0 | 0.1 (1.11%) |
| Sample 6 | 7.0 | 0.1 (1.43%) |
| Sample 7 | 4.0 | 0.0 (0.00%) |
| Sample 8 | 5.0 | 0.0 (0.00%) |
| Sample 9 | 9.0 | 0.2 (2.22%) |
| Sample 10 | 6.0 | 0.1 (1.67%) |

**BioPlex ToRC IgM – CMV IgM Specificity**

| Sample ID | CMV IgM (AI) Before DTT | DTT Treatment AI (% recovery) |
|---|---|---|
| Sample 1 | 25.0 | 0.1 (0.40%) |
| Sample 2 | 11.0 | 0.2 (1.82%) |
| Sample 3 | 8.5 | 0.1 (1.18%) |
| Sample 4 | 20.0 | 0.5 (2.50%) |
| Sample 5 | 15.5 | 0.2 (1.29%) |
| Sample 6 | 18.5 | 0.2 (1.08%) |
| Sample 7 | 21.0 | 0.2 (0.95%) |
| Sample 8 | 6.0 | 0.0 (0.00%) |
| Sample 9 | 16.5 | 1.2 (7.27%) |
| Sample 10 | 31.5 | 0.7 (2.22%) |