

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

**A. 510(k) Number:**

K091616

**B. Purpose for Submission:**

New device

**C. Measurand:**

IgG antibodies specific to Measles, Mumps, Rubella and Varicella Zoster virus (VZV).

**D. Type of Test:**

Multiplex flow immunoassay (multiplexed fluoromagnetic bead assay)

**E. Applicant:**

Bio-Rad Laboratories Inc.

**F. Proprietary and Established Names:**

BioPlex™ 2200 MMRV IgG Kit  
BioPlex™ 2200 MMRV IgG Calibrator Set  
BioPlex™ 2200 MMRV IgG Control Set

**G. Regulatory Information:**

<b>Product code</b>	<b>Classification</b>	<b>Regulation section</b>	<b>Panel</b>
OPL: Multiplex immunoassay for Measles virus, Mumps virus, Rubella and Varicella Zoster virus	Class II	866.3510; Rubella Virus Serological Reagents	Microbiology
JIX: Calibrator, multi- analyte mixture	Class II	862.1150 - Calibrator	Clinical Chemistry
JJY: Multi- analyte controls, all kinds (assayed)	Class I	862.1660 - Quality Control Material (assayed and unassayed)	Clinical Chemistry

Note: The BioPlex™ 2200 MMRV IgG Kit is a multiplex immunoassay for the detection of IgG antibodies to Measles, Mumps, Rubella and VZV. This device is classified as Class II as described above and the new product code assigned for this device is listed under the regulation section for Rubella reagents. The classification of the panel follows that of the analyte which has the highest classification. 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. 866.3520; Rubeola (measles) Virus Serological Reagents (Microbiology Panel: Class I 510(k) Exempt). Product code (LJB), Enzyme linked immunoabsorbent assay, Rubeola
2. 866.3380; Mumps Virus Serological Reagents (Microbiology Panel: Class I 510(k) Exempt). Product code (LJY), Enzyme linked immunoabsorbent assay, Mumps
3. 866.3900; Varicella Zoster Virus Serological Reagents (Microbiology Panel: Class II). Product code (LFY), Enzyme linked immunoabsorbent assay, Varicella Zoster Virus

#### **H. Intended Use:**

1. Intended use(s):

##### **The BioPlex™ 2200 MMRV IgG kit**

The BioPlex™ 2200 MMRV IgG kit is a multiplex flow immunoassay intended for the qualitative detection of IgG antibodies to Measles, Mumps, Rubella, and Varicella-zoster virus (VZV) in human serum and EDTA or heparinized plasma. The BioPlex 2200 MMRV 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 Measles, Mumps, Rubella, and VZV. This kit is not intended for use in screening blood or plasma donors.

The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients, or for use at point of care facilities.

##### **The BioPlex 2200 MMRV IgG Calibrator Set**

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

##### **The BioPlex 2200 MMRV IgG Control Set**

The BioPlex 2200 MMRV IgG Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and BioPlex 2200 MMRV IgG Reagent Pack in the clinical laboratory. The performance of the BioPlex 2200 MMRV IgG Control Set has not been established with any other Measles, Mumps, Rubella, or Varicella-zoster virus (VZV) IgG antibody assays.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

The Bio-Rad BioPlex 2200 System

**I. Device Description:**

The BioPlex 2200 MMRV IgG kit is a fully automated multiplexed micro particle bead based immunoassay for the qualitative detection of IgG antibodies to Measles, Mumps, Rubella, and VZV in human serum and EDTA or heparinized plasma using the Luminex flow cytometry technology. It consists of a reagent kit and the Bioplex 2200 Instrument. The BioPlex 2200 MMRV IgG Kit consists of a Reagent Pack which contains the; bead set containing dyed beads coated with Measles (Rubeola), Mumps, Rubella, and VZV antigens plus an Internal Standard bead (ISB), a Serum Verification bead (SVB), and a Reagent Blank bead (RBB) in buffer with Glycerol and protein stabilizers (bovine), ProClin™ 300 (0.3%), sodium benzoate (0.1%) and sodium azide (< 0.1%) as preservatives. Phycoerythrin conjugated murine monoclonal anti-human IgG antibody and phycoerythrin conjugated murine monoclonal anti-human FXIII antibody, in buffer with protein stabilizers (bovine), ProClin™ 300 (0.3%), sodium benzoate (0.1%) and sodium azide (< 0.1%) as preservatives, and sample diluent; containing buffer with protein stabilizers (bovine and murine). ProClin™ 300 (0.3%), sodium benzoate (0.1%) and sodium azide (< 0.1%) as preservatives. The device requires other materials which are provided independently from Bio-Rad and these include the BioPlex 2200 Instrument and Software System, the Bioplex 2200 sheath fluid and wash solution.

The BioPlex 2200 MMRV IgG Calibrator Set consists of three distinct serum based calibrators in three vials. They are made from defibrinated plasma with added known concentrations of Measles, Mumps, Rubella and VZV antibodies from human disease state plasma. The calibrators are used for the qualitative calibration of the device. The BioPlex 2200 MMRV IgG Control Set includes a negative control and a multi-analyte positive control, intended to be used as assayed quality control material with the BioPlex MMRV IgG Test.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

There is no single predicate device for the panel as a whole; the following is a list of the predicate devices for each of the analytes in the panel. These predicate devices are used as comparators to assess the performance of the device and determine the substantial equivalence. The final classification of the new multiplexed device follows that of the predicate with the highest classification.

- BioMerieux VIDAS Measles IgG
- BioMerieux VIDAS Mumps IgG
- Bio-Rad Rubella IgG EIA
- BioMerieux VIDAS Varicella-Zoster IgG (VZV)

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

The measles and mumps comparator devices are 510(k) exempt and therefore there is no 510(k) number. The 510(k) numbers for the Rubella and VZV predicate devices are K961053 and K923122 respectively.

3. Comparison with predicates:

1. Comparison with BioMerieux VIDAS Measles IgG (MSG)- 510(k) Exempt

<b>Similarities</b>		
Item	Device	Predicates
Intended Use	Intended for the qualitative detection of IgG antibodies to <b>Measles, Mumps, Rubella, and Varicella-zoster virus (VZV)</b>	Intended for use for the qualitative detection of IgG antibodies to measles (Rubeola) virus
Measurand	<b>Measles IgG</b> , in addition to Mumps, Rubella and VZV IgG	Measles IgG
Detection	Qualitative detection	Qualitative Detection

<b>Differences</b>		
Item	Device	Predicate
Matrices	Serum, EDTA or Heparinized Plasma	Serum
Analytes detected	Multiple Analytes	Single Analyte
Calibrators	Multiple calibrators	Standard
Controls	Negative Control and multi-analyte Positive Controls	Negative and positive control
Technology	Multiplexed flow immunoassay,	Enzyme immunoassay sandwich method with

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	using Antigen-coated paramagnetic microbead reagent.	fluorescent detection (ELFA), using Antigen coated solid phase receptacles
Indications	This kit is intended as an aid in the determination of serological status to Measles, Mumps, Rubella, and VZV.	Non specified

2. Comparison with BioMerieux VIDAS Mumps IgG (MPG) - 510(k) Exempt

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicates</b>
Intended Use	Intended for the qualitative detection of IgG antibodies to Measles, <b>Mumps</b> , Rubella, and Varicella-zoster virus (VZV)	Intended for use for the qualitative detection of IgG antibodies to Mumps virus
Measurand	<b>Mumps IgG</b> , in addition to Measles, Rubella and VZV IgG	Mumps IgG
Detection	Qualitative detection	Qualitative Detection

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Matrices	Serum, EDTA or Heparinized Plasma	Serum
Analytes detected	Multiple Analytes	Single Analyte
Calibrators	Multiple calibrators	Standard
Controls	Negative Control and multi-analyte Positive Control	Negative and positive control
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Enzyme immunoassay sandwich method with fluorescent detection (ELFA), using Antigen coated solid phase receptacles
Indications	Aid in the determination of serological status to Measles, Mumps, Rubella, and VZV.	Aid in the diagnosis of mumps and to provide epidemiological information on mumps.

Bio-Rad Rubella IgG EIA (K961053)

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicates</b>
Intended Use	Intended for the qualitative detection of IgG antibodies to Measles, Mumps, <b>Rubella</b> , and Varicella-zoster virus (VZV)	Intended for the qualitative, semi-quantitative, and quantitative detection of human IgG antibodies to <b>rubella virus</b> .
Indications	Aid in the determination of serological status to Measles, Mumps, Rubella, and VZV.	Aid in the assessment of the patient's immunological response to rubella, and as a qualitative screening test to determine immune status of individuals, including women of childbearing age.
Measurand	Rubella IgG, In addition to Measles, Mumps and VZV IgG	Rubella IgG
Detection	Qualitative detection	<b>Qualitative</b> , Semi-quantitative and Quantitative
Calibrators	Multiple Calibrators	Calibrators

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Matrices	Serum, EDTA or Heparinized Plasma	Serum
Detection	Qualitative	Qualitative, Semi-quantitative and Quantitative
Analytes detected	Multiple Analytes	Single Analyte
Controls	Negative Control and multi-analyte Positive Control	Negative control, low and high positive controls
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Enzyme immunoassay with fluorescent detection (EIA), using Antigen coated 96 well micro plate

BioMerieux VIDAS Varicella-Zoster IgG (VZV) - K923122

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicates</b>
Intended Use	Intended for the qualitative detection of IgG antibodies to Measles, Mumps, Rubella, and <b>Varicella-zoster virus (VZV)</b>	Intended for the qualitative detection of IgG antibodies to <b>Varicella-zoster virus</b>
Indications	Aid in the determination of serological status to Measles, Mumps, Rubella, and VZV.	Aid in the determination of immunological experience with Varicella-zoster virus.
Measurand	<b>VZV IgG</b> , in addition to Mumps, Rubella and Measles IgG	VZV IgG
Detection	Qualitative detection	Qualitative Detection

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Matrices	Serum, EDTA or Heparinized Plasma	Serum
Analytes detected	Multiple Analytes	Single Analyte
Calibrators	Multiple calibrators	Standard
Controls	Negative Control and multi-analyte Positive Control	Negative and positive control
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Enzyme immunoassay sandwich method with fluorescent detection (ELFA), using Antigen coated solid phase receptacles

**K. Standard/Guidance Document referenced (if applicable):**

1. NCCLS *In- vitro* guideline; Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2).
2. NCCLS *In- vitro* guideline; User Demonstration of Performance for Precision and Accuracy; Approved Guideline (EP15-A).
3. CEN *In- vitro* guideline; Stability Testing of In Vitro Diagnostic Reagents (13640).
4. CLSI *In- vitro* guideline; Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP05-A2).

5. CLSI *In- vitro* guideline; Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition (EP07-A2).

6. CLSI *In- vitro* guideline; Evaluation of the Linearity of Quantitative Measurement (EP06-A).

The following FDA guidance documents were also referenced in this submission:

1. 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>

2. Guidance for Industry and FDA Staff - Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests <http://www.fda.gov/cdrh/osb/guidance/1620.html>

<http://www.fda.gov/cdrh/osb/guidance/1620.html>

#### **L. Test Principle:**

The BioPlex 2200 MMRV IgG kit employs a panel of four antigen-coated fluoromagnetic beads with unique fluorescent signatures to identify the presence of IgG class antibodies to Measles, Mumps, Rubella, and VZV 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 antihuman 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). Additionally, 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 MMRV IgG Calibrator Set calibrates the instrument. The BioPlex 2200 MMRV IgG Reagent Pack is calibrated using a set of three (3) distinct serum based calibrators. Calibrators are used in a test system to establish points of reference that are used in the determination of qualitative numeric measurement of IgG antibodies to Measles, Mumps, Rubella, and Varicella-zoster virus (VZV) in human serum and EDTA or heparinized plasma. The MMRV IgG Calibrator Set should be loaded and assayed at minimum in duplicate every 30 days or with each new Re-agent Pack lot.

The BioPlex 2200 MMRV IgG Control Set includes a negative control as well as a positive control containing antibodies present for analytes within the BioPlex 2200

MMRV IgG Reagent Pack. The positive control is manufactured to give positive results for antibodies to Measles, Mumps, Rubella, and VZV with values above the cut-off for each specific assay. The negative control is manufactured to give negative results, with values below the cut-off for each specific assay. The mean values were derived from replicate analyses and should fall within the corresponding standard deviation. A BioPlex 2200 MMRV IgG Control Lot Data disk is available to load the necessary value assignment data into the instrument. The result for each of the tested antibodies is expressed as an antibody index (AI). For Mumps, Measles, and VZV, an AI of  $\leq 0.8$  indicates a negative result. An equivocal result is reported for an AI of either 0.9 or 1.0. AI results  $\geq 1.1$  are reported as positive. For Rubella, an AI of  $\leq 0.7$  indicates a negative result. An equivocal result is reported for an AI of either 0.8 or 0.9. AI results  $\geq 1.0$  are reported as positive.

#### **M. Performance Characteristics (if/when applicable):**

##### 1. Analytical performance:

###### *a. Precision/Reproducibility:*

Separate internal and external studies were conducted to evaluate the precision of the BioPlex 2200 MMRV IgG kit assays on the BioPlex 2200 Multi-Analyte Detection System. The internal precision study was conducted in-house by Bio-Rad Laboratories in accordance with CLSI EP5-A2. The internal Precision Study of the BioPlex 2200 MMRV IgG assay was assessed using serum, EDTA plasma and heparin plasma samples containing IgG class antibodies to Measles, Mumps, Rubella and (or) VZV. Reproducibility was determined by calculating the within-run (intra-assay), between-run (inter-assay), between day (inter-assay) and total precision using a reproducibility panel prepared at Bio-Rad Laboratories (Benicia, CA) consisting of analyte positive patient samples. Testing was conducted internally at Bio-Rad Laboratories. Twenty days of precision data were collected for the reagent set. Two runs were performed each day spaced by at least two hours. Assay calibration was conducted at the start of the study and all samples were randomized for each run. Controls were run daily to qualify the run. Each sample was run in duplicate per run for a total of eighty data points per sample for the twenty day analysis (forty data points for the ten day analysis). Data analysis was based on CLSI EP5-A2 (Evaluation of Precision Performance of Clinical Chemistry Devices). The reproducibility panel of patient samples included at least one high negative sample near the assay decision point, two low positive samples and two high positive samples (see Table 1.) Each sample was prepared by blending serum or plasma with like matrix to obtain the target analyte concentration. Multiple serum or plasma samples were used to make the panels. The BioPlex 2200 MMRV IgG reagent kit demonstrated acceptable precision and met the manufacturing design inputs, when tested against a precision sample set containing samples below, near or above the assay cut-off with the exception of one VZV sample with an AI value of 0.3

AI, which gave a within run CV of 14.4%. The within run imprecision for samples with AI >0.5 in all sample matrices ranged from 3.2% to 10.1% for Measles, 2.4% to 6.4% for Mumps, 2.9% to 7.2% for Rubella and 2.7% to 6.8% for VZV IgG. Total run imprecision for samples with AI >0.5 in all sample matrices ranged from 3.2% to 10.3% for Measles, 1.8% to 7.5% for Mumps, 3.9% to 7.6% for Rubella and 2.7% to 8.4% for VZV IgG.

The external reproducibility study was conducted in accordance with CLSI EP15-A2 during the external clinical evaluation at each of three clinical study sites. The reproducibility of each of the assays in the BioPlex 2200 MMRV IgG kit was assessed using three sample panels of different matrices (serum, EDTA plasma, and sodium heparin plasma). The positive samples of the panels were prepared at Bio-Rad Laboratories by pooling one or more antibody positive patient samples for one or more of the analytes in the BioPlex 2200 MMRV IgG Test. The panel consisted of members with varying levels of antibodies to the analytes in the BioPlex 2200 MMRV IgG kit, and a positive control (antibody positive for all analytes). Reproducibility testing was performed at 3 U.S. clinical trial sites using three reagent lots of BioPlex 2200 MMRV IgG Reagent Packs, BioPlex 2200 MMRV IgG Calibrator Set and the BioPlex 2200 MMRV IgG Control Set. Each site evaluated 1 lot of the BioPlex 2200 MMRV IgG kit. Each of the panel members and a positive and negative control was tested in quadruplicate on 1 run per day over 5 days at each of 3 sites (4 replicates x 1 run x 5 days = 20 replicates per panel member per site = 60 total replicates for 3 sites). The data were analyzed for intra-assay and inter-assay reproducibility according to the Clinical and Laboratory Standards Institute (CLSI) guidance EP15-A2 (Vol. 25, No. 17). The mean Antibody Index (AI), standard deviation (SD), and percent coefficient of variation (%CV) for each panel member were calculated. The data for the serum panel is represented below:

**Reproducibility: BioPlex 2200 Measles IgG Serum**

Measles IgG Panel Members	Sample N	Grand Mean (AI)	Within-Run		Between-Day		Between-Site*		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	60	2.5	0.112	4.4	0.138	5.5	0.185	7.3	0.256	10.2
High Positive 2	60	2.1	0.083	3.9	0.126	6.0	0.250	11.9	0.292	13.9
Low Positive 1	60	1.5	0.077	5.3	0.057	3.9	0.212	14.6	0.233	16.0
Low Positive 2	60	1.8	0.077	4.4	0.132	7.4	0.186	10.4	0.241	13.5
Near Cutoff 1	60	0.9	0.087	9.8	0.063	7.2	0.075	8.5	0.131	14.8
Near Cutoff 2	60	0.9	0.066	7.3	0.066	7.4	0.000	0.0	0.093	10.4

High Negative 1	60	0.7	0.042	5.7	0.035	4.8	0.065	8.8	0.085	11.5
High Negative 2	60	0.5	0.031	6.0	0.017	3.4	0.033	6.4	0.048	9.4
Positive Control	60	3.0	0.091	3.1	0.119	4.0	0.332	11.2	0.364	12.3

\* Between site includes between lot variance.

Reproducibility: BioPlex 2200 Mumps IgG Serum

Mumps IgG Panel Members	Sample N	Grand Mean (AI)	Within-Run		Between-Day		Between-Site*		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	60	2.7	0.083	3.1	0.078	2.9	0.160	5.9	0.196	7.2
High Positive 2	60	3.1	0.108	3.4	0.104	3.3	0.283	9.0	0.321	10.2
Low Positive 1	60	1.7	0.060	3.6	0.044	2.6	0.058	3.5	0.094	5.7
Low Positive 2	60	1.7	0.062	3.6	0.069	3.9	0.075	4.3	0.119	6.9
Near Cutoff 1	60	0.8	0.047	5.6	0.035	4.2	0.024	2.8	0.064	7.6
Near Cutoff 2	60	0.9	0.044	5.0	0.022	2.5	0.042	4.8	0.065	7.4
High Negative 1	60	0.5	0.024	4.7	0.011	2.1	0.005	1.0	0.026	5.3
High Negative	60	0.7	0.036	5.3	0.036	5.3	0.026	3.8	0.057	8.4
Positive Control	60	2.6	0.061	2.4	0.069	2.7	0.169	6.6	0.193	7.5

\* Between site includes between lot variance.

Reproducibility: BioPlex 2200 Rubella IgG Serum

Rubella IgG Panel Members	Sample N	Grand Mean (AI)	Within-Run		Between-Day		Between-Site*		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	60	1.9	0.083	4.4	0.051	2.7	0.163	8.7	0.190	10.1
High Positive 2	60	2.8	0.092	3.3	0.083	3.0	0.403	14.5	0.421	15.2
Low Positive 1	60	1.4	0.068	5.0	0.073	5.3	0.045	3.3	0.110	8.0
Low Positive 2	60	1.8	0.083	4.7	0.099	5.6	0.167	9.5	0.211	12.1
Near Cutoff 1	60	0.8	0.039	5.0	0.043	5.5	0.039	5.1	0.070	9.0
Near Cutoff 2	60	0.8	0.051	6.2	0.033	4.1	0.066	8.2	0.090	11.0
High Negative 1	60	0.5	0.029	5.4	0.016	3.0	0.048	9.0	0.058	10.9

High Negative	60	0.5	0.029	5.8	0.037	7.4	0.033	6.6	0.057	11.5
Positive Control	60	2.1	0.068	3.3	0.096	4.6	0.191	9.1	0.225	10.7

\* *Between site includes between lot variance.*

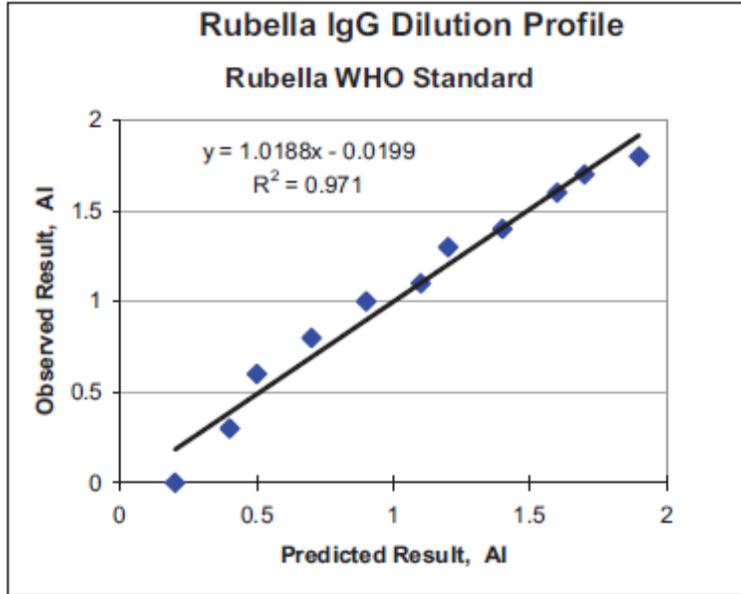
Reproducibility: BioPlex 2200 VZV IgG Serum

VZV IgG Panel Members	Sample N	Grand Mean (AI)	Within-Run		Between-Day		Between-Site*		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive 1	60	2.8	0.070	2.4	0.055	1.9	0.238	8.4	0.254	9.0
High Positive 2	60	3.5	0.083	2.4	0.103	3.0	0.323	9.3	0.349	10.0
Low Positive 1	60	1.1	0.047	4.3	0.069	6.3	0.058	5.3	0.101	9.3
Low Positive 2	60	1.2	0.055	4.5	0.064	5.2	0.000	0.0	0.084	6.8
Near Cutoff 1	60	0.8	0.039	4.9	0.067	8.5	0.000	0.0	0.077	9.8
Near Cutoff 2	60	1.0	0.049	4.7	0.075	7.3	0.000	0.0	0.090	8.7
High Negative 1	60	0.7	0.024	3.4	0.030	4.2	0.021	2.9	0.043	6.1
High Negative 2	60	0.5	0.035	7.3	0.051	10.6	0.022	4.7	0.066	13.6
Positive Control	60	2.4	0.058	2.4	0.062	2.5	0.174	7.1	0.194	7.9

\* Between site includes between lot variance.

*b. Linearity/assay reportable range:*

The test is a qualitative assay and linearity data is not required. However, for Rubella the lower range of the assay and the range around the cut-off (0 – 18 IU/ml) is quantitative. The company demonstrated the linearity for this assay range using a titration of the World Health Organization (WHO) anti-Rubella immunoglobulin, 1st International Standard, 1996 (RUBI-1-94). Dilution linearity was assessed in the range of 0 - 18 IU/mL (0 - 1.8 AI). The results were analyzed based on the recommendations in CLSI protocol EP6-A (Vol. 23, No. 16) and are shown in the figure below.



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

The test is a qualitative assay. However, for Rubella the lower range of the assay and the range around the cut-off (0 – 20 IU/ml) and the MMRV IgG calibrators are traceable to the World Health Organization (WHO) anti-Rubella immunoglobulin, 1st International Standard, 1996. The percent recovery for the international standard is represented in the table below.

Expected IU/mL	BioPlex 2200	
	Measured Mean AI (1.0 AI= 10 IU/ml)	Mean Percent of Expected
0.0	0.0	--
5.0	0.6	121
7.5	0.8	109
10.0	1.0	99
12.5	1.1	91
15.0	1.3	88
17.5	1.4	82
20.0	1.6	80

d. *Detection limit:*

Not applicable as this assay is a qualitative assay.

e. *Analytical specificity:*

## Cross-Reactivity

A cross-reactivity study was performed to determine if samples from various disease states and other potentially cross-reacting agents interfere with test results when tested with the BioPlex 2200 MMRV IgG kit. Samples known to be high positive samples for each of 13 potentially cross reactive antibodies listed in the table below were evaluated with the BioPlex 2200 MMRV IgG assays. Due to the high prevalence of IgG antibodies to Measles, Mumps, Rubella, and VZV in the population as a result of vaccination, and in order to properly evaluate the potential cross-reactivity, all samples were pre-tested by commercially available Measles, Mumps, Rubella and VZV assays. The samples which were negative by the commercially available assay were further tested by the BioPlex 2200 MMRV IgG kit. The following table summarizes negative agreement between the BioPlex 2200 MMRV IgG assays and the corresponding commercially available Measles, Mumps, Rubella and VZV assays within each of the thirteen cross-reactant panels. The results demonstrate that the various disease state samples evaluated do not cross-react with the 4 antigens in the BioPlex 2200 MMRV IgG kit. The potential cross reactivity with ANA IgG antibodies and HCV antibodies was not fully evaluated due to limitations of the sample size tested.

Potential Cross-Reactant	Number of Negative BioPlex 2200 Results / Number of Negative Commercially Available Assay Results							
	Measles		Mumps		Rubella		VZV	
	N	Negative Agreement	N	Negative Agreement	N	Negative Agreement	N	Negative Agreement
ANA IgG	5	5/5	5	5/5	2*	2/2	5	5/5
CMV IgG	10	10/10	10	10/10	10	10/10	10	10/10
EBV VCA IgG	10	10/10	10	10/10	10	10/10	10	10/10
HBsAb IgG	10	10/10	10	10/10	10	10/10	10	10/10
HCV Ab	3*	3/3	3	3/3	1*	1/1	1*	1/1
HSV-1 IgG	10	10/10	10	10/10	10	10/10	10	10/10
HSV-2 IgG	10	10/10	10	10/10	10	10/10	10	10/10
Measles IgG	-	N/A	10	10/10	10	10/10	10	10/10
Mumps IgG	10	10/10	-	N/A	10	10/10	10	10/10
Parvovirus B19 IgG	10	10/10	10	10/10	10	10/10	10	10/10
Rubella IgG	10	10/10	10	10/10	-	N/A	10	10/10
Toxoplasma IgG	10	10/10	6	6/6	8	8/8	10	10/10
VZV IgG	10	10/10	10	10/10	10	10/10	-	N/A

\* Potential cross-reactivity was not well assessed due to the limitation of the sample size tested.

## Interfering Substances

Testing for interfering substances was conducted according to CLSI Protocol EP7-A2 (Vol. 25, No. 27). No significant interference was observed in any of the substances tested. Serum component interference ranged from a -7.1% to 3.8% difference relative to non-spiked controls. Exogenous substance interference ranged from a -4.0% to 4.2% difference relative to non-spiked controls. The following substances, listed in the table below, were tested at the corresponding concentrations. Test and control samples were evaluated in alternating order in replicates of five each using the BioPlex 2200 MMRV IgG Kit. This sequence was repeated twice for a total of ten replicates per interferent and calculations were made based on EP7-A2 guidelines. Serum components were considered interfering if sample results deviated by more than  $\pm 20\%$  ( $\pm$  two standard deviations of the minimal within run precision criterion of 10% C.V.) relative to the value determined in the absence of the interferent. CLSI guidelines (EP7-A2) define interference as an effect that exceeds the normal assay variability and where incremental error caused by the substance is large enough to affect clinical decision making.

<b>Substance</b>	<b>Concentration</b>
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
Triglyceride	3300 mg/dL
Beta Carotene	0.6 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
Sodium Citrate	1000 mg/dL

*f. Assay cut-off:*

The cut-off values and assignment of the calibrators are determined by performing concordance and Receiver Operator Characteristic (ROC) analysis, using predicate results as the standard. This analysis was used to assist in optimizing negative and positive agreement (relative sensitivity and specificity). Based on the results, calibrator values were adjusted such that the cut-off values equal to 1.0 AI for Measles, Mumps, Rubella and VZV IgG assays. The calibration for Rubella was set based on calibration to the WHO RUBI-1-94 Rubella standard. Testing was conducted internally at Bio-Rad Laboratories. An equivocal zone of approximately +/- 10% was applied to the BioPlex 2200 MMRV IgG assays based on a separate analysis of assay precision at the established cut-off.

A total of 842 samples were evaluated to confirm cut-off values. Predicate testing for Measles, Mumps and VZV was performed at Bio-Rad Quality Systems Division (Irvine, CA) using VIDAS assay kits manufactured by BioMérieux (Durham, NC). Predicate testing for Rubella was performed at Bio-Rad BioPlex Division (Benicia, CA) using the Bio-Rad (Sera Quest) EIA kits manufactured by Quest International (Miami, FL). ROC Analysis was performed for each analyte using this population of samples. For the purpose of establishing a cut-off, predicate equivocal samples were not used during ROC analysis.

BioPlex 2200 MMRV IgG assays met or exceeded the concordance specifications established as Design Inputs of  $\geq 95\%$  positive agreement as well as  $\geq 96\%$  negative agreement for all analytes. The results of concordance testing and ROC analysis validate the cut-off that was established for each of the analytes in the BioPlex 2200 MMRV IgG assays.

2. Comparison studies:

*a. Method comparison with predicate device:*

Comparative Testing: Prospective

Performance of the MMRV IgG kit was evaluated against corresponding commercially available Measles, Mumps, Rubella, and VZV immunoassays. Serum samples from pregnant women (N = 396) and serum samples with a Measles, Mumps, Rubella, or VZV IgG test ordered (N = 1183) were tested at 3 U.S. clinical trial sites. Serum samples with a Measles, Mumps, Rubella, or VZV IgG test ordered included serum samples submitted for routine testing (N = 790) and serum samples for pre-employment evaluation (N = 393). Results for all populations are shown in the tables below. Equivocal results obtained for Mumps and Measles on the commercially available EIAs were further tested on two additional commercially available EIAs to obtain a final

result. The consensus results of two out of the three comparator tests are presented in the tables for Mumps and Measles, below. Equivocal results obtained for Rubella on the commercially available EIA were re-tested on the commercially available EIA, following the manufacturer's recommendations, and the re-test results are presented in the tables below for Rubella, below.

Pregnant Women		BioPlex 2200 MMRV IgG						
		Pos (+)	Eqv	Neg (-)	Total	Pos (+) % Agreement	Neg (-) % Agreement	
Commercially Available EIA	Measles IgG	Pos (+)	362	9	9	380	93.3% (362/388)	100% (8/8)
		Eqv*	0	0	8	8		
		Neg (-)	0	0	8	8		
		Total	362	9	25	396		
	Mumps IgG	Pos (+)	349	10	11	370	94.3% (349/370)	96.2% (25/26)
		Eqv*	0	0	0	0		
		Neg (-)	0	1	25	26		
		Total	349	11	36	396		
	Rubella IgG	Pos (+)	369	6	2	377	97.9% (369/377)	73.7% (14/19)
		Eqv**	0	0	0	0		
		Neg (-)	3	2	14	19		
		Total	372	8	16	396		
	VZV IgG	Pos (+)	348	8	4	360	95.1% (348/366)	100% (30/30)
		Eqv	0	0	6	6		
		Neg (-)	0	0	30	30		
		Total	348	8	40	396		

\* Results obtained by a consensus of two out of three commercially available EIAs.

\*\* Result after re-testing with the commercially available EIA following the manufacturer's recommendations.

Measles, Mumps, Rubella, or VZV Test Ordered			BioPlex 2200 MMRV IgG					
			Pos (+)	Eqv	Neg (-)	Total	Pos (+) % Agreement	Neg (-) % Agreement
Commercially Available EIA	Measles IgG	Pos (+)	1042	25	26	1093	94.7% (1042/1100)  95% CI 93.2 - 95.9%	80.2% (65/81)  95% CI 70.3 - 87.5%
		Eqv*	2	2	7	11		
		Neg (-)	4	10	65	79		
		Total	1048	37	98	1183		
	Mumps IgG	Pos (+)	1006	51	49	1106	90.4% (1006/1113)  95% CI 88.5 - 92.0%	91.0% (61/67)  95% CI 81.8 - 95.8%
		Eqv*	0	3	7	10		
		Neg (-)	1	5	61	67		
		Total	1007	59	117	1183		
	Rubella IgG	Pos (+)	1060	30	27	1117	94.8% (1060/1118)  95% CI 93.3 - 96.0%	86.2% (56/65)  95% CI 75.7 - 92.5%
		Eqv**	0	0	1	1		
		Neg (-)	4	5	56	65		
		Total	1064	35	84	1183		
	VZV IgG	Pos (+)	1047	16	36	1099	94.0% (1047/1114)  95% CI 92.4 - 95.2%	98.4% (60/61)  95% CI 91.3 - 99.7%
		Eqv	1	8	15	24		
		Neg (-)	0	0	60	60		
		Total	1048	24	111	1183		

\* Results obtained by a consensus of two out of three commercially available EIAs.

\*\* Result after re-testing with the commercially available EIA following the manufacturer's recommendations.

### Comparative Testing: Retrospective

Performance of the MMRV IgG kit was evaluated against corresponding commercially available Measles, Mumps, Rubella, and VZV immunoassays using retrospective serum samples negative for antibodies to Measles (N = 93), Mumps (N = 96), Rubella (N = 268), or VZV (N = 143). The negative samples for Measles and Mumps were selected using a consensus of two out of three commercially available EIAs. Negative samples for Rubella and VZV were selected using the corresponding predicate device. The results are presented below.

Retrospective Negative Samples: BioPlex 2200 MMRV IgG vs. EIA

Retrospective Measles, Mumps, Rubella, or VZV Negative Samples		BioPlex 2200 MMRV IgG					
		Pos (+)	Eqv	Neg (-)	Total	Neg (-) % Agreement	
Commercially Available EIA	Measles IgG (N = 93)	Pos (+)	0	0	0	0	100% (93/93)  95% CI 96.0 - 100%
		Eqv	0	0	0	0	
		Neg (-)	0	0	93	93	
		Total	0	0	93	93	
	Mumps IgG (N = 96)	Pos (+)	0	0	6	6	100% (83/83)  95% CI 95.6 - 100%
		Eqv	0	0	7	7	
		Neg (-)	0	0	83	83	
		Total	0	0	96	96	
	Rubella IgG (N = 268)	Pos (+)	0	0	0	0	95.9% (257/268)  95% CI 92.8 - 97.7%
		Eqv	0	0	0	0	
		Neg (-)	3	8	257	268	
		Total	3	8	257	268	
	VZV IgG (N = 143)	Pos (+)	0	0	0	0	100% (143/143)  95% CI 97.4 - 100%
		Eqv	0	0	0	0	
		Neg (-)	0	0	143	143	
		Total	0	0	143	143	

Correlation with CDC Rubella Evaluation Serum Panel

The performance of the BioPlex 2200 MMRV IgG kit was assessed using a masked, characterized Rubella IgG serum panel from the CDC. The panel consisted of 82 positive samples and 18 negative samples. The results are presented as a means to convey further information on the performance of the test kit and do not imply endorsement of the assay by the CDC. The results are presented below.

CDC Samples	Reference Result	BioPlex 2200 Pos (+)	BioPlex 2200 Neg (-)
Rubella IgG	Pos (+)	81	1*
	Neg (-)	0	18

## CDC Rubella Low Positive Control Testing

A lyophilized CDC low positive control serum was prepared by and tested at one clinical trial site. The control was tested neat and diluted 1/2. Each dilution was run in duplicate. The results are presented below.

Dilution	Mean (AI)
Neat – Prepared per package insert	2.3
1/2 Dilution	1.4

### *b. Matrix comparison:*

The following three studies were conducted to demonstrate agreement and precision for each matrix as well as specimen transport conditions and analyte stability

- BioPlex 2200 MMRV IgG Matrix comparison study: this study was conducted in accordance with CLSI EP9-A2 and demonstrates that there is no or minimal assay effect when EDTA and heparinized plasma are compared to serum.

- BioPlex 2200 MMRV IgG Sample Stability Study: this study provides the basis for fresh and frozen sample storage recommendations as well as the maximum number of freeze/thaw cycles.

- BioPlex 2200 MMRV IgG Sample Stability (Special Study) Report: this study provides additional validation of the prospectively collected serum samples used during the clinical trial, on the BioPlex 2200 MMRV IgG and predicate assays. The clinical samples were collected prospectively and frozen at -20C for testing at a future date. This study validates this storage practice for the BioPlex 2200 MMRV IgG and predicate assays.

In the matrix comparison study, matched serum and plasma (EDTA and heparin) samples drawn from 50 individual donors were acquired from commercial sources. All samples were evaluated in replicates of two. Plasma AI values were compared to matched serum AI values. All assays in the MMRV IgG kit and all lots pass the slope specification of  $1.0 \pm 0.2$ ; intercept specification of  $\pm 0.2$ , and correlation ( $r$ ) of  $\geq 0.98$ . The S.E.E (standard estimate of error) was less than 0.22 for all assays. The data has demonstrated that both EDTA and heparin plasma are acceptable matrices for the MMRV IgG assay<sup>3</sup>.

### 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:

See section (1f).

5. Expected values/Reference range:

The prevalence and expected values for each of the MMRV IgG assays were determined in a prospective study using serum samples from pregnant women (N = 396); and serum samples with a Measles, Mumps, Rubella, or VZV IgG test ordered for routine testing. The routine testing population included samples from pre-employment screening (N=393) and samples from subjects with a Measles test ordered (N=197), Mumps test ordered (N=199), Rubella test ordered (N=199), or VZV Test ordered (N=195).

**Pregnant Women**

Result	N	Age	Pos (%)	Eqv (%)	Neg (%)
Measles IgG	59	14 - 20	56 (94.9%)	1 (1.7%)	2 (3.4%)
	336	21 - 47	305 (90.8%)	8 (2.4%)	23 (6.8%)
	396	Total*	362 (91.4%)	9 (2.3%)	25 (6.3%)
Mumps IgG	59	14 - 20	54 (91.5%)	1 (1.7%)	4 (6.8%)
	336	21 - 47	295 (87.8%)	10 (3.0%)	31 (9.2%)
	396	Total*	349 (88.1%)	11 (2.8%)	36 (9.1%)
Rubella IgG	59	14 - 20	57 (96.6%)	0 (0.0%)	2 (3.4%)
	336	21 - 47	314 (93.5%)	8 (2.4%)	14 (4.2%)
	396	Total*	372 (93.9%)	8 (2.0%)	16 (4.0%)
VZV IgG	59	14 - 20	51 (86.4%)	1 (1.7%)	7 (11.9%)
	336	21 - 47	296 (88.1%)	7 (2.1%)	33 (9.8%)
	396	Total*	348 (87.9%)	8 (2.0%)	40 (10.1%)

\*The total includes one sample from a subject of unknown age.  
 Note: Due to rounding, numbers across columns may not total 100%.

Measles, Mumps, Rubella, or VZV Test Ordered

Result	Gender	N	Age	Pos (%)	Eqv (%)	Neg (%)	Total N
Measles IgG	F	16	8 - 20	14 (87.5%)	1 (6.3%)	1 (6.3%)	590
	M	14	8 - 20	11 (78.6%)	0 (0.0%)	3 (21.4%)	
	F	232	21 - 87	209 (90.1%)	3 (1.3%)	20 (8.6%)	
	M	328	21 - 87	297 (90.5%)	12 (3.7%)	19 (5.8%)	
	Total			531 (90.0%)	16 (2.7%)	43 (7.3%)	
Mumps IgG	F	19	8 - 20	17 (89.5%)	2 (10.5%)	0 (0.0%)	592
	M	18	8 - 20	16 (88.9%)	0 (0.0%)	2 (11.1%)	
	F	270	21 - 87	239 (88.5%)	9 (3.3%)	22 (8.1%)	
	M	285	21 - 87	252 (88.4%)	11 (3.9%)	22 (7.7%)	
	Total			524 (88.5%)	22 (3.7%)	46 (7.8%)	
Rubella IgG	F	27	8 - 20	22 (81.5%)	0 (0.0%)	5 (18.5%)	592
	M	11	8 - 20	8 (72.7%)	2 (18.2%)	1 (9.1%)	
	F	311	21 - 87	278 (89.4%)	8 (2.6%)	25 (8.0%)	
	M	243	21 - 87	210 (86.4%)	10 (4.1%)	23 (9.5%)	
	Total			518 (87.5%)	20 (3.4%)	54 (9.1%)	
VZV IgG	F	18	8 - 20	14 (77.8%)	0 (0.0%)	4 (22.2%)	588
	M	19	8 - 20	13 (68.4%)	0 (0.0%)	6 (31.6%)	
	F	254	21 - 87	229 (90.2%)	5 (2.0%)	20 (7.9%)	
	M	297	21 - 87	270 (90.9%)	7 (2.4%)	20 (6.7%)	
	Total			526 (89.5%)	12 (2.0%)	50 (8.5%)	

Note: Due to rounding, numbers across columns may not total 100%.

**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.