510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k072358

B. Purpose for Submission:
New assay and instrument

C. Measurand:
Anti-myeloperoxidase antibodies (MPO)
Anti-serine proteinase-3 antibodies (PR3)
Anti-glomerular basement membrane antibodies (GBM)

D. Type of Test:
Semi-quantitative multiplex flow immunoassay

E. Applicant:
Bio-Rad Laboratories, Inc.

F. Proprietary and Established Names:
Bioplex 2200 Vasculitis kit – Proprietary Name
Anti-neutrophil cytoplasmic antibody (ANCA) test system (MPO and PR3) and antibodies to glomerular basement membrane measurement device (GBM) – Established Names

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 – device and calibrator
   Class I – quality control
3. Product code:
   MOB, Anti-neutrophil cytoplasmic antibody (ANCA) test system (MPO and PR3)
   MVJ, Antibodies to glomerular basement membrane measurement device (GBM)
   JIX, Multi-analyte calibrator mixture
   JJY, Multi-analyte controls, all kinds (assayed and unassayed)
4. Panel:
   Immunology - 82

H. Intended Use:

1. Intended uses:
   The BioPlex 2200 Vasculitis kit is a flow multiplex immunoassay intended for the semi-quantitative detection of IgG autoantibodies to Myeloperoxidase (MPO), Proteinase 3 (PR3) and Glomerular Basement Membrane (GBM) in human serum. In conjunction with clinical findings, the test system is used as an aid in the diagnosis of anti-neutrophil cytoplasmic antibodies (ANCA) - associated vasculitides: Microscopic Polyangiitis (MPA), Necrotising Glomerulonephritis, Churg-Strauss Syndrome, Wegener's Granulomatosis and the autoimmune renal disorder, Goodpasture's syndrome.
   The BioPlex 2200 Vasculitis kit is intended for use with the Bio-Rad BioPlex 2200 System.

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

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

2. Indication(s) for use:
   Same as Intended use.
3. Special conditions for use statement(s):
   Prescription Use only
4. Special instrument requirements:
   BioPlex 2200 Instrument System

I. Device Description:

BioPlex 2200 Vasculitis Reagent Pack (Catalog No. 665-1850) contains supplies sufficient for 100 tests and contains the following components.

Vial	Description
Bead Set	One 10 mL vial, containing dyed beads coated with Myeloperoxidase (MPO), Proteinase-3 (PR3) and Glomerular Basement Membrane (GBM); an Internal Standard bead (ISB), a Serum Verification bead (SVB), and a Reagent Blank bead (RBB) in a buffer supplemented with Glycerol, protein stabilizers, and preservatives.
Conjugate	One 5 mL vial, containing phycoerythrin conjugated murine monoclonal anti-human IgG and phycoerythrin conjugated murine monoclonal anti-human factor XIII in phosphate buffer supplemented with murine and bovine protein stabilizers. ProClin® 300 (0.3%), sodium benzoate (0.1%) and sodium azide (<0.1%) are added as preservatives.
Sample Diluent	One 10 mL vial, containing bovine and murine protein stabilizers in triethanolamine buffer. ProClin® 300 (0.3%), sodium benzoate (0.1%) and sodium azide (<0.1%) are added as preservatives.

Additional Required Items, available separately from the sponsor.

	Description
663-1800	BioPlex 2200 Vasculitis Calibrator Set: Four 500 µL vials, each containing human antibodies to MPO, PR3 and GBM, in a human serum matrix made from defibrinated plasma. All calibrators contain ProClin® 300 (0.3%), sodium benzoate (0.1%) and sodium azide (<0.1%) as preservatives.

	Description
663-1830	BioPlex 2200 Vasculitis Control Set: Two 1.5 mL Positive Control serum vials, each containing human antibodies to MPO, PR3 and GBM, in a human serum matrix made from defibrinated plasma; and two (2) 1.5 mL Negative Control serum vials, in a human serum matrix made from defibrinated plasma. All controls contain ProClin ® 300 (0.3%), sodium benzoate (0.1%) and sodium azide (<0.1%) as preservatives.
660-0817	BioPlex 2200 Sheath Fluid: Two 4 L bottles containing Phosphate Buffered Saline (PBS). ProClin® 300 (0.03%) and sodium azide (<0.1%) are added as preservatives.
660-0818	BioPlex 2200 Wash Solution: One 10 L bottle containing Phosphate Buffered Saline (PBS) and Tween 20. ProClin® 300 (0.03%) and sodium azide (<0.1%) are added as preservatives.
660-0000	BioPlex 2200 Instrument and Software System

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Phadia Varelisa MPO ANCA EIA
   Phadia Varelisa PR3 ANCA EIA
   INOVA QUANTA Lite GBM ELISA
   INOVA NOVA Lite, ANCA, Ethanol Fixed Slides
2. Predicate 510(k) number(s):
   k041040, k041043, k984336, and k961340
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indication for Use	Aid in diagnosis of ANCA-related vasculitides in conjunction with clinical symptoms	Same
Antigens	Purified MPO, PR3, and GBM	For immunoassays, same antigens
Assay type	Semi-quantitative immunoassay	Semi-quantitative immunoassay for 3 of 4 predicate assays
Analyte detected	IgG autoimmune antibodies to three autoimmune antigens	Same

Differences		
Item	Device	Predicate
Number of analytes	Multiple (3)	Single

Differences		
Item	Device	Predicate
simultaneously detected		
Enzyme conjugate	Phycoerythrin conjugated	Horse radish peroxidase conjugated
Signal detected	Fluorescence	Visual color for 3 of predicate assays
Assay technology	Flow cytometric determination of fluorescently labeled microbeads	Spectrophotometric enzyme immunoassay for 3 predicates; immunofluorescence for one predicate
Solid surface coupling antigens	Magnetic microbeads containing both visual color for identification of analyte and different color for detection of antibody binding	Wells of microwells containing a single antigen only or cell surface containing indistinguishable antigens for immunofluorescent detection of antibody binding

Three of the 4 predicate devices detect individual entities to aid in the diagnosis of anti-neutrophil cytoplasmic autoantibody (ANCA) associated small blood vessel vasculitides. Such vasculitides include Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and kidney-localized forms of these diseases. The inclusion of glomerular basement membrane disease is due to the clinical observation that such patients may have ANCA, though not all ANCA producing patients have autoantibodies to glomerular basement membrane. The reason to include the fourth predicate device is due to the observation, largely a clinical consensus, that the combined use of enzyme immunoassays to these antigens when combined with immunofluorescence detection of ANCA, reflected in this predicate, gives the highest specificity and reasonable sensitivity for ANCA-associated vasculitides.

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

Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP5-A2)
Interference Testing in Clinical Chemistry; Approved Guideline (EP7-A)
Evaluation of Linearity (EP6-a)
FDA guidance (May 11, 2005) Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices

L. Test Principle:

The kit uses multiplex flow immunoassay, an assay technology resembling traditional enzyme immunoassay, but permits simultaneous detection and identification of several analytes in a single assay. Three (3) different populations of magnetic microbeads are coated with antigens associated with vasculitis disease (MPO, PR3 and GBM). The BioPlex 2200 Instrument System combines an aliquot of patient sample, sample diluent, and bead reagent into a reaction vessel. The mixture is incubated at 37°C. After a wash cycle, anti-human IgG antibody, conjugated to phycoerythrin (PE), is added to the beads and this mixture is incubated at 37°C. The excess conjugate is removed in another wash cycle, and the beads are

re-suspended in wash buffer. The bead mixture then passes through the fluorescence detector. The fluorescence detector utilizes flow cytometric hardware technology to detect both the identity of the beads and the amount of antibody captured by the bead-bound antigen. Raw data is calculated in relative fluorescence intensity (RFI). Beads used in the assay have embedded within them a ratio of fluorescent dyes whose final spectral characteristics are unique to that population of beads. Varying ratios of fluorescent dyes changes the spectral characteristics of different bead populations. It is therefore possible to coat one bead population with a certain analyte, combine the beads into a single reagent, and detect each population of beads and its bound analyte at analysis. The preferred biological specimen type used in the assay is human serum.

Three additional dyed beads, an Internal Standard Bead (ISB), a Serum Verification Bead (SVB) and a Reagent Blank Bead (RBB) 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 instrument is calibrated using a set of four (4) distinct calibrator vials, supplied separately. The calibrator vials represent four (4) different antibody concentrations and establish semi-quantitative calibration. The result for each of these antibodies is expressed as an antibody index (AI). The detected anti-neutrophil cytoplasmic autoantibodies are human IgG.

The Control Set includes a negative control as well as a multi-analyte positive control containing antibodies present for analytes within the kit. The positive control is manufactured to give positive results, with values above the cut-off for each specific bead. The negative control is manufactured to give negative results, with values below the cut-off for each specific bead.

The recommended minimum 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.

Statistical quality control (QC) tests can be applied periodically to determine if assays are performing as expected. These tests assess any significant changes in the mean and standard deviation of the results for the analyte, beyond the inherent variations of the assay when processed on the instrument. Kit quality control analysis is performed by processing control samples and analyzing the mean and standard deviation of the analyte results over defined intervals. This activity is monitored with Levey-Jenning graphs and Westgard Rules.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Separate internal and external studies were conducted to evaluate the reproducibility of the proposed assay on the BioPlex 2200 instrument. The internal reproducibility study was conducted in-house by the sponsor. The external reproducibility study was conducted at each of the three clinical study sites.

   Internal Precision Study

   Precision of the assay was assessed in serum samples for antibodies to each of the three analytes (MPO, PR3, GBM). Precision was determined by calculating the within-run (intra-assay), between run (inter-assay), between-day (Inter-assay), and Total Precision. A precision panel for each analyte was prepared. The panel members for each assay included at least one negative sample (<0.2 AI), one high

negative sample (0.5 to 0.8 AI), two near cut-off samples (0.9 to 1.1 AI), two low positive samples (1.3 to 2.0 AI), and two positive samples (3.0 to 5.0 AI). Each panel member was prepared by spiking analyte negative serum with one or more positive sample for one or more analytes.

Twenty days of precision were collected over a 23 days period for one reagent lot. Each sample was run in duplicate per run for a total of eighty (80) data points per sample. Two runs were performed each day with at least two hours separating each run. All samples were randomized for each run and assay calibration was performed at the start of the study. Controls were run daily to quality the run. Data analyses are based on CLSI EP5-A2 Evaluation of Precision Performance of Clinical Chemistry Devices.

Precision specifications for samples at or above the cut-off point were:

Within Run: CV ≤ 10%
Between Run: CV ≤ 15%
Between Days: CV ≤ 15%
Total Precision: CV ≤ 20%

The following results for samples having an AI greater than 0.6 were obtained (Summary precision for negative sample, < 0.2 AI, not calculated):

GBM, MPO and PR3 Precision Performance (%CV) of Samples > 0.6 AI					
Precision Parameter	CV Specification	Precision Limit	GBM	MPO	PR3
Within Run	≤10%	Minimum	2.2%	2.5%	2.2%
		Maximum	4.2%	7.1%	4.2%
Total	≤20%	Minimum	4.1%	4.2%	5.3%
		Maximum	6.8%	7.9%	6.4%
Between Run	≤15%	Minimum	2.3%	0.0%	0.0%
		Maximum	4.3%	6.1%	4.4%
Between Day	≤15%	Minimum	1.7%	0.0%	3.1%
		Maximum	4.7%	4.3%	4.4%

The following results for the positive control were obtained (Summary precision for negative control, < 0.2 AI, not calculated):

Precision Parameter	CV Specification	GBM	MPO	PR3
Within Run	≤10%	3.7%	3.5%	3.9%
Total	≤20%	5.9%	6.7%	6.1%
Between Run	≤15%	1.9%	4.4%	3.9%
Between Day	≤15%	4.2%	3.7%	2.6%

Precision for all the samples met the acceptance criteria. The within-run precision ranged from 2.2% to 7.1%, between run precision ranged from 0.0% to 6.1%, and total run precision ranged from 4.1% to 7.9% across all assays.

External Reproducibility Study

In order to assess the reproducibility of the BioPlex 2200 Vasculitis Kit, a 10 member reproducibility serum panel was prepared at Bio-Rad Laboratories and provided to three sites for testing. Two lots of the Reagent Pack, two lots of the Calibrator Set and two lots of Control Set were used to evaluate reproducibility. Two of the three sites evaluated one lot and the third site evaluated a separate second lot of the kit. Each of the panel members and Control Set were tested in duplicate on two runs per day over 3 days (2 replicates x 2 runs x 3 days = 12 replicates per panel member per site = 36 total replicates for 3 sites).

Each positive panel member of the 10 member reproducibility panel was prepared by combining one or more patient samples positive for antibodies to MPO, PR3 and GBM. Of the 10 panel members, two members had high levels of antibodies to MPO, PR3 and GBM, two members had lower levels of antibodies to MPO, PR3 and GBM, and two members had antibody levels near the cutoff for MPO, PR3 and GBM. There were also one high negative and one low negative panel member. In addition, 1 positive control and 1 negative control was included and tested as panel members. Each panel member described above was made in serum (N=10). Table 3 presents the 10 member reproducibility panel and the target Antibody Index of each member.

Panel Member	Target AI		
	GBM	MPO	PR3
High Positive 1	4.2	3.3	3.9
High Positive 2	4.9	5.0	4.3
Low Positive 1	1.3	1.2	1.3
Low Positive 2	1.7	1.7	1.5
Near Cutoff 1	1.0	0.8	1.1
Near Cutoff 2	1.2	1.1	1.1
Negative 1	0.7	0.4	0.7
Negative 2	0.1	0.0	0.0
Positive Control	3.2	2.6	2.6
Negative Control	0.0	0.0	0.0

The between-site %CV for GBM ranged from 0.0% to 6.0%, for MPO ranged from 0.0% to 8.2%, and for PR3 ranged from 0.0% to 10.9%. The average between-site and total %CV values for each of the 10 samples for the three analytes are as follows:

Analytes	Samples	Sample N	Mean AI	Between-Site*		Total	
				SD	%CV	SD	%CV
anti-GBM	High Positive 1	36	4.3	0.204	4.7 %	0.322	7.5 %
	High Positive 2	36	4.8	0.280	5.9 %	0.377	7.9 %
	Low Positive 1	36	1.4	0.087	6.0 %	0.135	9.4 %
	Low Positive 2	36	1.7	0.047	2.8 %	0.116	6.9 %
	Near Cutoff 1	36	1.1	0.079	7.2 %	0.099	9.0 %
	Near Cutoff 2	36	1.2	0.081	6.7 %	0.110	9.2 %
	Negative 1	36	0.7	0.012	1.6 %	0.038	5.3 %
	Negative 2	36	0.2	0.000	0.0 %	0.000	0.0 %
	Positive Control	36	2.8	0.063	2.3 %	0.153	5.4 %
	Negative Control	36	0.2	0.000	0.0 %	0.000	0.0 %

Analytes	Samples	Sample N	Mean AI	Between-Site*		Total	
				SD	%CV	SD	%CV
anti-MPO	High Positive 1	36	4.0	0.326	8.2 %	0.456	*11.5 %*
	High Positive 2	36	5.5	0.254	4.6 %	0.462	8.4 %
	Low Positive 1	36	1.5	0.017	1.2 %	0.089	6.2 %
	Low Positive 2	36	1.9	0.025	1.3 %	0.170	8.7 %
	Near Cutoff 1	36	1.0	0.048	4.6 %	0.082	7.9 %
	Near Cutoff 2	36	1.3	0.000	0.0 %	0.075	5.6 %
	Negative 1	36	0.6	0.048	8.2 %	0.070	*11.9 %*
	Negative 2	36	0.2	0.000	0.0 %	0.000	0.0 %
	Positive Control	36	2.9	0.102	3.5 %	0.223	7.7 %
	Negative Control	36	0.2	0.000	0.0 %	0.000	0.0 %
anti-PR3	High Positive 1	36	4.2	0.076	1.8 %	0.326	7.8 %
	High Positive 2	36	4.5	0.267	6.0 %	0.376	8.4 %
	Low Positive 1	36	1.4	0.033	2.4 %	0.108	7.9 %
	Low Positive 2	36	1.5	0.085	5.5 %	0.157	*10.2 %*
	Near Cutoff 1	36	1.2	0.027	2.2 %	0.088	7.3 %
	Near Cutoff 2	36	1.1	0.000	0.0 %	0.073	6.4 %
	Negative 1	36	0.8	0.042	5.3 %	0.074	9.3 %
	Negative 2	36	0.2	0.000	0.0 %	0.000	0.0 %
	Positive Control	36	2.3	0.032	1.4 %	0.167	7.3 %
	Negative Control	36	0.2	0.024	*10.9 %*	0.041	*18.8 %*

The total %CV for the MPO analyte ranged from 0.0 % to 11.5 % , total %CV for the PR3 analyte ranged from 0.0% to 18.8% and total %CV for the GBM analyte ranged from 0.0 % to 9.4 %. The proposed assay met the general specification for all 3 analytes for total imprecision (< 20%CV).

b. Linearity/assay reportable range:

A dilution linearity study was conducted internally for the three analytes contained in the kit. The claimed assay range is 0.2 AI to 8 AI. The methods used to evaluate dilution/linearity used a range from 0.2 to 9.9 AI. The extended assay range was implemented to demonstrate linearity beyond the highest calibrator. Five high positive serum samples were purchased for each analyte represented in the kit. The samples were selected at the high end of the assay range. However, some analytes contained very high concentrations and so high samples with values significantly above the assay range (>20%) were initially diluted with negative serum to bring the value within the range of the assay. All high samples with values within the assay range were then diluted with negative serum using the following dilutions (% high patient sample): 80%, 60%, 40%, 20%, 10%, and 0%. Each sample was tested in 4 replicates. Based on the CLSI guidelines EP6-A, (Vol. 23 No. 16, Evaluation of the Linearity of Quantitative Measurement procedure: Statistical Approach) linear and polynomial regression analysis of sample AI vs. dilution was performed to determine if the dilution curve exhibits a statistically significant nonlinear regression. The assessment of a non-linear curve is only a test of statistical significance, and indicates that nonlinearity has been detected. It does not mean that the effect of this non-linearity is enough to affect patient results. If the linear or non-linear regression

coefficients are significant, then calculated values obtained from the linear regression are compared to calculated values obtained from either the second order polynomial curve or the third order polynomial curve. The differences in the results between the recovered and expected values (%difference) are then compared to the acceptance criteria to determine acceptance. The acceptance criteria were: All dilutions must recover with < 2SD or <20% of the expected result which is the clinical acceptance range.

All of the patient samples exhibited non-linear curves on dilution for all three analytes. In this analysis, the expected concentration was assumed to be the concentration calculated by division of the observed undiluted concentration with the reciprocal of the dilution, assuming perfect technical dilution. The regression analyses utilized the observed AI for each analyte as the dependent y-variable vs. the expected AI as the independent x-variable.

The deviation from linearity of the AI was within the sponsor's clinical acceptance criteria since the %deviation from linearity by the non-linear dilution curves was 20% or less for all dilutions, except for the highest dilution. The percent deviation from linearity for all samples at all dilutions ranged from -33% to 13%. Dilution of samples with the lowest AI (highest dilution) displayed the greatest %deviation from linearity. The following table illustrates the %deviation from linearity by the non-linear dilution.

dilution	mean % deviation from linearity		
	GBM	MPO	PR3
10	-30.9%	-24.3%	-26.4%
5	-1.7%	-1.9%	-1.7%
2.5	11.0%	8.3%	8.9%
1.67	8.1%	6.6%	6.8%
1.25	1.6%	1.6%	1.5%
1	-5.5%	-4.5%	-4.5%
All dilutions	**-2.9%**	**-2.4%**	**-2.6%**

This indicates that on average, the expected AI value will be 3% below the observed value when the expected value is the concentration determined from the reciprocal of the dilution. This difference in concentration is modest and likely not clinically significant. The highest dilution had the greatest percent deviation from linearity and is not unusual for an assay of this type. The assay detects autoantibodies, typically low affinity antibodies, and displays difficulty when diffusion controlled binding conditions occur, such as under high dilution and low autoantibody concentration conditions.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No reference standard for anti-MPO, anti-PR3, or anti-GBM exists. Calibrators and controls are prepared for use and assigned arbitrary values during assay development.

d. *Detection limit:*
No information available. The reagent blank bead included in the kit is not coated with MPO, PR3, or GBM antigens so it would not be expected to capture

autoantibody. However, the signal from this bead could be a random as well as a non-random signal. A non-random signal would indicate binding of non-specific material or antibody to beads and subsequent occurrence of an assay signal. A limit of blank determination would measure the amount of random fluorescence present in a specimen without any autoantibody or non-specific binding. If the random and non-specific binding signal is subtracted from the fluorescence signal of specimens from normal and diseased subjects then there would be little need to assess the random signal (signal limit of a blank specimen).

e. Analytical specificity:

Separate interfering substances and cross reactivity studies were conducted internally at Bio-Rad to evaluate the potential interference of specific endogenous and exogenous substances and to determine whether or not cross-reactivity occurs in the presence of non-vasculitis reactive IgG antibodies with the assay.

Interfering Substances

For the interfering substances study, human serum samples that were previously characterized as positive for the various markers at concentrations near the middle of the assay range (1.5 to 3.5 AI) were supplemented with various interfering substances. A control was made for each interfering substance by spiking the pooled sera with the solvent or solution used to make the interfering substance spiking solution. The test sample and control were run using two reagent lots in replicates of 5 per lot (10 total). Calculations were established using CLSI EP7-A2. Potential interference was defined as any substance that exhibited a % difference from the control greater than the pre-established specification of 20%. Interference was evaluated with the following interferents at the stated concentrations.

Endogenous Substances	Exogenous Substances
Hemoglobin 500mg/dL	Ascorbic Acid 3 mg/dL
Bilirubin (unconjugated) 20mg/dL	Sodium Citrate 1000 mg/dL
Bilirubin (conjugated) 30 mg/dL	EDTA 800 mg/dL
Triglycerides 3500mg/dL	Lithium Heparin 8000 units /dL
Protein (total) 12.9 g/dL	Sodium Heparin 8000 units/dL
Beta-Carotene 0.6mg/dL	
Cholesterol 500mg/dL	
Red Blood Cells 0.4% Concentration	
Gamma-globulin 6g/dL	

All three analytes met interference specifications for endogenous components and exogenous substances. The degree of endogenous interference ranged from -8.0% to 0.0% for GBM, -2.7% to 2.7% for MPO and -4.2% to 4.5% for PR3 relative to controls. Similarly, the degree of exogenous interference was 0.0% for GBM, ranged from -2.6% to 0.0% for MPO, and -4.0% to 0.0% for PR3 relative to their respective controls.

Cross Reactivity

For the cross reactivity study, human serum samples that were previously characterized as positive for the various cross-reactants were evaluated for possible cross reactivity in the assay. In order to evaluate whether reactivity was due to cross-reactivity or reactivity to MPO, PR3 or GBM antibodies, the samples were also tested

with commercially available predicate EIA tests. Cross reactivity was defined as >20% of the samples being positive by the proposed assay and those samples being confirmed negative by the corresponding commercially available microplate enzyme immunoassays. A panel of ten specimens high positive for each test cross reactant (~2 times cut-off) were evaluated for possible cross -reactivity in the proposed assay, with the exception of seven tissue transglutaminase positive samples and eight Mitochondrial M2 positive samples due to limited availability of these samples. Patient samples were obtained from commercial sources as well as an in-house sample bank. The following lists the cross-reactive antibodies tested.

Test Cross Reactant	Sample size (N)
ANA	10
Anti Saccharomyces Cervisiae antibodies IgG (ASCA)	10
Cardiolipin	10
CMV IgG	10
dsDNA	10
EBV IgG	10
Gliadin IgG	10
HBV IgG	10
Histone	10
HSV1 IgG	10
Measles IgG	10
Mitochondrial	8
Mumps IgG	10
Parvovirus antibody	
Rhematoid Factor (RF)	10
Rubella IgG	10
Thyroglobulin (TG)	10
Thyroid Peroxidase (TPO)	10
tTG (tissue transglutaminase) IgG	7
VZV IgG	10
Anti smooth muscle cell antibodies (ASMA)	10
HCV IgG	10
HIV antibodies	10
HSV2	10

The following table summarizes the reactivity in the proposed assay and a commercially available test method. Only cross-reactants showing any reactivity are listed. Cross-reactant samples not listed showed no reactivity in either assay.

BioPlex 2200 and Predicate Scoring of Cross Reactive Samples					
Cross Reactant	N	Result Type	GBM	MPO	PR3
ASCA	10	BioPlex2200 (+)	0	0	1
		Predicate, (+)	0	0	1
		Discrepants	0	0	0

BioPlex 2200 and Predicate Scoring of Cross Reactive Samples					
Cross Reactant	N	Result Type	GBM	MPO	PR3
Cardiolipin	10	BioPlex2200 (+)	0	0	0
		Predicate, (+)	0	1	0
		Discrepants	0	1	0
Histone	10	BioPlex2200 (+)	0	2	2
		Predicate, (+)	0	1	0
		Discrepants	0	1	2
Mumps IgG	10	BioPlex2200 (+)	0	0	1
		Predicate, (+)	0	0	1
		Discrepants	0	0	0
Parvovirus antibodies	10	BioPlex2200 (+)	0	0	0
		Predicate, (+)	0	1	0
		Discrepants	0	1	0
VZV IgG	10	BioPlex2200 (+)	0	0	1
		Predicate, (+)	0	0	1
		Discrepants	0	0	0

The table lists 1 to 2 of 10 samples cross-reactive in the proposed assay. Only histone, cardiolipin, and Parvovirus reactivity indicate a suspicion since there was positive cross-reactivity in the proposed assay and not the other commercially available test or vice versa. Due to the low number of tested samples, it is likely that the proposed assay lacked reactivity for Parvovirus antibody and cardiolipin while having 10% of tested samples had reactivity in the other commercially available EIA due to random chance. For histone, the proposed assay had cross-reactivity in 2 of 10 samples for 2 analytes. It is not possible to determine based on this information if the patient supplying the sample had anti-histone antibodies that cross react in the proposed assay or had antibodies to the analytes in the proposed assay. It often occurs that autoimmune patients with other autoimmune diseases have antibody reactivity to MPO, PR3, and GBM.

f. Assay cut-off:

A final cut-off of 1.0 AI was sought for all analytes based on an evaluation of 356 serum samples with the assay and corresponding commercially available microplate EIA tests using the results of these predicate assays as the reference outcome. Of the 356 serum samples, 315 were negative for GBM antibodies, 259 for MPO antibodies and 195 for PR3 antibodies by the assay. Discordant results were confirmed by re-testing samples using predicate and the proposed assay. ROC analysis was performed for each analyte using this population of samples. For the purpose of establishing a cut-off, equivocal samples on the commercially available microplate EIA tests were not used during ROC analysis or in evaluating the concordance statistics.

The sponsor used the following assays as the reference outcome for an ROC determination. The cutoffs and indeterminate ranges used for each assay are also included.

Predicate Device	Cut-Off	Indeterminate Range
GBM INOVA	21.0	N/A
MPO Varelisa	Ratio <1.0	Ratio 1.0 – 1.4
PR3 Varelisa	Ratio <1.0	Ratio 1.0 – 1.4

The sponsor also lists sample size specifications and concordance specifications for the predicate assays as follows:

Assay	Number of Samples		Agreement with Predicate
	Positive	Negative	Overall
GBM	≥40	≥100	95%
MPO	≥40	≥100	95%
PR3	≥40	≥100	93%

The overall agreement is calculated from the ROC analysis at each cutoff value of the proposed assay as part of the determination. The "sensitivity" value in the ROC is therefore percent positive agreement while the "specificity" value is the percent negative agreement. The cutoff values for each analyte with the above specifications are one choice for a cutoff or range of cutoff values, though not clinically based. A series of tables summarizes results for each analyte. For the GBM analyte, the following are shown:

			Predicate – INOVA-EIA				
Assay Name	Cut-off	Bioplex Method	Positives	Negatives	Total	Predicate-equivocal	Total samples
GBM	1.0	Positives	39	2	41		
		Negatives	2	313	315	0	356
		Total	41	315	356		

	Estimate	95% Confidence Interval	
% Positive Agreement	95.1 % (39/41)	83.9 %	98.7 %
% Negative Agreement	99.4 % (313/315)	97.7 %	99.8 %
% Overall Agreement	98.9 % (352/356)	97.1%	99.6 %

Test Receiver Operator Characteristic (ROC) curves of BioPlex 2200 GBM vs. INOVA GBM Score (n = 356; AUC = 0.999 ± 0.0010; p <0.0001; 95% CI of area 0.997 to 1.000)

cut-off	Sensitivity	Specificity	TP	TN	FP	FN	Overall Agreement
-	100.0%	0.0%	41	0	315	0	
0.00	100.0%	75.2%	41	237	78	0	78.1
0.10	100.0%	95.2%	41	300	15	0	95.8
0.20	97.6%	97.5%	40	307	8	1	97.5
0.30	97.6%	98.7%	40	311	4	1	98.6
0.40	97.6%	99.4%	40	313	2	1	99.2
0.80	95.1%	99.4%	39	313	2	2	98.9
1.00	**95.1%**	**99.7%**	**39**	**314**	**1**	**2**	**99.2**
1.50	92.7%	99.7%	38	314	1	3	98.9
1.70	92.7%	100.0%	38	315	0	3	99.2
1.80	90.2%	100.0%	37	315	0	4	98.9
1.90	85.4%	100.0%	35	315	0	6	98.3

At the sponsor's choice of 1.0 AI as a cutoff, the overall agreement was 99.2%, substantially higher than the 95% minimum chosen as a specification for the analyte GBM.

For the MPO analyte the following are shown.

			Predicate – Varelisa-EIA				
Assay Name	Cut-off	Bioplex Method	Positives	Negatives	Total	Predicate-equivocal	Total samples
MPO	1.0	Positives	84	2	86		
		Negatives	7	257	264	6	356
		Total	91	259	350		

	Estimate	95% Confidence Interval	
%Positive Agreement	92.3 % (84/86)	85.0 %	96.2 %
%Negative Agreement	99.2 % (257/265)	97.2 %	99.8 %
Overall Agreement	97.4 % (341/350)	95.2 %	98.6 %

Test Receiver Operator Characteristic (ROC) curves BioPlex 2200 MPO vs. Varelisa MPO Score (n = 350; AUC = 0.996 ± 0.0024; p <0.0001; 95% CI of area 0.991 to 1.000)

cut-off	Sensitivity	Specificity	TP	TN	FP	FN	Overall Agreement
-	100.0%	0.0%	91	0	259	0	
0.00	100.0%	72.6%	91	188	71	0	79.7
0.10	98.9%	92.3%	90	239	20	1	94.0
0.20	96.7%	95.0%	88	246	13	3	95.4
0.30	96.7%	96.5%	88	250	9	3	96.6
0.40	95.6%	97.7%	87	253	6	4	97.1
0.50	94.5%	98.5%	86	255	4	5	97.4
0.60	93.4%	98.8%	85	256	3	6	97.4
0.70	92.3%	99.2%	84	257	2	7	97.4
1.00	**91.2%**	**99.2%**	**83**	**257**	**2**	**8**	**97.1**
1.10	91.2%	99.6%	83	258	1	8	97.4
1.40	91.2%	100.0%	83	259	0	8	97.7

cut-off	Sensitivity	Specificity	TP	TN	FP	FN	Overall Agreement
1.70	89.0%	100.0%	81	259	0	10	97.1
1.80	86.8%	100.0%	79	259	0	12	96.6

At the sponsor's choice of 1.0 AI as a cutoff, the overall agreement was 97.1%, substantially higher than the 95% minimum chosen as a specification for the analyte MPO.

For the PR3 analyte the following are shown.

			Predicate – Varelisa-EIA				
Assay Name	Cut-off	Bioplex Method	Positives	Negatives	Total	Predicate-equivocal	Total samples
PR3	1.0	Positives	136	9	145		
		Negatives	9	186	195	16	356
		Total	145	195	340		

	Estimate	95% Confidence Interval	
%Positive Agreement	93.8% (136/145)	88.6%	96.7%
%Negative Agreement	95.4% (186/195)	91.5%	97.6%
Overall Agreement	94.7% (322/340)	91.8%	96.6%

Test Receiver Operator Characteristic (ROC) curves BioPlex 2200 PR3 vs. Varelisa PR3 Score (n = 340; AUC = 0.988 ± 0.0043; p <0.0001; 95% CI of area 0.980 to 0.996)

cut-off	Sensitivity	Specificity	TP	TN	FP	FN	Overall agreement
-	100.0%	0.0%	145	0	195	0	
0.00	100.0%	42.1%	145	82	113	0	66.8
0.10	99.3%	81.5%	144	159	36	1	89.1
0.20	97.9%	90.3%	142	176	19	3	93.5
0.30	96.6%	91.3%	140	178	17	5	93.5
0.40	96.6%	91.8%	140	179	16	5	93.8
0.50	96.6%	92.8%	140	181	14	5	94.4
0.60	96.6%	93.3%	140	182	13	5	94.7
0.70	95.9%	93.8%	139	183	12	6	94.7
0.80	93.8%	94.4%	136	184	11	9	94.1
0.90	**93.8%**	**95.4%**	**136**	**186**	**9**	**9**	**94.7**
1.10	91.7%	95.4%	133	186	9	12	93.8
1.20	91.7%	95.9%	133	187	8	12	94.1
1.30	91.0%	95.9%	132	187	8	13	93.8
1.40	91.0%	96.4%	132	188	7	13	94.1
1.80	90.3%	96.4%	131	188	7	14	93.8
1.90	89.0%	96.4%	129	188	7	16	93.2
2.00	87.6%	97.4%	127	190	5	18	93.2

At the sponsor's choice of 1.0 AI as a cutoff, the overall agreement was 94.7%. Using the final cut-off of 1.0 AI, overall agreements of 98.9%, 97.4% and 94.7% were observed for the GBM, MPO and PR3 assays.

2. Comparison studies:

a. Method comparison with predicate device:

Left-over serum specimens were obtained from routine clinical care/analysis. Samples were collected based on inclusion/exclusion criteria per sample population. Mayo Clinic study provided samples for 4 of 5 study populations meeting the study inclusion criteria through a review of sample records.

(1) For the "Unselected Patient Samples Previously Tested on FDA-cleared predicate tests (anti-MPO, anti-PR3 and/or anti-GBM)", Mayo staff verified through a review of sample records what samples had been tested for one or more anti-MPO, anti-PR3 and/or anti-GBM tests. These were not selected on test value but based on the use of one of the predicate tests. 300 specimens were selected.

(2) For "Retrospective Known Positive Samples for anti-MPO", Mayo staff verified that samples enrolled in this population had a positive anti-MPO EIA result through review of sample records. 100 specimens were selected.

(3) For "Retrospective Known Positive Samples for anti-PR3", Mayo staff verified that samples enrolled in this population had a positive anti-PR3 EIA result through review of sample records. 100 specimens were selected.

(4) For "Retrospective Known Positive Samples for anti-GBM", Mayo staff verified that samples enrolled in this population had a positive anti-GBM EIA result through review of sample records. 100 specimens were selected.

Bio-Rad Laboratories provided one of 5 study populations. Three hundred (300) normal blood donor samples were purchased from one vendor and eighteen anti-GBM known positive samples were purchased from another vendor by Bio-Rad Laboratories and provided to Mayo Clinic for testing.

The following are listed as inclusion/exclusion criteria for sample selection from left-over serum specimens:

- The sample is from a normal blood donor (purchased and provided by Bio-Rad). Or
- The patient sample is left-over remnant from prior routine clinical testing with a FDA-cleared predicate test (MPO, PR3 and/or GBM) and is not selected based on the test value. Or
- The sample is retrospective and known positive for anti-MPO on an FDA-cleared predicate test. Or
- The sample is retrospective and known positive for anti-PR3 on an FDA-cleared predicate test. Or
- The sample is retrospective and known positive for anti-GBM on an FDA-cleared predicate test.
- **and** the sample is serum and is $\geq$1.0 mL.

Exclusion Criteria

- The sample does not meet the inclusion criteria.
- The sample is from an individual previously enrolled in the study.
- Incorrect sample matrix (plasma).
- Sample known to be frozen and thawed > 3 times at time of testing.
- The sample is grossly hemolyzed.

Testing of samples was performed at The Mayo Clinic, San Francisco General Hospital, and the sponsor's testing lab. Samples tested in the study were stored at -20°C or colder prior to being entered in the study. The samples were thawed and centrifuged prior to testing in the proposed assay. Testing occurred within 1 – 2 days after the samples were thawed. Samples were stored at 2-8°C after thawing and prior to testing. Samples tested on the predicate assays were stored and tested the same as above. Samples purchased by Bio-Rad Laboratories were received frozen on dry ice and stored at ≤ -20°C prior to shipping the samples to the site for testing. These samples were stored frozen (≤ -20°C or colder) prior to testing. After thawing, the samples were tested within 7 days. Samples were stored at 2-8°C after thawing. Mayo Clinic provided and tested 521 samples that were stored at 2-8°C for greater than 7 days. Because this was outside the allowable storage conditions, the samples were excluded from the study. A total of 1348 samples were tested. Eleven samples were re-tested after initial error messages were obtained. Seven samples had "Serum Verification Bead (SVB) signal too low" errors that did not resolve on repeat testing. Three samples had multiple error messages including "Insufficient Bead Events to Calculate Result" errors that did resolve on repeat testing. One sample had "Assay Dispense Sample Volume Not Met" error that did resolve on repeat testing. The repeat test result was used in the data analysis. Thus, four samples that were valid test results on re-testing were included in analysis.

Three hundred (300) normal blood donors and three hundred (300) unselected patient samples previously tested with the predicate assays were tested with the BioPlex 2200 Vasculitis kit. Seven of the 300 normal blood donor samples were excluded due to "Serum Verification Bead (SVB) signal too low" analysis error during BioPlex 2200 Vasculitis kit testing. The BioPlex 2200 Vasculitis kit was further evaluated by testing 227 retrospective samples positive for anti-MPO (N=100), anti-PR3 (N=100), and anti-GBM (N=27). All samples were also tested by the corresponding commercially available microplate EIA methods. In addition, the anti-MPO and anti-PR3 positive samples were tested by an ANCA IFA method using ethanol-fixed slides.

The following table lists the testing of normal blood donors, unselected patient samples, and positive samples using the proposed assay and the predicate EIA or ANCA immunofluorescence assay for MPO.

Varelisa MPO ANCA EIA	BioPlex 2200 MPO			pANCA IFA	BioPlex 2200 MPO		
	Positive	Negative	Total		Positive	Negative	Total
Positive	97	8	105	Positive	83	6	89
Equivocal	3	0	3	Negative	10	1	11
Negative	7	578	585	Total	93	7	100
Total	107	586	693				

%positive agreement with EIA = 92.4% (95% confidence interval 85.5% to 96.7%);
% negative agreement with EIA = 98.8% (95% confidence interval 97.6% to 99.5%);
% overall agreement with EIA = 97.4% (95% confidence interval 95.9% to 98.4%%).
%positive agreement with IFA = 93.3%;

% negative agreement with IFA = 9%;
%overall agreement with IFA 84% (95% confidence interval 75.6% to 89.9%).

The following table lists the testing of normal blood donors, unselected patient samples, and positive samples using the proposed assay and the predicate EIA or ANCA immunofluorescence assay for PR3.

Varelisa PR3 ANCA EIA	BioPlex 2200 PR3			cANCA IFA	BioPlex 2200 PR3		
	Positive	Negative	Total		Positive	Negative	Total
Positive	84	0	84	Positive	93	5	98
Equivocal	9	1	10	Negative	2	0	2
Negative	10	589	599	Total	95	5	100
Total	103	590	693				

%positive agreement with EIA = 100% (95% confidence interval 95.7 to 100%);
% negative agreement with EIA = 98.3% (95% confidence interval 97.0% to 99.1%);
% overall agreement with EIA = 97.1% (95% confidence interval 95.6% to 98.2%).
%positive agreement with IFA = 94.9%;
%negative agreement with IFA = 0%;
%overall agreement with IFA 93% (95% confidence interval 86.2% to 96.6%)

The following table lists the testing of normal blood donors, unselected patient samples, and positive samples using the proposed assay and the predicate EIA or ANCA immunofluorescence assay for GBM.

		BioPlex 2200 GBM		
		Positive	Negative	Total
INOVA QuantaLite GBM EIA	Positive	16	5	21
	Negative	3	596	599
	Total	19	601	620

%positive agreement = 76.2% (95% confidence interval 52.8% to 91.8%).
% negative agreement = 99.5% (95% confidence interval 98.5% to 99.9%.
% overall agreement = 98.7% confidence interval 97.5% to 99.4%).

The following table summarizes the %agreement for each analyte.

%Agreement	MPO with EIA (with IFA)	PR3 with EIA (with IFA)	GBM with EIA
%Positive agreement	92.4% (93.3%)	100% (94.9%)	76.2%
%Negative agreement	98.8% (9%)	98.3% (0%)	99.5%
%Total agreement	97.4% (84%)	97.1% (93%)	98.7%

The low %Negative agreement of MPO and PR3 with the immunofluorescence assay (9% and 0%) is likely due to the lack of negative samples (see tables above) since a substantial number of samples were positive in the immunofluorescence assay.

Testing of both EIA and IFA for each analyte is not compared with a clinical diagnosis. To the extent that the predicate assays correlate with clinical disease and to the extent that the overall agreement of the proposed assay with the predicate tests is sufficiently high, this data indicates substantially equivalence and reasonable effectiveness at diagnosis of clinical diseases stated in the Indications for use.

b. Matrix comparison:
Not necessary as the only matrix is human serum.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.
   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.
4. Clinical cut-off:
   Not applicable.
5. Expected values/Reference range:
   Values were determined in 300 serum samples from normal blood donors utilized as part of the comparison studies. Seven samples with BioPlex 2200 analysis error message (Serum Verification Bead – signal too low) which did not resolve upon repeat testing were excluded from the data analysis. Therefore, 293 samples were evaluated. Results of <1.0 AI are reported as negative and results of 1.0 AI or greater are reported as positive.

BioPlex Result	Positive # (%)	Negative # (%)
Anti-MPO	0/293 (0.0%)	293/293 (100.0%)
Anti-PR3	0/293 (0.0%)	293/293 (100.0%)
Anti-GBM	2/293 (0.7%)	291/293 (99.3%)

Additionally, values were determined in three hundred (300) serum samples from unselected patient samples previously tested with the assay. The results of this testing are as follows:

BioPlex Result	Positive		Negative	
	N	%	N	%
Anti-GBM	1	0.3 %	299	99.7 %
Anti-MPO	14	4.7 %	286	95.3 %
Anti-PR3	8	2.7 %	292	97.3 %

N. Instrument Name:
BioPlex 2200 System

O. System Descriptions:

The BioPlex 2200 System consists of the instrument, family of assays, and software. The instrument is an automated, floor-standing analyzer providing an integrated method for random access (or STAT) heterogeneous, multiplexed immunoassays on serum or plasma specimens. The instrument contains:

- sample handling, scheduling, and processing modules with associated reagent assay packs
- signal detector module
- automated cleaning and maintenance modules
- external computer with software to control instrument, reagent assays, error detection and handling, maintenance, and result analysis
- associated fluid storage, transfer, and removal
- connection interface with laboratory information systems

The detector module uses a proprietary flow cytometer-based multiplex technology to simultaneously assess multiple related analytes.

1. Modes of Operation:
   The instrument performs regular and STAT handling of assays and reagents.
2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ___X_____ or No _____
3. Specimen Identification:
   A barcode reader reads specimen identifying barcodes.
4. Specimen Sampling and Handling:
   A sample handling module performs specimen sampling and transfer of specimen and reagents for the designated assay. Assays are designated via a work request input from the user using a computer menu system.
5. Calibration:
   To adjust differing instrument conditions and reagent lots, assay calibration is performed at regular intervals. Calibration is performed for each new reagent lot, expiration of assay calibration curve (after 30 days), or after sponsor performed field service.
6. Quality Control:
   An Internal Standard bead functions as a correction of fluoresce intensity to detect and compensate for Detector Module fluctuations. Serum verification bead functions to detect the presence of serum in the specimen and the unexpected dilutions of specimens. A reagent blank bead functions as a non-coated bead to identify non-specific binding of materials. Non-specific binding possibly may give an unacceptably high signal. These three beads function as internal quality control measures.
   Positive and negative control materials are provided separately and are used in each assay run to monitor performance of the assay. Materials are handled as specimens in the assay. Standard laboratory quality control analysis (Levy-Jennings charts using Westgard rules), is available for periodic assessment. These controls function as external quality control measures.
   The recommended minimum frequency of quality control monitoring is once every 24-hour testing period. This interval may vary depending upon national, state, and local requirements and regulations or upon local laboratory policy.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The

"Performance Characteristics" Section above:

Calibrators and controls

Control Set

The BioPlex 2200 Vasculitis Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and BioPlex 2200 Vasculitis Reagent Pack in the clinical laboratory. The controls are provided in liquid form in a human serum matrix made from defibrinated plasma containing preservatives including ProClin® 300 (0.3%) and Sodium Azide (0.095%). The control set includes individual 1.5 mL vials of negative level and 1.5 mL vials of multi-analyte positive level, instructions for use, and assignment of values sheet.

The positive controls contain known analyte concentrations of Myeloperoxidase, Proteinase 3, and Glomerular Basement Membrane derived from human disease state plasma. The Negative Controls are prepared exclusively from the negative serum matrix. The Positive Controls are prepared by blending human disease state serum with the negative serum matrix. The recommended minimum frequency for performing quality control is once every 24-hour testing period and with each new lot of Reagent Pack. The vials can be directly loaded into the instrument sample racks and processed with the Reagent Pack in the same manner as patient samples. All the vials have a pre-affixed barcode, a unique lot number and a QC prefix identifying it as a quality control sample. Quality control data can be entered manually, based on the data contained in the Assignment of Value Sheet or via CD-ROM. The Data CD-ROM is available to load the necessary value assignment data into the instrument. The CD-ROM has an XML file, which contains assigned values for the controls, lot specific information such as expiration date, lot numbers and barcode ID.

Calibrator Set

The Calibrator Set is intended for the calibration of the Reagent Pack. The Reagent Pack is calibrated using a set of four (4) distinct serum base calibrators. The Calibrators are provided in a human serum matrix made from defibrinated plasma with added known analyte concentrations of Myeloperoxidase, Proteinase 3, and Glomerular Basement Membrane derived from human disease state plasma, and added preservatives including ProClin® 300 (0.3%) and Sodium Azide (0.095%). The Calibrators are manufactured independently from the controls. The calibrators are prepared from defibrinated human plasma where the total IgG and IgM are depleted. Calibrator assignment is established from replicate analyses using a master set of calibrators and a specific lot of Reagent Packs on multiple BioPlex 2200 instruments. There are no known certified reference materials available for any of the analytes in the Calibrator Set.

The calibrator set includes four distinct calibrator vials, instructions for use, and a lot specific assigned calibrator values sheet. A Calibrator Lot Data CD-ROM is required to load the necessary value assignment data into the instrument.

The calibrators are directly loaded into the BioPlex 2200 instrument sample racks and before control or patient samples are processed, a multi-level series of calibrators is processed, generating calibration curves for each analyte. Plotting Fluorescence Ratio (FR) against analyte concentration, calibration curves are used to quantify analyte concentrations of subsequent patient and control samples. The Calibrator Set should be loaded and assayed at minimum in duplicate every 30 days or with each new lot of Reagent Pack.

A study was conducted to assess the onboard calibration stability for the BioPlex 2200 Vasculitis kit. Calibration curve stability is the ability to quantify controls and samples over

a period of time without a significant shift in quantitation. At day 0 multiple reagent packs from a single lot were opened and placed on the analyzer. The reagent lot is calibrated and controls are run to qualify the calibration curve. Calibrators and controls are run as samples in duplicate every 7 days up to 35 days and quantified using the calibration curve from day 0. Controls are run in 10 replicates on day 0 to establish a control mean. When controls and calibrators are evaluated as samples, the results can be associated with a Serum Verification Bead (SVB) sample error. Samples with results associated with these errors were not repeated. However, no results are obtained for any sample that elicited sample dispense and reagent dispense errors. At each time point control values are measured against a range (established as two standard deviation from the mean control value from day 0 run). The calibration curve is no longer acceptable if control values fall outside this range in consecutive runs.
The data indicates that onboard calibration curves were stable for 35 days for all three assays, supporting a 30 day stability claim. Additional calibration curve stability studies will be conducted after 3, 6, 12, 18, 24, 30 and 36 months. The data appear adequate for the current calibration curve stability claim.

Carryover
The BioPlex 2200 has one sample dispense probe for dispensing all patient samples. The purpose of the sample probe carryover study was to quantify the amount of analyte that might carry over on the sample probe from a high concentration sample into a subsequent low concentration sample, causing a falsely elevated result.
The maximum allowable analyte carryover from the sample probe is 0.1 AI. Results are reported by the BioPlex 2200 instrument to one decimal place (0.1 AI), so carryover below this level is not measurable. Each of the three analytes was tested with a sample having a high concentration. The high sample concentrations are:

Vasculitis Analyte	Sample ID	Concentration (AI)
MPO	15625	30.0
PR3	15647	730.0
GBM	15627	110.0

For all the analytes tested, the low concentration sample was the Negative Control spiked with the Positive Control. Supplementation was done to slightly elevate the concentration of the low concentration sample and avoid quantitations <0.2 AI. The high concentration sample was followed by three (3) aliquots of the low concentration sample. Aliquot #1 was tested in a single replicate, aliquot #2 was tested in single replicate, and aliquot #3 was tested in triplicate, producing five (5) replicates of the low concentration sample. This sequence was repeated three more times, for a total of four repeating blocks. The sponsor notes that future assay kits on the BioPlex 2200 can have sample dispense volumes of 50 µL. The higher sample volume will increase the risk of sample probe carryover. The sponsor chose to test carryover in the Vasculitis Kit using a high concentration sample volume of 50 µL. The low concentration sample pool volume remained unchanged at 5 µL. The study was performed on three different instruments per analyte. The mean was calculated for the first

low sample replicates following after the high concentration sample. This was the "test" group and had the greatest risk of carryover. The mean was calculated of the low concentration replicates #3-5. This was the "control" group, where no carryover was expected.

Carryover was calculated as: Carryover (AI) = Mean AI_{Test} – Mean $AI_{Control}$

A t-test (2 tailed) was performed on the test group vs. the control group to see if it was statistically significant. Sample carryover met specifications for all the analytes (< 0.1 AI).

The sponsor additionally tested the reagent dispensing probe used to dispense all assay reagents. The purpose of the reagent carryover study was to quantify the effect of reagent that might carry over on the reagent probe from one reagent dispense into a subsequent reagent dispense, causing a falsely suppressed or elevated result. The sponsor believed that significant carryover might occur when the proposed assay is performed after the EBV IgM assay. The EBV IgM assay contains in its Sample Diluent Reagent goat-anti-human-IgG. Though the goat anti-human IgG antibody is necessary for the neutralization of human IgG in the IgM assay, there is a risk that the anti-human IgG antibody might carry over into other assays and cause interference. The proposed assay is a high-risk assay because it contains mouse-anti-human-IgG-PE conjugate. A single run on one instrument was sufficient to test all analytes in the proposed assay. The run was repeated on two additional instruments. Forty-five replicates of the proposed assay's Positive Control were tested in the run. The instrument dispensing software and hardware movement created 13-19 opportunities among the 45 replicates for the dispense of the EBV anti-human IgG reagent to be followed immediately by the dispense of the proposed assay reagent. The instrument log was obtained after the completion of the run, and was analyzed to classify the results into one of two groups:

- Potential carryover- replicates *with* opportunities for the high-risk carryover combination.
- Baseline - replicates *without* opportunities for the high-risk carryover combination.

In both the groups the mean, SD, and CV were calculated. The %Carryover Effect and effect ratio were calculated as follows:

$$\%CarryoverEffect = \frac{Mean_{Carryover} - Mean_{baseline}}{Mean_{baseline}} \times 100 \quad EffectRatio = \frac{\%CarryoverEffect}{\%CV_{baseline}}$$

The specification for this assessment was: Any quantitation bias induced by reagent probe carryover (the observed carryover effect) must be ≤ ±10%, or ≤2.0x CV (analogous to ≤2.0x SD), from the control mean, whichever is greater. In an attached report summary, the sponsor lists the following results:

Analyte	Instrument #	Baseline		Carryover		Carryover Effect (%)	Effect ratio
		Result (AI)	%CV	Result (AI)	%CV		
GBM	PU31	2.6934	3.4	2.7154	3.2	0.8%	0.2
	PU49	2.7621	4.3	2.7832	4.2	0.8%	0.2
	PU57	3.0694	3.4	3.0906	3.1	0.7%	0.2
MPO	PU31	2.5993	3.9	2.6110	3.6	0.4%	0.1
	PU49	2.6114	4.8	2.6234	5.3	0.5%	0.1
	PU57	2.9730	3.8	3.0045	3.9	1.1%	0.3
PR3	PU31	2.1762	3.7	2.1790	2.9	0.1%	0
	PU49	2.1459	4.3	2.1708	4.7	1.2%	0.3
	PU57	2.5582	3.7	2.5759	4.3	0.7%	0.2

The %carryover effect for all three analytes was approximately 1% or less. The results met the specification for this carryover.

Q. Proposed Labeling:

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

R. Conclusion:

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