

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k130528

B. Purpose for Submission:

New Device

C. Measurand:

IgM antibodies to Cardiolipin (CL)
IgM antibodies to Beta-2-Glycoprotein I (β 2GPI)

D. Type of Test:

Semi-quantitative multiplex flow, bead-based immunoassay

E. Applicant:

Bio-Rad Laboratories, Inc.

F. Proprietary and Established Names:

BioPlex® 2200 APLS IgM kit
BioPlex® 2200 APLS IgM Calibrator set
BioPlex® 2200 APLS IgM Control set

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5660 – Multiple autoantibodies immunological test system
21 CFR §862.1150 – Calibrator
21 CFR § 862.1660 – Quality Control Material (assayed and unassayed)

2. Classification:

Class II (Assays, Calibrator)
Class I (Controls)

3. Product code:

MID, System Test, Anti-Cardiolipin Immunological
MSV, Antibodies, β 2- Glycoprotein I (β 2-GPI)
JIX, Calibrator, Mult-Analyte Mixture
JJX, Single (specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

Immunology (82) (Assays)
Chemistry (75) (Calibrators, Controls)

H. Intended Use:

1. Intended use(s):

The BioPlex® 2200 APLS IgM kit is a multiplex flow immunoassay intended for the semi-quantitative detection of IgM antibodies to Cardiolipin (CL) and Beta-2 Glycoprotein I (β 2GPI) in human serum and plasma (lithium heparin, sodium heparin, and sodium citrate). In conjunction with clinical findings, the test systems are used as an aid in the diagnosis of primary Antiphospholipid Syndrome (APS) and those secondary to systemic lupus erythematosus (SLE) or SLE-like disorders.

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

The BioPlex® 2200 APLS IgM Calibrator Set is intended for the calibration of the corresponding BioPlex® 2200 APLS IgM Reagent Pack.

The BioPlex® 2200 APLS IgM Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex® 2200 Instrument and BioPlex® APLS IgM Reagent Pack in the clinical laboratory. The performance of the BioPlex® APLS IgM Control Set has not been established with any other Antiphospholipid assay.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For Prescription Use only

4. Special instrument requirements:

BioRad BioPlex® 2200 Instrument and Software Version 4.0

I. **Device Description:**

BioPlex® 2200 Antiphospholipid Syndrome (APLS) IgM kit includes the following components:

- One (1) 100 mL vial of Bead Set containing two different populations of dyed beads coated with Cardiolipin (CL) and Beta-2-Glycoprotein I (β 2GPI), an Internal Standard Bead (ISB), a Serum Verification Bead (SVB) and a Reagent Blank Bead (RBB) in a MOPS (3-[N-Morpholino] propanesulfonic acid) buffer supplemented with glycerol and protein stabilizers (bovine); ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) as preservatives.
- One (1) 5 mL-vial of Conjugate containing phycoerythrin conjugated murine monoclonal anti-human IgM antibody and phycoerythrin conjugated murine monoclonal anti-human FXIII antibody in MOPS buffer supplemented with protein stabilizer (bovine), and ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) as preservatives,
- One (1) 10-mL vial of Sample Diluent containing buffer with protein stabilizers (bovine and murine), and ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) as preservatives.

BioPlex® 2200 APLS IgM Calibrator set contains seven vials of human antibodies to CL or β 2GPI in a human serum matrix made from defibrinated plasma with ProClin 300 ($\leq 0.3\%$) and sodium azide ($< 0.1\%$) as preservatives.

BioPlex® 2200 APLS IgM Control set contains four 1.5-mL vials of Positive controls of human antibodies to CL or β 2GPI and two vials of Negative Controls in a human serum matrix made from defibrinated plasma; and, in a human serum matrix made from defibrinated plasma with ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) as preservatives.

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

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

HemosIL AcuStar anti-Cardiolipin IgM, k092181

HemosIL AcuStar anti- β 2 Glycoprotein-I IgM, k091556

2. Comparison with predicate:

Similarities			
Item	Device: BioPlex® 2200 APLS IgM Kit	Predicate: HemosIL AcuStar anti-Cardiolipin IgM	Predicate: HemosIL AcuStar anti- β 2 Glycoprotein-I IgM
Intended Use	Semi-quantitative detection of IgM antibodies to Cardiolipin (CL) and Beta-2 Glycoprotein I (β 2GPI) in human serum and plasma as an aid in the diagnosis of primary Antiphospholipid Syndrome (APS) and those secondary to systemic lupus erythematosus (SLE) or SLE-like disorders.	Semi-quantitative measurement of anti-Cardiolipin (aCL) IgM antibodies in human citrated plasma or serum on the ACL AcuStar, as an aid in the diagnosis of thrombotic disorders related to primary and secondary Antiphospholipid Syndrome (APS)	Semi-quantitative measurement of Anti- β 2 Glycoprotein-I (Anti- β 2GPI) IgM antibodies in human plasma or serum on the ACL AcuStar, as an aid in the diagnosis of thrombotic disorders related to primary and secondary Antiphospholipid Syndrome (APS)
Sample Type	Serum and plasma (lithium heparin, sodium heparin, and sodium citrate)	Serum or citrated plasma	Serum or citrated plasma
Capture Antigen	Purified Cardiolipin antigen (tetraoleoyl succinylcardiolipin (ammonium salt))	Bovine Cardiolipin	Not Applicable
	Purified human β 2GPI	Same	Same

Similarities			
Item	Device: BioPlex® 2200 APLS IgM Kit	Predicate: HemosIL AcuStar anti-Cardiolipin IgM	Predicate: HemosIL AcuStar anti- β2 Glycoprotein-I IgM
Assay Type	Semi-Quantitative detection	Same	Same
Analyte Detected	Human IgM antibodies to Cardiolipin	Same	Not Applicable
	Human IgM antibodies to β2GPI	Not Applicable	Same
Cut-off	Anti-CL: 20 MPL U/mL	Anti-CL: Same – 20 U/mL	Anti-CL: Not Applicable
	Anti-β2GPI: 20 U/mL	Anti-β2GPI: Not Applicable	Anti-β2GPI: Same – 20 U/mL
Controls	Two levels, Sold separately	Same	Same

Differences			
Item	Device: BioPlex® 2200 APLS IgM Kit	Predicate: HemosIL AcuStar anti- Cardiolipin IgM	Predicate: HemosIL AcuStar anti- β2 Glycoprotein-I IgM
Assay Technology	Automated multiplex flow immunoassay	Two-step chemiluminescent immunoassay	Two-step chemiluminescent immunoassay
Conjugate Antibody	Phycoerythrin conjugated murine monoclonal anti-human IgM	Isoluminol-labeled anti-human IgM antibody	Isoluminol-labeled anti-human IgM antibody
Specimen Type	Serum and plasma (citratd and heparin)	Serum and plasma (citratd only)	Serum and plasma (citratd only)
Signal Detection	Fluorescence	Chemiluminescent	Chemiluminescent
Solid Phase	Antigen-coated paramagnetic microbead reagent	Antigen-coated magnetic particles	Antigen-coated magnetic particles
Control	One Negative and one Positive Controls (sold separately)	One low and one high control (Sold separately)	One low and one high control (Sold separately)
Calibrator(s)	4 levels of Calibrator (sold separately)	Two calibrator levels (included in test kit)	Two calibrator levels (included in test kit)

Differences			
Item	Device: BioPlex® 2200 APLS IgM Kit	Predicate: HemosIL AcuStar anti- Cardiolipin IgM	Predicate: HemosIL AcuStar anti- β2 Glycoprotein-I IgM
Assay Range	Anti-CL: 0.2 – 112 MPL U/mL	Anti-CL: 1.0 – 15480 U/mL	Anti-CL: Not Applicable
	Anti-β2GPI: 0.6 – 112 U/mL	Anti-β2GPI: Not Applicable	Anti-β2GPI: 1.1 – 16820 U/mL
Quantitation	Results are determined from a standard calibration curve utilizing a point-to-point calculation.	Assay utilizes a stored Master 4 Parameter Logistic Curve (4PLC) fit adjusted with two lot dependent calibrator levels	Assay utilizes a stored Master 4 Parameter Logistic Curve (4PLC) fit adjusted with two lot dependent calibrator levels
Instrumentation	Bio-Rad BioPlex® 2200 System	HemosIL AcuStar	HemosIL AcuStar

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

CEN 13640:2002, Stability Testing of In Vitro Diagnostic Reagents

EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition.

EP06-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline, Second Edition.

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

EP09-A2IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Second Edition (Interim Revision) (used for matrix comparison).

EP12-A2, User Protocol for Evaluation of Qualitative test Performance, Approved Guideline, Second Edition.

EP14-A2, Evaluation of Matrix Effects, Approved Guideline, Second Edition

EP15-A2, User Verification of Performance for Precision and Trueness, Approved Guideline, Second Edition.

EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantification, Approved Guideline.

L. Test Principle:

The BioPlex® 2200 APLS IgM kits use multiplex flow immunoassay, a methodology similar to traditional EIA; however, this method permits simultaneous detection and identification of many antibodies in a single tube. In the BioPlex APLS assays, two different populations of dyed beads are coated with antigens. One bead population is coated with β2-glycoprotein I and a second population is coated with both cardiolipin and β2-glycoprotein I. Three additional populations of fluorescent beads function as assay controls. The system combines an aliquot of patient sample, sample diluent, and bead reagent into a reaction vessel and

incubates the mixture at 37°C. After a wash cycle to remove unbound antibody, the secondary conjugate containing either phycoerythrin conjugated murine monoclonal anti-human IgM and phycoerythrin conjugated murine monoclonal anti-human FXIII antibody (a control) is added and the mixture is incubated at 37°C. Excess conjugate is removed in another wash cycle and the beads are re-suspended in wash buffer. The bead mixture then passes through the detector. The identity of the dyed beads is determined by the fluorescence of the dyes, and the amount of antibody captured by the antigen is determined by the fluorescence of the attached phycoerythrin. Raw data are calculated in relative fluorescence intensity (RFI).

Three additional dyed beads, Internal Standard Bead (ISB), Serum Verification Bead (SVB), and a Reagent Blank Bead (RBB) are present in each reaction mixture to verify detector response, the addition of serum to the reaction vessel, and the absence of significant non-specific binding in serum, respectively.

The anti-phospholipid assays are calibrated using a set of calibrators supplied separately by Bio-Rad Laboratories. Results are calculated for both of the antibodies and are compared against their own respective cut-off. For anti-β₂-glycoprotein I, the results are provided in units/mL (U/mL). The anti-cardiolipin results are similar except that the units are MPL-U/mL. The negative/positive assay cutoff for the two analytes of the BioPlex® 2200 APLS IgM kit is 20 units.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision testing of the BioPlex® 2200 APLS IgM kit on the BioPlex® 2200 instrument was performed in accordance with CLSI EP5-A2. Two serum and heparinized plasma panels consisting of samples spanning the measuring range were assayed in replicate twice daily over 20 days (n=80) except for the mid negative samples were run over 10 days (n=40). The data were analyzed at an internal Bio-Rad site for within-run, between-run, between-day, and total precision and the standard deviation and percent coefficient of variation are summarized below:

Anti-Cardiolipin: Serum Samples

Precision Sample	N	Mean MPL-U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Mid Negative	40	6.1	0.2	3.0%	0.1	1.6%	0.1	1.5%	0.2	3.7%
Mid Negative	40	6.9	0.2	2.8%	0.1	1.6%	0.0	0.0%	0.2	3.2%
Mid Negative	40	9.1	0.3	2.8%	0.2	2.1%	0.0	0.0%	0.3	3.5%
Mid Negative	40	10.7	0.4	3.3%	0.1	1.1%	0.1	1.0%	0.4	3.6%

Precision Sample	N	Mean MPL-U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Negative Near Cut-off	80	14.5	0.8	5.3%	0.5	3.6%	0.6	3.8%	1.1	7.4%
Negative Near Cut-off	80	15.6	0.9	6.0%	0.2	1.1%	0.1	0.4%	1.0	6.1%
Near Cut-off	80	17.5	1.1	6.3%	0.2	1.0%	0.8	4.5%	1.4	7.9%
Near Cut-off	80	19.6	1.3	6.4%	0.4	2.1%	0.9	4.3%	1.6	8.0%
Low Positive	80	22.6	1.0	4.5%	0.8	3.5%	1.0	4.6%	1.7	7.3%
Low Positive	80	22.7	1.3	5.7%	0.8	3.4%	1.1	4.8%	1.9	8.2%
High Positive	80	73.6	5.5	7.5%	1.5	2.1%	1.8	2.4%	6.0	8.1%
High Positive	80	79.1	3.7	4.7%	0.0	0.0%	2.1	2.7%	4.3	5.4%
Pos Control	80	62.9	2.3	3.7%	1.9	3.0%	1.2	2.0%	3.2	5.2%

Anti-Cardiolipin: Heparin Samples

Precision Sample	N	Mean MPL-U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Mid Negative	40	6.8	0.2	2.7%	0.1	2.1%	0.0	0.7%	0.2	3.4%
Mid Negative	40	7.4	0.2	3.0%	0.1	1.3%	0.1	1.4%	0.3	3.6%
Mid Negative	40	10.4	0.3	3.1%	0.3	2.5%	0.0	0.0%	0.4	4.0%
Mid Negative	40	10.7	0.3	2.8%	0.0	0.0%	0.2	2.1%	0.4	3.5%

Precision Sample	N	Mean MPL-U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Negative Near Cut-off	80	13.6	0.6	4.6%	0.2	1.6%	0.4	3.2%	0.8	5.8%
Negative Near Cut-off	80	14.0	0.8	5.4%	0.2	1.3%	0.6	4.4%	1.0	7.1%
Near Cut-off	80	18.2	1.0	5.3%	0.7	3.9%	0.2	1.1%	1.2	6.7%
Near Cut-off	80	19.0	1.3	6.9%	0.3	1.3%	0.6	3.0%	1.4	7.6%
Low Positive	80	21.5	1.3	6.1%	0.4	1.8%	0.0	0.0%	1.4	6.3%
Low Positive	80	23.4	0.8	3.4%	0.7	2.8%	0.9	3.9%	1.4	5.9%
High Positive	80	72.7	3.2	4.4%	1.4	1.9%	2.1	2.9%	4.1	5.6%
High Positive	80	72.8	2.8	3.8%	2.2	3.1%	0.0	0.0%	3.6	4.9%

Anti- β 2GPI: Serum Samples

Precision Sample	N	Mean U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Mid Negative	40	8.8	0.3	3.0%	0.1	0.7%	0.0	0.0%	0.3	3.1%
Mid Negative	40	9.0	0.3	3.1%	0.3	2.8%	0.0	0.0%	0.4	4.2%
Mid Negative	40	12.8	0.5	3.5%	0.4	2.9%	0.0	0.0%	0.6	4.6%
Mid Negative	40	15.4	0.6	4.1%	0.2	1.2%	0.3	1.7%	0.7	4.6%

Precision Sample	N	Mean U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Negative Near Cut-off	80	17.4	0.6	3.3%	0.2	1.3%	0.1	0.7%	0.6	3.7%
Negative Near Cut-off	80	17.7	0.7	3.8%	0.4	2.1%	0.4	2.1%	0.9	4.8%
Near Cut-off	80	20.6	0.9	4.2%	0.0	0.0%	0.5	2.6%	1.0	4.9%
Near Cut-off	80	21.4	0.8	3.6%	0.4	2.0%	0.6	2.7%	1.1	4.9%
Low Positive	80	23.5	0.7	3.1%	0.7	2.7%	0.7	2.9%	1.2	5.1%
Low Positive	80	23.5	0.9	3.9%	0.5	2.0%	0.9	3.7%	1.4	5.8%
High Positive	80	79.5	5.7	7.2%	2.5	3.2%	1.1	1.4%	6.3	8.0%
High Positive	80	85.5	3.9	4.6%	0.0	0.0%	2.0	2.4%	4.4	5.2%
Pos Control	80	52.1	2.3	4.4%	1.7	3.2%	1.4	2.7%	3.2	6.0%

Anti- β 2GPI: Heparin Samples

Precision Sample	N	Mean U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Mid Negative	40	8.4	0.3	3.1%	0.2	1.8%	0.0	0.3%	0.3	3.7%
Mid Negative	40	8.6	0.3	3.3%	0.1	1.5%	0.1	1.0%	0.3	3.8%
Mid Negative	40	12.2	0.5	3.7%	0.3	2.7%	0.0	0.0%	0.6	4.6%
Mid Negative	40	13.1	0.4	2.8%	0.0	0.0%	0.2	1.5%	0.4	3.2%

Precision Sample	N	Mean U/mL	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
Negative Near Cut-off	80	16.8	0.5	3.0%	0.2	1.4%	0.4	2.5%	0.7	4.2%
Negative Near Cut-off	80	17.0	0.7	3.9%	0.0	0.0%	0.5	2.7%	0.8	4.7%
Near Cut-off	80	20.3	0.6	3.0%	0.6	2.9%	0.0	0.2%	0.9	4.2%
Near Cut-off	80	20.9	0.8	3.9%	0.0	0.0%	0.5	2.3%	0.9	4.5%
Low Positive	80	23.3	0.9	3.9%	0.3	1.1%	0.0	0.0%	1.0	4.1%
Low Positive	80	24.0	0.6	2.4%	0.4	1.7%	0.6	2.6%	0.9	3.9%
High Positive	80	78.6	2.9	3.7%	2.3	2.9%	0.0	0.0%	3.7	4.7%
High Positive	80	79.0	3.5	4.5%	1.5	1.9%	1.4	1.8%	4.1	5.2%

A second smaller repeatability study was performed following EP15-A2 recommendations using all four sample matrices (serum, lithium heparin, sodium heparin and sodium citrate) to determine the within-run, between run and total precision of the BioPlex® 2200 APLS IgM kit. Studies were conducted internally at Bio-Rad. Each matrix had a least two samples that were negative, 2 near the cut-off, 2 low positive and 2 high positive for a total of 8 samples per matrix. Samples were tested in quadruplicate over 5 days for a total of 20 measurements per sample. Precision results for all samples (both serum and plasma) are summarized below:

Anti- β 2GPI IgM: Serum and Plasma

Precision Sample	N	Concentration Range U/mL	Within Run (% CV)		Between Run (% CV)		Total (% CV)	
			Min	Max	Min	Max	Min	Max
Negative	20	12 – 17.5	2.9%	4.9%	1.0%	5.9%	3.2%	7.6%
Near Cut-off	20	18.5 – 22.4	2.3%	7.8%	0.8%	6.5%	2.9%	10.1%
Low Positive	20	52.8 – 56.8	4.2%	8.3%	0.0%	3.0%	4.3%	8.3%
High Positive	20	92.9 – 101.5	2.7%	7.3%	0.0%	3.3%	3.6%	7.3%

Anti- aCL: Serum and Plasma

Precision Sample	N	Concentration Range MPL-U/mL	Within Run (% CV)		Between Run (% CV)		Total (% CV)	
			Min	Max	Min	Max	Min	Max
Negative	20	12.2 – 18.3	3.2%	5.9%	1.9%	6.9%	3.7%	9.1%
Near Cut-off	20	7.8 – 21.3	4.0%	8.0%	0.0%	7.1%	4.0%	10.7%
Low Positive	20	35.0 – 43.5	3.7%	8.1%	0.0%	3.5%	4.5%	8.6%
High Positive	20	64.6 – 80.9	2.7%	7.7%	0.0%	3.6%	4.2%	7.7%

A reproducibility study was performed to estimate the reproducibility between instrument, within run, between run and total precision. Precision was evaluated using five patient samples spanning the measuring range of both the β 2GPI IgM kit and aCL IgM kit using 3 reagent lots in 2 runs with 10 replicates per run on three Bioplex 2200 instruments for total of 60 data points per sample.

Anti-B2GPI IgM Lot-to-Lot Reproducibility Data:

Precision Sample	N	Mean (U/mL)	Within Run		Between Run		Between Lot		Total	
			SD	%CV	SD	%SD	SD	%CV	SD	%CV
APSQSM03	60	16.5	0.51	3.1	0.38	2.3	0.92	5.6	1.12	6.8
APSQSM04	60	18.6	0.65	3.5	0.74	4.0	2.44	13.1	2.63	14.1
APSQSM06	60	22.9	0.72	3.2	0.56	2.5	1.40	6.1	1.67	7.3
APSQSM07	60	26.6	0.92	3.5	0.69	2.6	2.11	7.9	2.41	9.1
APSQSM14	60	98.0	3.88	4.0	3.03	3.1	10.01	10.2	11.16	11.4

Anti-CL IgM Lot-to-Lot Reproducibility Data:

Precision Sample	N	Mean (MPL-U/mL)	Within Run		Between Run		Between Lot		Total	
			SD	%CV	SD	%SD	SD	%CV	SD	%CV
APSQSM03	60	15.0	0.42	2.8	0.42	2.8	0.59	3.9	0.84	5.6
APSQSM04	60	14.6	0.46	3.1	0.59	4.0	1.55	10.6	1.72	11.8

Precision Sample	N	Mean (MPL-U/mL)	Within Run		Between Run		Between Lot		Total	
			SD	%CV	SD	%SD	SD	%CV	SD	%CV
APSQSM06	60	23.6	0.73	3.1	0.78	3.3	1.31	5.6	1.69	7.2
APSQSM07	60	26.2	0.86	3.3	0.60	2.3	1.50	5.7	1.83	7.0
APSQSM14	60	91.9	3.40	3.7	2.63	2.9	2.73	3.0	5.09	5.5

b. Linearity/assay reportable range:

Six APLS aCL and six aβ2GPI IgM positive patient samples (three high and three mid-range) were tested to demonstrate linearity. These samples were diluted with immunodepleted serum according to CLSI EP06-A. Each sample and dilution was evaluated in replicates of four using one APLS IgM lot on one instrument. Linear and polynomial regression analysis of APLS IgM recovery vs. sample dilution was performed to determine if the dilution curves exhibit statistically significant non-linear regression based on the CLSI guideline EP06-A.

The regression parameters (slope, intercept and r^2) of the observed values vs. predicted values are show below.

APLS IgM Assays	Sample	Conc.	Dilution range	Slope	Intercept	r^2
aCL IgM (MPL-U/mL)	1	49.5	0.6 – 49.5	1.0000	-0.0010	0.9931
	2	57.4	0.1 – 57.4	1.0005	-0.0117	0.9914
	3	54.1	0.1 – 54.1	0.9992	0.0184	0.9915
	4	93.9	0.1 – 93.9	0.9999	0.0174	0.9954
	5	92.3	0.1 – 92.3	0.9995	0.0203	0.9985
	6	100.0	0.6 – 100.0	0.9998	0.0226	0.9925
aβ2GPI IgM (U/mL)	1	54.2	0.6 – 54.2	0.9996	0.0099	0.9942
	2	49.9	0.1 – 49.9	1.0009	-0.0319	0.9933
	3	53.1	0.1 – 53.1	0.9996	0.0089	0.9952
	4	92.5	0.6 – 92.5	0.9996	0.0167	0.9978
	5	111.1	0.1 – 111.1	0.9998	0.0258	0.9980
	6	100.3	0.2 – 100.3	1.0003	-0.0275	0.9985

The assay measuring range reported by each APLS IgM assay is shown below.

BioPlex® 2200 APLS Assay	Assay reportable range
aCL IgM	0.2 to 112.0 MPL-U/mL
aβ2GPI IgM	0.2 to 112.0 U/mL

Over-Range (OR) results may be generated for values greater than the reportable measuring range and results are reported as > 112 MPL-U/mL for aCL and > 112 U/mL for aβ2GPI IgM.

High dose hook effect:

Not Applicable

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

Traceability:

There is no international or certified reference material available for APLS aCL and aβ2GPI IgM. The calibrators are assigned relative arbitrary units (U/mL for aβ2GPI, and MPL-U/mL for aCL).

Value Assignment:

The calibrators are manufactured independently from the controls, and are stabilized with <0.3% ProClin® 300, <0.1% sodium benzoate, and <0.1% sodium azide. Calibrator assignment is established for matched lots of BioPlex® 2200 APLS IgM kit and calibrators using a master set of calibrators as reference and replicate analyses on multiple BioPlex® 2200 instruments. The BioPlex® 2200 APLS IgM Reagent Kit is calibrated using a set of four distinct serum based calibrators for aCL and aβ2GPI IgM, which are used to establish points of reference for determining the presence of APLS IgM in human specimens.

The negative control has been tested to give results with values below the cut-off for each assay. The positive control is prepared by blending human disease state serum with negative serum matrix and is manufactured to give results with values above the assay cut-off. The value assignment of the Control Set is derived by testing each control on three BioPlex® 2200 Analyzers with at least two kit lots. For each control level, three vials are tested in replicates of five using each of the kit lots and each analyzer. This testing is performed on three analyzers for a total of 45 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 mean value of the control is calculated using data from all reagent lots, and the acceptable range of is based on the inter-assay precision specification (i.e. 2x inter-assay precision).

The target values and ranges (in parenthesis) for the BioPlex® 2200 APLS IgM Calibrator and Control sets are listed below.

Calibrator Level	BioPlex® 2200 APLS IgM	
	aCL IgM MPL-U/mL	aβ2GPI IgM U/mL
1	≤ 6	≤ 6
2	14	14
3	28	28
4	112	112

Control Level	BioPlex® 2200 APLS IgM	
	aCL IgM MPL-U/mL	aβ2GPI IgM U/mL
Negative	≤ 10	≤ 10
Positive	56	56

Stability: Stability studies have been performed to support the following claims:

Calibrator and Control: BioPlex® 2200 APLS IgM Control and Calibrator Sets: Calibrator Open Vial Stability (2 to 8°C), 30 days from first opening; Control Open Vial Stability (2 to 8°C), 60 days from first opening; Calibration Curve On-board Stability, 30 days; Calibrators and Controls Real Time Stability (2 to 8°C), 24 months; labeled as until expiration date; Calibrators and Controls Accelerated Stability (2 to 8°C), 2.5 years predicted; Calibrators and Controls Freeze-thaw (-20 or -70°C), 5-freeze thaw cycles.

Kit Stability: BioPlex® 2200 APLS IgM Kit: Real Time (unopened) Kit Stability, 24 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.

Sample Stability: Sample stability studies were also performed: Sample stability fresh (2 to 8°C), 7 days; Sample stability frozen (-20 or -70°C), 24 months; Sample Freeze-thaw (-20 or -70°C), up to 3-freeze thaw cycles acceptable.

d. Detection limit:

To determine detection limits for the BioPlex® 2200 IgM kit, three high titer negative serum samples (negative below cut-off) ranged from 14.53 to 16.91 U/mL for aβ2GPI IgM and 8.73 to 14.48 MPL-U/mL for aCL IgM were diluted in various increments in analyte free serum. Each dilution plus analyte free serum was assayed daily in replicates of ten for a period of five days (50 data points total per dilution) on one BioPlex 2200 instrument.

All testing was conducted internally at Bio-Rad using equations from CLSI EP17-A to determine analytical sensitivity. LoD was calculated as “LoD = LoB + cβSDs”. For this calculation, the LoB was calculated at the 95th percentile of negative samples. cβ is the 95th percentile of the standard Gaussian distribution with a correction factor applied to account for the biased estimate of the population standard deviation ($1.645/(1-1/(4xf))$), where f is the degrees of freedom of the estimated standard deviation SDs. LoQ = LoD if the %CV of LoD is less than or equal to 20%;

otherwise, LoQ was calculated from the regression line of the LoD standard deviation versus the analyte concentration. The results of LoQ, LoD and LoB are summarized in the table below:

BioPlex® 2200 APLS IgM Assay	LoB	LoD	LoQ
aCL (MPL-U/mL)	0.0974	0.13	0.2
aβ2GPI (U/mL)	0.1044	0.13	0.2

e. *Analytical specificity:*

Interfering Substances:

An interfering substances study was conducted to evaluate the potential interference of specific endogenous and exogenous substances with the BioPlex® 2200 APLS IgM assay according to CLSI EP7-A2. Samples were prepared by blending a pool of negative human serum with samples positive for aCL IgM and aβ2GPI IgM to achieve approximate values of 10, 20, 60 and 100 MPL for aCL IgM and 10, 20, 60, 100 U/mL for aβ2GPI IgM with interferent or blank. Test and control samples were evaluated in alternating order in replicates of ten each. Substances are considered interfering if their presence in a sample results in more than ± 20% deviation in quantitation relative to the value determined in the absence of the substance. No interference was observed with any of the substances tested. The substances and the maximum levels tested are shown in the table below:

Substance	Concentration
Hemoglobin	≤ 500 mg/dL
Bilirubin, Unconjugated	≤ 20 mg/dL
Bilirubin, Conjugated	≤ 30 mg/dL
Cholesterol	≤ 500 mg/dL
Red Blood Cells	≤ 0.4% (v/v)
Gamma Globulin	≤ 6 g/dL
Triglycerides	≤ 3300 mg/dL
Protein (total)	≤ 12 g/dL
Beta-Carotene	≤ 0.6 mg/dL
Ascorbic Acid	≤ 3 mg/dL
Lithium Heparin	≤ 8000 units/dL
Sodium Heparin	≤ 8000 units/dL
Sodium Citrate	≤ 1000 mg/dL

Cross-Reactivity:

A cross-reactivity study was performed to determine if samples from individuals with various disease states and other potentially cross-reactants interfere with test results from the BioPlex® 2200 APLS IgM kit. Samples from individual with known disease states for potential cross reactivity listed in the table below were evaluated with the BioPlex® 2200 APLS IgM kit. This cohort is different from samples used in

the method comparison and clinical studies. The table below shows the number (N) of samples containing potential cross reactants as disease state evaluated by the BioPlex® APLS IgM. The cross reactivity was obtained as the positivity rate from the ratio of the number of samples scored positive by the BioPlex® APLS IgM assays to the total number of cross reactant samples evaluated. A positivity rate must be \leq 20% for a minimum of 10 samples for each non-APS disease state to be considered not cross reactive.

Cross Reactive Disease State	N	aCL IgM		a β 2GPI IgM	
		# Positive	% Positive	# Positive	% Positive
Systemic Lupus Erythematosus	34	2	5.9%	2	5.9%
Scleroderma	20	3	15.0%	3	15.0%
Sjogrens	22	0	0.0%	0	0.0%
Crohn's Disease	21	2	9.5%	2	9.5%
Ulcerative Colitis	20	1	5.0%	1	5.0%
Rheumatoid Arthritis	12	1	8.3%	1	8.3%
Syphilis	15	0	0.0%	0	0.0%

f. Assay cut-off:

The cutoff value and assignment of the calibrators are determined by performing concordance testing and Receiver Operator Characteristic (ROC) analysis. The study to determine the APLS IgM assay cutoff is comprised of two sample groups – one clinical cohort has 103 samples from patients diagnosed as primary and secondary APS and 123 from non-APS disease control donors. It was later confirmed by testing 208 samples from apparently normal healthy donors.

The cut-off was established to achieve a clinical specificity of 99% while accepting the resultant clinical sensitivity. The criteria for choosing a cutoff at the 99th percentile of a normal healthy population is derived from the “International Consensus Statement on an Update of the Classification Criteria for Definite Antiphospholipid Syndrome (APS)”, Journal of Thrombosis and Haemostasis (2006) 4, 295.

A cutoff of 20 MPL-U/mL for aCL IgM and 20 U/mL for a β 2GPI IgM was established.

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the BioPlex® 2200 APLS IgM kit was evaluated including 199 patients diagnosed with primary or secondary APS, 346 patients with other rheumatic or non-APS disease. Results in the measuring range and 10% of diluted total samples of both the new and the predicate immunoassays are compared.

Results are summarized in the tables below:

		Predicate IgM Kit (aCL: 1.0 – 15480 U/mL)		
		Positive	Negative	Total
aCL IgM (0.2–112 MPL-U/mL)	Positive	77	1*	78
	Negative	28**	403	431
	Total	105	404	509

* 1 Scleredoma

** 2 Gout, 2 Scleroderma, 4 SLE, 3 UC 4 RA, 6 PAPS, 7 SAPS

aCL IgM Positive Agreement = 73.3% (77/105) (95% CI: 64.2– 80.9%)

aCL IgM Negative Agreement = 99.5% (403/404) (95% CI: 98.6 – 100.0%)

aCL IgM Overall Agreement = 94.3% (480/509) (95% CI: 91.9 – 96%)

		Predicate IgM Kit (aβ2GPI: 1.1 – 841 U/mL)		
		Positive	Negative	Total
aβ2GPI IgM (0.2–112 U/mL)	Positive	79	15*	94
	Negative	4**	233	237
	Total	83	248	331

* 3 Scleroderma, 1 RA, 6 PAPS, 5 SAPS

** 1 Osteoarthritis, 1 Scleroderma, 1 PAPS, 1 SAPS

aβ2GPI IgM Positive Agreement = 95.2% (79/83) (95% CI: 88.3 – 98.1%)

aβ2GPI IgM Negative Agreement = 94.0% (233/248) (95% CI: 90.3 – 96.3%)

aβ2GPI IgM Overall Agreement = 94.3% (312/331) (95% CI: 91.2 – 96.3%)

b. Matrix comparison:

Testing for matrix effects was conducted using more than 38 matched sets of serum and sodium (Na) citrate, lithium (Li) heparin, and sodium heparin plasma samples drawn from the same donor in accordance with CLSI EP9-A2. The samples were spiked with aCL IgM or aβ2GPI IgM positive sera as necessary in order to assemble a panel of samples to cover the measuring range of the assay. All samples were evaluated in singlicate in separate runs on two different BioPlex 2200 instruments. Plasma values were compared to matched serum values. Anticoagulants were considered non-interfering if the plasma result was within 20% of the serum result which was equivalent to the linear regression of aβ2GPI IgM or aCL IgM values from matched serum versus plasma samples has a slope of 1.00 ± 0.2 , a y-intercept of 0.0 ± 6.0 and a correlation coefficient between 0.980 and 1.000. The regression correlation parameters for the slopes, intercepts and correlation coefficient (r) are summarized below:

Matrix Comparison	N	BioPlex® APLS Assay	Slope (95% CI)	Intercept (95% CI)	R
Lithium Heparin vs. Serum	38	aCL IgM	1.0106 (0.9808, 1.0405)	-0.3053 (-2.0421, 1.4315)	0.9962
	38	aβ2GPI IgM	0.9991 (0.9682, 1.0301)	0.3054 (-1.5299, 2.1405)	0.9958
Sodium Heparin vs. Serum	38	aCL IgM	1.0369 (0.9786, 1.0953)	-0.6412 (-4.0349, 2.7525)	0.9864
	38	aβ2GPI IgM	1.0271 (0.9750, 1.0779)	-0.2328 (-3.3212, 2.8555)	0.9889
Sodium Citrate vs. Serum	38	aCL IgM	1.0494 (1.0190, 1.0779)	-0.9676 (-2.7381, 0.8029)	0.9963
	38	aβ2GPI IgM	1.0354 (1.0082, 1.0627)	-0.4738 (-2.0890, 1.1414)	0.9970

3. Clinical studies:

a. *Clinical Sensitivity and specificity:*

The clinical studies involved testing 545 specimens including 199 from patients diagnosed with primary or secondary APS and 346 from non-APS disease control patients. The BioPlex® 2200 APLS IgM sensitivity and specificity are shown below:

		Clinical Diagnosis		
		Positive	Negative	Total
BioPlex® 2200 APLS aCL IgM	Positive	67	11	78
	Negative	132	335	467
	Total	199	346	545

aCL IgM Sensitivity = 33.7% (67/199) (95% CI: 27.5 – 40.5%)

aCL IgM Specificity = 96.8% (335/346) (95% CI: 94.4 – 98.2%)

		Clinical Diagnosis		
		Positive	Negative	Total
BioPlex® 2200 APLS aβ2GPI IgM	Positive	80	14	94
	Negative	119	332	451
	Total	199	346	545

aβ2GPI IgM Sensitivity = 40.2% (80/199) (95% CI: 33.6 – 47.1%)

aβ2GPI IgM Specificity = 96.0% (332/346) (95% CI: 93.3 – 97.6%)

The results of the BioPlex® 2200 APLS IgM kit in each of disease category are shown below.

Disease Category	Number Enrolled	aCL IgM		aβ2GPI IgM	
		# pos	% pos	# pos	% pos
Primary APS (PAPS)	123	43	35.0%	50	40.7%
Secondary APS (SAPS)	76	24	31.6%	30	39.5%
Total Diagnosed APS	199	67	33.7%	80	40.2%
Submitted for APS testing	243	11	4.5%	14	5.8%
Apparently Healthy Subject	300	7	2.3%	3	0.01%
Systemic Lupus Erythematosus	101	4	3.96%	5	4.95%
Scleroderma	24	1	4.2%	3	12.5%
Sjogrens	21	1	4.8%	0	0.0%
Crohn's Disease	21	1	4.8%	1	4.8%
Ulcerative Colitis	18	0	0.0%	0	0.0%
Rheumatoid Arthritis	90	3	3.33%	4	4.44%
Syphilis	15	0	0.0%	0	0.0%
CREST	3	0	0.0%	0	0.0%
Fibromyalgia	19	0	0.0%	0	0.0%
Gout	14	0	0.0%	0	0.0%
Inflammatory Arthritis	4	0	0.0%	0	0.0%
Osteoarthritis	11	1	9.1%	1	9.1%
Wegeners Granulomatosis	5	0	0.0%	0	0.0%

b. Other clinical supportive data (when a. is not applicable):

Not applicable

4. Clinical cut-off:

See Assay Cutoff

5. Expected values/Reference range:

Three hundred samples from apparently healthy donors including 132 males ranging in age from 7 to 85 and 168 females ranging in age from 14 to 83 were tested with BioPlex® 2200 APLS IgM kit. The number of positive, mean value and 99th percentile of the BioPlex® APLS IgM results are shown below.

APLS Assay	N (%Positive)	Mean	99 th percentile
aCL IgM	7 (2.3%)	3.0 MPL-U/mL	27.9 MPL-U/mL
aβ2GPI IgM	3 (1.0%)	2.5 U/mL	19.4 U/mL

Results of < 20.0 MPL-U/mL for aCL < 20.0 U/mL for aβ2GPI are reported as negative and results > 20.0 MPL-U/mL for aCL and > 20.0 U/mL for aβ2GPI are reported as positive.

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.