

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K080008

B. Purpose for Submission:

New device

C. Measurand:

Toxoplasma gondii IgG, Rubella IgG and Cytomegalovirus (CMV) IgG

D. Type of Test:

Multiplex flow immunoassay (multiplexed fluoromagnetic bead assay)

E. Applicant:

BIO-RAD LABORATORIES, INC.

F. Proprietary and Established Names:

BioPlex™ 2200 ToRC IgG Kit

BioPlex™ 2200 ToRC IgG Calibrator Set

BioPlex™ 2200 ToRC IgG Control Set

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
Multiplex flow immunoassay, <i>T.gondii</i> , rubella and CMV (OMI)	Class II	866.3510 - Rubella virus Serological Reagents.	Microbiology
Calibrator, multi-analyte mixture (JIX)	Class II	862.1150 - Calibrator.	Clinical Chemistry
Multi-analyte controls, all kinds (assayed and unassayed) (JJY)	Class I	862.1660 - Quality Control Material (Assayed and Unassayed).	Clinical Chemistry

H. Intended Use:

1. Intended use(s):

BioPlex™ 2200 ToRC IgG Kit

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

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

This kit is intended as an aid in the determination of serological status to *T. gondii*, Rubella and CMV. This kit is not intended for use in screening blood or plasma donors.

Performance characteristics for *T. gondii* and Rubella have not been evaluated in immunocompromised or immunosuppressed individuals. Performance characteristics for CMV 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 a point of care.

BioPlex™ 2200 ToRC IgG Calibrator Set

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

BioPlex™ 2200 ToRC IgG Control Set

The BioPlex 2200 ToRC IgG Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and BioPlex ToRG IgG Reagent Pack in the clinical laboratory. The performance of the BioPlex 2200 ToRC IgG Control Set has not been established with any other *Toxoplasma gondii*, Rubella or Cytomegalovirus (CMV) IgG antibody assays.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

Prescription

4. Special instrument requirements:

The BioPlex 2200 ToRC IgG kit is intended for use with the BioPlex 2200 System instrument and software. The software currently available for the BioPlex 2200 System instrument is Software Version 2.0 which was previously cleared for marketing with K063866 (BioPlex 2200 Syphilis IgG Kit).

I. Device Description:

The BioPlex™ 2200 ToRC IgG kit uses multiplex flow immunoassay, to detect IgG antibodies to *Toxoplasma gondii* (*T. gondii*), Rubella, and Cytomegalovirus (CMV). The device uses 3 different populations of dyed beads coated with cell lysates bearing *T. gondii*, Rubella, or CMV antigens, together with 3 additional dyed beads; Internal Standard Bead (ISB), Serum Verification Bead (SVB) and a Reagent Blank Bead (RBB).

The BioPlex 2200 ToRC IgG Calibrator Set consists of; for *T. gondii* and Rubella, six vials, representing six different levels of antibody concentrations used for quantitative calibration and for CMV, four vials, representing four different antibody concentrations, used for qualitative and semi-quantitative calibration.

The BioPlex 2200 ToRC IgG Control Set includes a negative control and two multi-analyte positive controls. A Low Positive Control which contains antibodies for *T. gondii*, Rubella and CMV and a High Positive Control which contains antibodies for *T. gondii* and Rubella.

J. Substantial Equivalence Information:

Predicate Device 1: bioMérieux, Inc. VIDAS® TOXO IgG II (K993319)

Component	Similarities	
	Device	Predicate
Measurand	Toxoplasma IgG	Toxoplasma IgG
Detection	Quantitative detection	Quantitative detection
Intended Use	aid in the determination of serological status to <i>T. gondii</i> ,	aid in determination of immune status.
	Differences	
	Device	Predicate
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Two-step enzyme immunoassay sandwich method with fluorescent detection (ELFA), using Antigen-coated solid phase receptacles
Matrices	Serum, EDTA or Heparinized plasma	Serum
Antigen used	Partially purified cell lysates of <i>T. gondii</i> , Rubella, and CMV	Membrane and cytotoxic Toxoplasma antigen (RH Sabin Strain)
Controls	Negative Control and multi-analyte Positive Controls	Negative Control and Positive Control specific for <i>T. gondii</i>
calibrators	Multiple calibrators	Single
Analytes	multiple	Single

Predicate Device 2: **bioMérieux, Inc. VIDAS® Rubella IgG (K902925)**

Component	Similarities	
	Device	Predicate
Measurand	Rubella IgG	Rubella IgG
Intended Use	Quantitative detection	Qualitative detection
Intended Use	aid in the determination of serological status to <i>T. gondii</i> ,	for the detection of IgG antibodies to Rubella virus in human sera.
	Differences	
	Device	Predicate
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Two-step enzyme immunoassay sandwich method with fluorescent detection (ELFA), using Antigen-coated solid phase receptacles
Matrices	Serum, EDTA or Heparinized plasma	Serum
Controls	Negative Control and multi-analyte Positive Controls	Negative Control and Positive Control specific for Rubella
calibrators	Multiple calibrators	Single
Analytes	multiple	Single

Predicate Device 3: **bioMérieux, Inc. VIDAS® CMV IgG (K920661)**

Component	Similarities	
	Device	Predicate
Measurand	CMV IgG	CMV IgG
Matrices	Serum, EDTA or heparinized plasma	Serum
Intended Use	Qualitative detection of CMV IgG to aid in the determination of serological status to CMV	Semi-Quantitative detection of CMV IgG for use in determination of CMV immunological experience from a single serum sample or as an aid in diagnosis of current CMV infection through evaluation of paired sera
	Differences	
	Device	Predicate
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Two-step enzyme immunoassay sandwich method with fluorescent detection (ELFA), using Antigen-coated solid phase receptacles
antigen	Partially purified cell lysates of <i>T. gondii</i> , Rubella, and CMV	Purified and inactivated CMV antigen (Strain AD 169)
Controls	Negative Control and multi-analyte Positive Controls	Negative Control and Positive Control specific for CMV
calibrators	Multiple calibrators	Single
Analytes	Multiple	Single

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

STANDARDS	
Title and Reference Number	
Detection and Quantitation of Rubella IGG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of Test Products in the Clinical Laboratory; Approved Guideline (1997) (I/LA6-A)	
Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP5-A2)	
Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)	

Other Standards	
GUIDANCE	
Document Title	Web Page
Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff	http://www.fda.gov/cdrh/ode/guidance/1567.html
Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff	http://www.fda.gov/cdrh/oivd/guidance/1588.html

L. Test Principle:

BioPlex 2200 ToRC IgG kit employs a panel of three antigen-coated fluoromagnetic beads with unique fluorescent signatures to identify the presence of IgG class antibodies to *T. gondii*, Rubella, and CMV antigens in a two step assay format.

Step 1: The system combines an aliquot of patient sample with sample diluent and bead reagent then agitates the mixture at 37°C.

Step 2: Immobilized IgG antibodies are bound to a phycoerythrin (PE)-labeled anti-human IgG conjugate and detected by flow cytometry.

The fluorescence of the dyes determines the identity of the beads and the fluorescence of the PE label determines the amount of antibody captured by the antigen. The device calculates the results in relative fluorescence intensity (RFI).

Additional the ISB beads, SVB beads and a RBB beads are present in each reaction mixture to verify detector response, the addition of serum or plasma to the reaction vessel and the absence of significant non-specific binding in serum or plasma.

The BioPlex 2200 ToRC IgG Calibrator Set calibrates the instrument. For *T. gondii* and Rubella, the calibrators provide quantitative calibration and results for patient samples expressed in IU/mL. For *T. gondii*, results of ≤ 9 IU/mL are reported as negative, 10 - 11

IU/mL are reported as equivocal and ≥ 12 IU/mL are reported as positive. For Rubella, results of ≤ 7 IU/mL are reported as negative, 8 and 9 IU/mL are reported as equivocal and ≥ 10 IU/mL are reported as positive. For CMV, the calibrators provide qualitative calibration, results with an antibody index (AI) ≤ 0.8 AI are reported as negative, results equal to 0.9 and 1.0 AI are reported as equivocal, and results ≥ 1.1 AI are reported as positive.

The BioPlex 2200 ToRC IgG Control Set (described above in device description) includes a negative control as well as two multi-analyte positive controls. The BioPlex ToRC IgG Positive Controls give positive results, with values above the cut-off for each specific analyte. The BioPlex ToRC IgG Negative Control gives negative results, with values below the cut-off for each specific analyte. The recommended frequency for performing quality control is once every 24-hour testing period. Performing quality control is also necessary after each new assay calibration and certain service procedures.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Separate internal and external reproducibility studies were conducted. The reproducibility studies were performed for serum, EDTA- plasma and heparinized plasma panels. The panels consisted of 10 samples for each of the 3 analytes; negative ($< 50\%$ of the cutoff), high negative (50-80% of the cutoff), peri- cutoff (90-110% of the cutoff), low positive (2-3x the cutoff), high positive (mid assay range) and very high positive (80-100% of the maximum assay range) samples for each of the analytes. Additionally, a positive control (positive for all three analytes), a negative control (negative for all 3 analytes) were tested.

External reproducibility evaluated at three U.S. sites. All samples and controls were tested in duplicate on 2 runs per day for 5 days at each of the sites (i.e. a total of 60 replicates per sample) using different lots of the Bio-Rad BioPlex 2200 ToRC IgG Reagent Pack and Calibrator Set.

For the internal reproducibility study, the same method repeated over 20 days (resulting in 80 replicates). In both studies, the data was analyzed for intra-assay and inter-assay reproducibility according to the principles described in the Clinical Laboratory Standards Institute (CLSI) guidance EP5-A2 (Vol. 24, No. 25). The standard deviation (SD) and percent coefficient of variation (% CV) were calculated.

For the internal reproducibility study, the within-run precision for positive samples ranged from 3.9% to 9.5% for *T. gondii* IgG, 3.0% to 5.5% for Rubella IgG, and 1.8% to 5.6% for CMV IgG. The total precision for positive samples ranged from 6.6% to 12.3% for *T. gondii* IgG, 5.2% to 9.9% for Rubella IgG, and 3.8% to 8.7% for CMV IgG.

For the external reproducibility study, the within-run precision for positive samples, in all matrices ranged from 3.7% to 7.7% for *T. gondii* IgG, 4.6% to 6.6% for Rubella IgG, and 2.5% to 5.8% for CMV IgG. The total precision for positive samples in all matrices ranged from 4.8% to 17.6% for *T. gondii* IgG, 7.2% to 10.3% for Rubella IgG and 4.4% to 12.1% for CMV IgG.

Additionally, the a panel of three Rubella IgG samples measuring at or near cut-off was used to assess the precision within run for Rubella at three U.S. clinical sites, the samples were tested in replicates of 40. The total precision was less than 10%

b. Linearity/assay reportable range:

The linearity of the ToRC IgG assay over the assay's reportable range was assessed following the methods described in the CLSI document EP6-A. The assay ranges are *T. gondii* (3 – 900 IU/mL), Rubella (1 – 250 IU/mL) and CMV (0.2 – 8.0 AI), and were established by examining the clinical relevance of reporting high value results and the ability of the calibration curve to discriminate sample dilutions. For each analyte 5 high positive specimens were serially diluted in negative serum, and tested using a single lot of the ToRC IgG kit in 4 replicates. Linear and polynomial regression analysis of IU/mL or AI vs. sample dilution was performed to determine if the dilution curves exhibit statistically significant non-linear regression. The results demonstrated acceptable dilution linearity, as all recoveries were within $\pm 20\%$ of the predicted value.

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

The BioPlex 2200 ToRC IgG assays report results as either IU/mL (*T. gondii* and rubella) or an antibody index, AI, (CMV). IU/mL values are calculated by using a Rodbard 4-PL curve fit through the six calibrator levels. AI values are calculated by using linear regression analysis through segments of the calibration curve.

The quantitative value assignments for the Toxo and Rubella portions of the assay were traced back to the WHO anti-Toxoplasma serum, 3rd International Standard (TOXM) and the WHO anti-Rubella immunoglobulin, 3rd preparation of the 1st International Standard, 1996 (RUBI-1-94) respectively. Dilutions of both standards were analyzed with the BioPlex 2200 ToRC IgG assay for *T. gondii* and Rubella IgG. Linearity was evaluated based on the principles described in CLSI EP6-A. Results obtained demonstrated acceptable dilution linearity, as all recoveries were within $\pm 20\%$ of the predicted value.

For Rubella, the recovered IU/mL at the standard value of 10 IU/mL (assay cut-off) measured 11.2 IU/mL or 112% of the expected value for the BioPlex 2200, which is well within the normal variation of the assay and standard preparation. The BioPlex Rubella IgG assay range is 1 to 250 IU/mL. Assay linearity through this range as demonstrated with patient samples as explained in section b, linearity and assay reportable range. Over quantitation of $>30\%$ was observed with the BioPlex Rubella assay at a standard value of 50 IU/mL. This over quantitation is not observed with patient samples and does not affect the clinical decision.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Interfering Substances

The manufacturer assessed the test's performance with potentially interfering substances (according to CLSI Protocol EP7-A2 (Vol. 25, No. 27) by spiking samples with the interferent or solvent (negative control) was at levels indicated in the table below. The positive samples consisted of 20 to 30 IU/mL for *T. gondii*, 25-40 IU/mL of Rubella and 4.0 to 6.0 AI for CMV (prepared by mixing a pool of negative human serum with samples positive for *T. gondii*, Rubella or CMV IgG). Test and control samples were evaluated in replicates of ten. The percent change in signal for all analytes ranged from -10.7 to 5.3%. No significant interference was observed for any of the interfering substances in either the positive or the negative sample.

Substance	Concentration
Hemoglobin	500 mg/dL
Bilirubin, Unconjugated	30 mg/dL
Bilirubin, Conjugated	30 mg/dL
Cholesterol	500 mg/dL
Red Blood Cells	0.4% (v/v)
Gamma Globulin	6 g/dL
Triglyceride	3500 mg/dL
Total Protein (albumin)	12 g/dL
Ascorbic Acid	3 mg/dL
Heparin Lithium	8000 units/dL
Heparin Sodium	8000 units/dL
EDTA	800 mg/dL

Cross-Reactivity

The manufacturer evaluated the potential cross-reactivity of the assay as follows: A panel of ten positive specimens for each of the potential cross reactant (as determined by an FDA cleared device), were evaluated for possible cross-reactivity with the ToRC IgG kit for each of the three assays. Due to the high prevalence of Rubella IgG antibodies in the normal population, the sponsor evaluated the test specimens on corresponding commercially available EIAs. Most of the samples were high positive for each of the potential cross-reacting agents. The study did not rule out potential cross reactivity for CMV with some of the agents. The majority of all samples that elicited a positive result were also confirmed positive by the corresponding commercially available EIA, indicating reactivity to ToRC IgG antibodies rather than cross reactivity with a potentially interfering factor. The results are presented in the table below:

Potential Cross-Reactants	N	Method	# of Positive and Discordant Determinations		
			<i>T. gondii</i> IgG	Rubella IgG	CMV IgG
ANA IgG	10	BioPlex 2200	4	9	8*
		Reference	4	10	8
		Discrepant	0	1	0
CMV IgG	10	BioPlex 2200	2	9	10
		Reference	2	9	10
		Discrepant	0	0	0
dsDNA	10	BioPlex 2200	4	9	9
		Reference	4*	9	9
		Discrepant	0	0	0
EBV VCA IgG	10	BioPlex 2200	1	9	3
		Reference	1	9	3
		Discrepant	0	0	0
hCG	10	BioPlex 2200	4	9	10
		Reference	4	9	10
		Discrepant	0	0	0
HIV IgG	10	BioPlex 2200	1	9	10
		Reference	1	9	10
		Discrepant	0	0	0
HSV-1 IgG	10	BioPlex 2200	0	10	7
		Reference	0	10	6*
		Discrepant	0	0	0
HSV-2 IgG	10	BioPlex 2200	3	10	8*
		Reference	3	10	9
		Discrepant	0	0	0
Influenza IgG	10	BioPlex 2200	5	7	7
		Reference	5	7	7
		Discrepant	0	0	0
Mumps IgG	10	BioPlex 2200	4	10	7
		Reference	4	10	7
		Discrepant	0	0	0
Multiple Myeloma IgG	10	BioPlex 2200	3	7	9
		Reference	3	8	7
		Discrepant	0	1	2
Parvovirus B19 IgG	10	BioPlex 2200	2	10	4
		Reference	2	10	5
		Discrepant	0	0	1
Rheumatoid Factor IgM	10	BioPlex 2200	1	10	9
		Reference	1	10	9
		Discrepant	0	0	0
Rubella IgG	10	BioPlex 2200	3	10	7
		Reference	3	10	7
		Discrepant	0	0	0
Rubeola (measles) IgG	10	BioPlex 2200	0	10	5
		Reference	0	10	5
		Discrepant	0	0	0
<i>T. gondii</i> IgG	10	BioPlex 2200	9	9	9
		Reference	9	9	9
		Discrepant	0	0	0
VZV IgG	10	BioPlex 2200	3*	10	7
		Reference	3*	10	7
		Discrepant	0	0	0

*One equivocal result was not included in the count and was not considered as a false positive or negative discrepant.

f. *Assay cut-off:*

The manufacturer established the cutoff values for the ToRC IgG panel using 877 patient serum samples. These samples were first tested on the predicate devices. Each sample was classified as positive, negative or equivocal for each of these assays. Samples equivocal on the predicate device were not used in ROC analysis or concordance statistics. Comparisons were made for each analyte with its respective predicate test (excluding equivocal samples) on a Receiver Operating Characteristics (ROC) analysis.

The BioPlex 2200 ToRC IgG assays report results as either IU/mL (*T. gondii* and rubella) or an antibody index, AI, (CMV). IU/mL values are calculated by using a Rodbard 4-PL curve fit through the six calibrator levels. AI values are calculated by using linear regression analysis through segments of the calibration curve.

Calibrators are prepared by blending defibrinated/delipidated human plasma units each with known antibody activities to the antigens of the BioPlex 2200 ToRC IgG panel in a human base matrix that does not contain IgG. Multiple calibrator levels are prepared using gravimetric/volumetric dilution. These calibrators are used to assay characterized patient samples using the BioPlex 2200 ToRC IgG panel. The cut-off value and assignment of the calibrators are determined by performing concordance and Receiver Operator Characteristic (ROC) analysis, using predicate results as the standard. Analyze-it software is used for the ROC analysis. This software does not employ an equivocal range for the on-test condition. However the BioPlex 2200 assays have equivocal ranges, thus there is a slight difference in the statistics generated using the Analyze-it software.

This analysis was used to assist in optimizing negative and positive agreement (relative sensitivity and specificity), as well as overall agreement. Based on the results, calibrator values were adjusted such that the cut-off value at time of market was equal to 12 IU/mL, 10 IU/mL, and 1.1 AI for *T. gondii*, rubella, and CMV IgG, respectively. Testing was conducted internally at Bio-Rad Laboratories.

The BioPlex 2200 *T. gondii*, rubella and CMV IgG assays exhibited acceptable positive, negative and overall agreement. ROC analysis displayed lower sensitivity at the set cut-off for rubella since the ROC analysis uses all BioPlex 2200 results including those in the equivocal range of which there were 33. The results of concordance testing and ROC analysis validate the defined cut-offs for each of the analytes in the BioPlex ToRC IgG panel.

2. Comparison studies:

a. *Method comparison with predicate device:*

The manufacturer compared the performance of the ToRC IgG kit to the VIDAS *T. gondii*, Rubella, and CMV immunoassays. U.S. clinical sites tested a combined: 300 prospective samples from pregnant women (150 U.S. and 150 Europe), 1200 prospective samples submitted for ToRC testing consisting of 400 samples for *T. gondii*, 400 samples

for Rubella, and 400 samples for CMV IgG testing, and 100 prospective samples from immunocompromised/AIDS patients submitted for CMV testing. The following tables show the combined results from all sites. Additionally a retrospective Rubella study using preselected samples based on there reactivity on an FDA cleared Rubella IgG detection device was performed. Two U.S. clinical sites tested a combined: 50 Rubella IgG low positive samples (10-20 IU/ml), 50 Rubella IgG high positive samples (> 20 IU/ml) and 130 collected Rubella IgG negative samples. The results are summarized below.

BioPlex Rubella IgG vs. EIA: Prospective

Antibody/Population				Predicate Rubella IgG Assay			BioPlex 2200 Agreement Excluding Equivocal Results				BioPlex 2200 Agreement Including Equivocal Results			
				Pos (+)	Neg (-)	Equivocal	Pos (+) % Agreement	95% Confidence Interval	Neg (-) % Agreement	95% Confidence Interval	Pos (+) % Agreement	95% Confidence Interval	Neg (-) % Agreement	95% Confidence Interval
BioPlex 2200 ToRC IgG	Rubella IgG	Pregnant Women (N = 300)	Pos (+)	276	0	0	97.2% (276/284)	94.5-98.6%	100% (8/8)	67.6-100%	94.5% (276/292)	91.3-96.6%	100% (8/8)	67.6-100%
			Neg (-)	8	8	2								
			Equivocal	6	0	0								
			Total	290	8	2								
	Clinical Samples Submitted for Testing (N = 400)		Pos (+)	358	0	1	96.5% (358/371)	94.1-97.9%	100% (12/12)	75.8-100%	92.7% (358/386)	89.7-94.9%	85.7%* (12/14)	61.1-96.0%
			Neg (-)	13	12	3								
			Equivocal	12	1	0								
			Total	383	13	4								

*Due to the low prevalence of Rubella IgG negative samples, a retrospective study was conducted and is presented in Table M.

BioPlex *T. gondii* IgG vs. EIA: Prospective

Antibody/Population				Predicate <i>T. gondii</i> IgG Assay			BioPlex 2200 Agreement			
				Pos (+)	Neg (-)	Equivocal	Pos (+) % Agreement	95% Confidence Interval	Neg (-) % Agreement	95% Confidence Interval
BioPlex 2200 ToRC IgG	<i>T. gondii</i> IgG	Total (N = 700)	Pos (+)	118	0	6	97.5% (118/121)	93.0-99.2%	98.8% (569/576)	97.5-99.4%
			Neg (-)	1	569	1				
			Equivocal	1	1	3				
			Total	120	570	10				

BioPlex CMV IgG vs. EIA: Prospective

Antibody/Population				Predicate CMV IgG Assay			BioPlex 2200 Agreement			
				Pos (+)	Neg (-)	Equivocal	Pos (+) % Agreement	95% Confidence Interval	Neg (-) % Agreement	95% Confidence Interval
BioPlex 2200 ToRC IgG	CMV IgG	Total (N = 700)	Pos (+)	394	2	2	99.0% (394/398)	97.4- 99.6%	98.7% (298/302)	96.6- 99.5%
			Neg (-)	4	298	0				
			Equivocal	0	0	0				
			Total	398	300	2				
		HIV+ (N = 100)	Pos (+)	86	0	0	100% (86/86)	95.8- 100%	100% (14/14)	76.8- 100%
			Neg (-)	0	14	0				
			Equivocal	0	0	0				
			Total	86	14	0				

BioPlex Rubella IgG vs. EIA: Retrospective

Preselected Samples		Predicate Rubella IgG Assay			BioPlex 2200 Agreement Excluding Equivocal Results				BioPlex 2200 Agreement Including Equivocal Results			
		Pos (+)	Neg (-)	Equivocal	Pos (+) % Agreement	95% Confidence Interval	Neg (-) % Agreement	95% Confidence Interval	Pos (+) % Agreement	95% Confidence Interval	Neg (-) % Agreement	95% Confidence Interval
BioPlex 2200 ToRC IgG Rubella IgG	Pos (+)	90	0	1	92.8% (90/97)	85.8- 96.5%	100% (124/124)	97.0- 100%	86.5%* (90/104)	78.7- 91.8%	99.2% (124/125)	95.6- 99.9%
	Neg (-)	7	124	2								
	Equivocal	5	0	1								
	Total	102	124	4								

*Most of the discordant samples were low positive or close to the cut-off for the predicate assay.

b. Matrix comparison:

Performance of the Bioplex ToRC IgG kit, with respect to matrix comparison, was demonstrated using clinically relevant samples in four separate studies: Serum vs. Plasma (Anticoagulant) Testing, Internal Reproducibility Study, Reproducibility at Multiple Sites (see reproducibility section, and Analytical Specificity (see Interfering

Substances).

In the serum vs. plasma experiments, the effects of EDTA and heparin anticoagulants on the measurement of *T. gondii*, Rubella and CMV IgG were assessed using 10 positive and 10 negative samples for the three analytes. Plasma values were compared to matched serum values and anticoagulant interference was scored for individual and mean differences. The data demonstrated no appreciable interference from EDTA or heparin in IgG positive or negative samples in any of the Bioplex ToRC IgG assays. Regression analyses of the matched serum and plasma pairs for all three assays, the slope offset of serum vs. plasma quantification was less than 5%, and the R² values were 0.9869 or greater, except for *T. gondii* where the R² value for serum vs. heparin plasma was 0.9482.

c. Comparison with CDC panels:

A correlation study was performed to evaluate the characteristics of the BioPlex ToRC IgG kit with well-characterized, masked serum panels provided by the Centers for Disease Control (CDC) for *T. gondii* (70 positives, 30 negatives), Rubella (82 positives, 18 negatives) and CMV (66 positives, 34 negatives). The BioPlex 2200 ToRC IgG kit correctly identified all panel members.

d. CDC Rubella Low Positive Control:

A lyophilized CDC low positive control serum was prepared and tested neat and diluted in duplicates at three clinical trial sites. The results of the neat and 1/2 diluted samples ranged from 25-36 IU/mL and 13-20 IU/mL, respectively.

e. Comparison with Rubella and CMV Seroconversion Panels:

Four commercially available seroconversion panels were tested with the BioPlex 2200 System ToRC IgG kit to evaluate performance of the Rubella IgG and CMV IgG assays. Each seroconversion panel member was tested once at Bio-Rad Laboratories and the results were compared to the results provided by the manufacturer. All results are expressed as specimen signal to cutoff ratios (S/CO). Ratios > 1 are considered reactive. The bleed where each method became reactive is highlighted in the tables below.

Rubella			
Panel	Bleed Day at which sero-conversion occurs		Difference between bleeds
	Bioplex 2200 ToRC	Assigned values	
1	22	22	0
2	21	24	2*

* The next bleed after day 21 was day 24.

CMV

Panel	Bleed Day at which sero-conversion occurs		Difference between bleeds
	Bioplex 2200 ToRC	Assigned values	
1	24	24	0
2	8	51	43*

* The next bleed after day 8 was day 51.

3. Clinical studies :

- Clinical Sensitivity*: Not applicable
- Clinical specificity*: Not applicable
- Other clinical supportive data (when a. and b. are not applicable)*:

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The observed prevalence for the ToRC IgG antibodies was determined individually and for each possible dual positive combination in the study populations (The pregnant women samples from the U.S. and Europe, samples from the routine testing population for *T. gondii*, Rubella, or CMV IgG and samples from HIV immunocompromised patients). The prevalence and the expected values for the ToRC IgG antibodies are presented by age and gender in the following tables.

Prevalence of Individual Assay Positive in Pregnant Women

Age	<i>T. gondii</i> IgG US		<i>T. gondii</i> IgG Europe		Rubella IgG		CMV IgG	
	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence
16-25	5/42	11.9	9/31	29.0	71/73	97.3	41/73	56.2
26-35	5/89	5.6	33/83	39.8	155/172	90.1	90/172	52.3
36-45	1/19	5.3	16/36	44.4	50/55	90.9	28/55	50.9
Total	11/150	7.3	58/150	38.7	276/300	92.0	159/300	53.0

Note: There was 1 equivocal result for *T. gondii* and 6 equivocal results for Rubella

Prevalence of Dual Assay Positive in Pregnant Women

Age	<i>T. gondii</i> IgG / Rubella IgG US		<i>T. gondii</i> IgG / Rubella IgG Europe		<i>T. gondii</i> IgG / CMV IgG US		<i>T. gondii</i> IgG / CMV IgG Europe		Rubella IgG / CMV IgG	
	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence
16-25	5/42	11.9	9/31	29.0	2/42	4.8	4/31	12.9	39/73	53.4
26-35	4/89	4.5	31/83	37.3	2/89	2.2	19/83	22.9	79/172	45.9
36-45	1/19	5.3	16/36	44.4	1/19	5.3	6/36	16.7	25/55	45.5
Total	10/150	6.7	56/150	37.3	5/150	3.3	29/150	19.3	143/300	47.7

Prevalence of Individual Assay Positive in Samples Submitted for ToRC IgG Testing

Age	Gender	<i>T. gondii</i> IgG		Rubella IgG		CMV IgG	
		Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence
1-10	Female	1/9	11.1	8/9	88.9	3/9	33.3
	Male	1/8	12.5	7/8	87.5	0/8	0.0
11-20	Female	2/63	3.2	54/63	85.7	34/63	54.0
	Male	1/31	3.2	28/31	90.3	16/31	51.6
21-30	Female	30/232	12.9	214/232	92.2	123/232	53.0
	Male	6/56	10.7	50/56	89.3	33/56	58.9
31-40	Female	31/250	12.4	222/250	88.8	125/250	50.0
	Male	19/83	22.9	63/83	75.9	54/83	65.1
41-50	Female	8/86	9.3	78/86	90.7	60/86	69.8
	Male	16/96	16.7	78/96	81.3	64/96	66.7
51-60	Female	5/57	8.8	54/57	94.7	37/57	64.9
	Male	26/99	26.3	91/99	91.9	62/99	62.6
61-70	Female	12/44	27.3	38/44	86.4	36/44	81.8
	Male	15/50	30.0	49/50	98.0	35/50	70.0
71+	Female	8/12	66.7	12/12	100.0	12/12	100.0
	Male	5/14	35.7	12/14	85.7	10/14	71.4
Unknown Age and/or Gender		1/10	10.0	8/10	80.0	6/10	60.0
Total		187/1200	15.6	1066/1200	88.8	710/1200	59.2

Note: There were 5 equivocal results for *T. gondii*, 31 equivocal results for Rubella, and 1 equivocal result for CMV.

Prevalence of Dual Assay Positive in Samples Submitted for ToRC IgG Testing

Age	Gender	<i>T.gondii</i> IgG/ Rubella IgG		<i>T.gondii</i> IgG/ CMV IgG		Rubella IgG /CMV IgG	
		Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence
1-10	Female	1/9	11.1	0/9	0.0	2/9	22.2
	Male	1/8	12.5	0/8	0.0	0/8	0.0
11-20	Female	2/63	3.2	2/63	3.2	28/63	44.4
	Male	1/31	3.2	1/31	3.2	14/31	45.2
21-30	Female	29/232	12.5	24/232	10.3	120/232	51.7
	Male	5/56	8.9	3/56	5.4	31/56	55.4
31-40	Female	30/250	12.0	17/250	6.8	115/250	46.0
	Male	15/83	18.1	13/83	15.7	43/83	51.8
41-50	Female	8/86	9.3	6/86	7.0	54/86	62.8
	Male	14/96	14.6	12/96	12.5	52/96	54.2
51-60	Female	5/57	8.8	4/57	7.0	35/57	61.4
	Male	24/99	24.2	15/99	15.2	57/99	57.6
61-70	Female	12/44	27.3	12/44	27.3	30/44	68.2
	Male	15/50	30.0	11/50	22.0	34/50	68.0
71+	Female	8/12	66.7	8/12	66.7	12/12	100.0
	Male	5/14	35.7	5/14	35.7	9/14	64.3
Unknown Age and/or Gender		1/10	10.0	1/10	10.0	4/10	40.0
Total		176/1200	14.7	134/1200	11.2	640/1200	53.3

Prevalence of CMV in Immunocompromised/AIDS Patient Samples Submitted for CMV IgG Testing

Age	Gender	CMV IgG	
		Pos/Total	% Prevalence
1-10	Female	0/0	0.0
	Male	1/1	100
11-20	Female	4/8	50.0
	Male	2/6	33.3
21-30	Female	2/2	100
	Male	5/5	100
31-40	Female	10/10	100
	Male	12/14	85.7
41-50	Female	8/8	100
	Male	21/23	91.3
51-60	Female	3/4	75.0
	Male	10/11	90.9

61-70	Female	4/4	100
	Male	4/4	100
71+	Female	0/0	0.0
	Male	0/0	0.0
Total		86/100	86.0

N. Proposed Labeling:

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

O. Conclusion:

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