510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k093954

B. Purpose for Submission:
New Device

C. Measurand:
Anti-cyclic citrullinated peptide (CCP) IgG autoantibodies

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

E. Applicant:
Bio-Rad Laboratories

F. Proprietary and Established Names:
BioPlex 2200 Anti-CCP Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5775, Rheumatoid Factor Immunological Test System
   21 CFR § 862.1150, Calibrator
   21 CFR § 866.1660, Quality control material (assayed and unassayed)
2. Classification:
   Class II (Device)
   Class II (Calibrator)
   Class I (Quality control)
3. Product codes:
   NHX, Antibodies, anti-cyclic citrullinated peptide (CCP)
   JIX, Calibrator, multi-analyte mixture
   JJY, Multi-analyte controls, all kinds (assayed)
4. Panel:
   Immunology (82)
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   The BioPlex 2200 Anti-CCP kit is a multiplex flow immunoassay intended for the semi-quantitative detection of IgG antibodies to Cyclic Citrullinated Peptide (CCP) in human serum or plasma (EDTA and sodium heparin). Detection of CCP antibodies is used as an aid in the diagnosis of rheumatoid arthritis and should be used in conjunction with other clinical findings and laboratory results.
   The BioPlex 2200 Anti-CCP kit is intended for use with the Bio-Rad BioPlex 2200 System.

   The BioPlex 2200 Anti-CCP Calibrator Set is intended for calibration of the BioPlex 2200 Anti-CCP Reagent Pack.

   The BioPlex 2200 Anti-CCP Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and

BioPlex Anti-CCP Reagent Pack in the clinical laboratory. The performance of the BioPlex 2200 Anti-CCP Control Set has not been established with any other immunoassays.

2. Indication(s) for use:
   Same as intended use
3. Special conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   BioPlex 2200 Multi-Analyte Detection System

I. Device Description:

The device components include the following: Bead Set containing dyed beads coated with Cyclic Citrullinated Peptide, Internal Standard, Serum Verification, and a Reagent Blank Bead, with glycerol and protein stabilizers; Conjugate containing phycoerythrin conjugated murine monoclonal anti-human IgG antibody and murine monoclonal anti-human FXIII antibody; Sample Diluent; Calibrator Set of 6 vials containing antibodies to Cyclic Citrullinated Peptide; Positive and Negative Controls; Sheath Fluid; Wash Solution; and the BioPlex 2200 Instrument and Software System (Instrument cleared in k041658).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Axis-Shield DIASTAT Anti-CCP
2. Predicate K number(s):
   k023285
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	BioPlex 2200 Anti-CCP Kit	**Axis-Shield DIASTAT Anti-CCP**
Intended Use	The BioPlex 2200 Anti-CCP kit is a multiplex flow immunoassay intended for the semi-quantitative detection of IgG antibodies to Cyclic Citrullinated Peptide (CCP) in human serum or plasma (EDTA and sodium heparin). Detection of CCP antibodies is used as an aid in the diagnosis of rheumatoid arthritis and should be used in conjunction with other clinical findings and laboratory results.	Same
Capture Antigen	Cyclic citrullinated peptide (CCP), second generation	Same
Assay Type	Semi-quantitative detection	Same
Analyte Detected	Human IgG antibodies to Cyclic Citrullinated Peptide	Same
Controls	Negative and positive controls	Same

Similarities		
Item	Device	Predicate
Quantitation	Results are determined from a standard calibration curve utilizing a four-parameter logistic (4-PL) curve fit algorithm	Same

Differences		
Item	Device	Predicate
	BioPlex 2200 Anti-CCP Kit	**Axis-Shield DIASTAT Anti-CCP**
Assay Technology	Automated Multiplex flow immunoassay	Manual, microtiter plate format, Enzyme-linked Immunosorbent assay (ELISA)
Conjugate Antibody	Phycoerythrin conjugated murine monoclonal anti-human IgG	Alkaline phosphatase labeled murine monoclonal antibody to human IgG
Substrate	None	Mg+2, phenolphthalein monophosphate (PMP)
Specimen Type	Serum and plasma (EDTA and heparin)	Serum and plasma (EDTA, lithium heparin, and sodium citrate)
Signal Detection	Fluorescence	Color, read at 550 nm
Solid Phase	Antigen-coated paramagnetic microbead reagent. Microbeads are infused with red and infrared fluorescent dyes for bead classification. Green fluorescence from the immunoassay label is used for analyte measurement.	Antigen-coated solid phase microtiter wells
Calibrator	6 levels	5 levels
Calibrator Range	0 – 300 U/mL	0 – 100 U/mL
Assay Type	Semi-Quantitative assay	Semi-quantitative and qualitative assay
Assay Range	0.5-300 U/mL	Up to 100 U/mL
Assay Cut-Off	3.0 U/mL	5 U/mL
Calibrators and Controls	Sold separately	Kit components

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

CLSI EP5-A, EP-7A, EP6-A, EP12-A2, EP14-A2, EP15-A2, and EP17-A

L. Test Principle:

The BioPlex 2200 Anti-CCP kit uses multiplex flow immunoassay, a methodology similar to traditional EIA; however, this method permits simultaneous detection and identification of many antibodies in a single tube. One population of fluorescent beads is coated with streptavidin-biotinylated CCP. Three 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 phycoerythrin conjugated murine monoclonal anti-human IgG 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 washed beads are re-suspended in wash buffer. The bead mixture then passes through the detector. The identity of the assay and control beads is determined by the fluorescence embedded in the surface of the bead and the amount of immobilized antibody is determined by the fluorescence of the anti-IgG reporter conjugate. The amount of immobilized analyte is determined by the median fluorescence intensity of the phycoerythrin reporter. Raw data are calculated in relative fluorescence intensity (RFI). The RFI is converted to U/mL using the calibration curve established by the 6 levels of BioPlex 2200 Anti-CCP Calibrators.

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

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:
   Separate CLSI EP5-A2 and EP15-A2 reproducibility studies were conducted to evaluate the performance of the BioPlex2200 Anti-CCP kit on the BioPlex2200 Instrument. Results from the CLSI EP5-A2 study are shown below. Multiple lots of the peptide antigen are tested to ensure consistency of the product. To immobilize CCP, streptavidin is covalently coupled to the bead surface using carbodiimide chemistry, and then biotinylated CCP is immobilized to the streptavidin surface. Evaluation of a well-characterized sample panel is employed as a functional test to verify the integrity of the CCP antigen and functional utility of the coupled bed. Several lots of biotinylated CCP were evaluated in order to ensure that the functionality and stability criteria in the development phase were met consistently. An internal reproducibility study was also performed using four development lots in order to evaluate within run, between run, and total variation of the assay. Patient samples were evaluated in 10 and 21 replicates, respectively, on three instruments twice per day. Results are within acceptance criteria.

   Per CLSI EP5-A2, three serum and plasma (EDTA and heparinized) panels composed of 10 samples each spanning the measuring range were assayed in replicate twice daily over 20 days (n=80). One positive and one negative control were included. The data were analyzed for within-run, between-run,

between-day, and total precision and the standard deviation and percent coefficient of variation are summarized below:

Anti-CCP Serum Panel			Within-Run		Between Run		Between Day		Total	
Sample	N	Mean (U/mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Negative 1	80	2.1	0.16	7.4	0.00	0.0	0.07	3.6	0.17	8.2
Negative 2	80	2.0	0.15	7.5	0.00	0.0	0.04	1.9	0.16	7.8
Near Cut-Off 1	80	2.8	0.14	5.2	0.07	2.5	0.13	4.7	0.21	7.5
Near Cut-Off 2	80	2.8	0.18	6.4	0.00	0.0	0.09	3.3	0.20	7.2
Low Positive 1	80	3.0	0.22	7.2	0.00	0.0	0.09	3.1	0.23	7.8
Low Positive 2	80	3.3	0.17	5.2	0.11	3.2	0.10	3.1	0.23	6.9
Positive 1	80	26.1	1.41	5.4	0.00	0.0	1.17	4.5	1.83	7.0
Positive 2	80	21.0	1.03	4.9	0.86	4.1	0.75	3.6	1.54	7.3
High Positive 1	80	128.5	8.08	6.3	0.00	0.0	2.12	1.6	8.35	6.5
High Positive 2	80	140.7	8.44	6.0	4.45	3.2	0.00	0.0	9.54	6.8
Pos. Control	80	15.0	1.04	7.0	0.00	0.0	0.51	3.4	1.16	7.8
Neg. Control	80	0.1	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
Anti-CCP EDTA Panel			**Within Run**		**Between Run**		**Between Day**		**Total**	
Sample	N	Mean (U/mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Negative 1	80	2.1	0.14	6.9	0.07	3.4	0.11	5.1	0.19	9.2
Negative 2	80	2.2	0.16	7.3	0.06	2.8	0.00	0.0	0.17	7.8
Near Cut-Off 1	80	3.0	0.21	7.1	0.08	2.7	0.07	2.2	0.24	7.9
Near Cut-Off 2	80	2.7	0.15	5.4	0.08	3.1	0.10	3.8	0.20	7.3
Low Positive 1	80	3.1	0.20	6.3	0.00	0.0	0.11	3.6	0.22	7.2
Low Positive 2	80	3.0	0.20	6.5	0.09	3.1	0.03	1.0	0.22	7.3
Positive 1	80	22.1	1.02	4.6	0.60	2.7	0.30	1.4	1.22	5.5
Positive 2	80	32.0	1.56	4.9	0.96	3.0	0.41	1.3	1.87	5.9
High Positive 1	80	133.5	10.35	7.8	0.00	0.0	3.30	2.5	10.86	8.1
High Positive 2	80	147.6	7.75	5.2	2.89	2.0	5.00	3.4	9.67	6.5
Anti-CCP Heparin Panel			**Within Run**		**Between Run**		**Between Day**		**Total**	
Sample	N	Mean (U/mL)	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Negative 1	80	2.2	0.14	6.5	0.06	2.6	0.07	3.3	0.17	7.7
Negative 2	80	2.3	0.15	6.6	0.07	3.0	0.04	1.5	0.17	7.4
Near Cut-Off 1	80	2.7	0.16	5.9	0.05	1.8	0.05	1.7	0.17	6.4
Near Cut-Off 2	80	2.9	0.19	6.7	0.10	3.4	0.10	3.4	0.24	8.2
Low Positive 1	80	3.5	0.21	6.0	0.04	1.0	0.12	3.3	0.24	6.9
Low Positive 2	80	3.7	0.24	6.5	0.08	2.1	0.00	0.0	0.25	6.8
Positive 1	80	21.0	1.18	5.6	0.37	1.8	0.38	1.8	1.29	6.2
Positive 2	80	23.7	1.55	6.5	0.41	1.7	0.37	1.6	1.65	6.9
High Positive 1	80	126.2	6.28	5.0	0.00	0.0	3.26	2.6	7.08	5.6
High Positive 2	80	132.5	6.57	5.0	4.27	3.2	0.00	0.0	7.84	5.9

b. Linearity/assay reportable range:
Five high anti-CCP IgG positive patient samples ranging from 240 to 314

U/mL were tested to evaluate linearity. These samples were diluted six times with immunodepleted serum per CLSI EP06-A. Each sample and dilution was evaluated in quadruplicate using one anti-CCP IgG lot on one instrument. Linear and polynomial regression analyses of anti-CCP IgG recovery U/mL *vs.* sample dilution were performed to determine if the dilution curves exhibit statistically significant non-linearity. The regression parameters of the observed values vs. predicted values are shown below for three of the samples within the reportable range. The BioPlex 2200 Anti-CCP IgG assay demonstrated linearity from 0 to 300 U/mL.

Sample (U/mL)	**Slope**	**Intercept**	**r^2**
Sample A (270.2)	1.0000	-0.0011	0.9985
Sample B (263.1)	0.9999	0.0106	0.9990
Sample C (240.9)	1.0002	-0.0166	0.9978

The percent difference between the observed and the predicated values from regression for each sample dilution is shown below.

Dilution ID	**Percent Sample**	**Percent Difference of Predicted**				
		Sample 1 (270.2 U/mL)	Sample 2 (314.5 U/mL)	Sample 3 (263.1 U/mL)	Sample 4 (240.9 U/mL)	Sample 5 (314.9 U/mL)
1	10%	1.9	-11.5	9.4	-4.1	-10.2
2	20%	2.7	-3.3	0.2	2.6	-2.1
4	40%	2.7	5.9	-2.7	3.8	5.9
6	60%	2.3	5.5	-3.4	2.4	5.0
8	80%	-3.1	-3.8	1.9	0.4	-3.6
10	100%	0.6	N/A	0.3	-1.8	N/A

The BioPlex 2200 system also offers an on-board dilution feature for testing over-range samples. The dilution prior to analysis was evaluated for 1:4, 1:10 and 1:100. Three different high positive anti-CCP samples for each dilution feature were also diluted manually to compare to onboard dilution by the BioPlex 2200. All samples were assayed in replicates of ten. Results for samples diluted onboard the BioPlex 2200 were displayed as the sample result multiplied by the dilution factor. Recovery of samples diluted onboard must be within ±20% of that of the same sample diluted manually and precision (U/mL CV) must be ≤ 10%.

The results below indicated that the onboard sample dilution feature of the BioPlex 2200 system can be used to dilute over-range samples 1:4, 1:10 or 1:100 for the anti-CCP assay. Onboard values shown are the reported values

divided by the dilution factor. The percent recovery is the percent ratio of the adjusted onboard dilution U/mL value to the manual dilution U/mL value.

Dilution	Sample	Manual U/mL	Onboard U/mL	Recovery	Manual CV	Onboard CV
1:100	1	16	17	104%	3.7%	4.3%
	2	15	15	103%	2.9%	3.6%
	3	11	11	100%	3.4%	3.7%
1:10	4	32	28	88%	4.0%	3.7%
	5	27	24	90%	2.5%	2.9%
	6	24	22	91%	1.6%	3.1%
1:4	7	98	92	93%	3.8%	4.7%
	8	84	73	87%	2.7%	3.2%
	9	73	69	94%	7.6%	2.8%

c. *Traceability, Stability, Expected values (controls, calibrators, or methods)*:
No international or certified reference material for anti-CCP IgG is available. The BioPlex 2200 Anti-CCP Calibrators are assigned relative units from predicate Axis-Shield DIASTAT Anti-CCP Calibrators. BioPlex 2200 Anti-CCP Calibrators are prepared by blending defibrinated and delipidated human plasma units with known anti-CCP IgG activity in a processed human serum matrix made from immunodepleted, defibrinated plasma. These calibrators are used to assay characterize patient samples with the BioPlex 2200 Anti-CCP IgG assay. Calibrator assignment is established from replicate analyses on multiple BioPlex 2200 instruments using a master set of calibrators and a specific lot of BioPlex 2200 Anti-CCP IgG Reagent Pack.

Both Negative and Positive Controls were made in a human serum matrix from defibrinated plasma. All antibodies are derived from human disease state plasma. 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 sponsor's acceptable range of ±15% is based on the inter-assay precision specification of 15%.

Stability
Stability studies have been performed to determine the open vial and shelf life stability for the Control and Calibrator Sets. The claims are as follows:
Open vial stability, 60 days from first opening
Shelf life stability, 24 months
Real-time stability, 9 months (current data support 9 months but testing will

continue for at least 30 months)
Accelerated stability, 2.5 years (current data support 2.5 years but testing will continue until all calibrator and control levels have been monitored for 30 months)
Real time and on-board kit stability of the BioPlex Anti-CCP Kit were also conducted. The minimum real-time stability specification for the kit is one year from the date of the manufacture, and the minimum on-board stability is 30 days.

d. Detection limit:
The Limit of Detection (LoD) of BioPlex 2200 Anti-CCP was determined by assaying low positive, high negative, and blank samples in replicates of 50. The LoD was calculated according to CLSI EP17-A. The samples were prepared from an anti-CCP low positive control which was then diluted in 25% increments in negative serum (IgG depleted) to a concentration level from 4.02 to 0.04 U/mL. Each dilution was assayed daily in replicates of ten for a period of five days. The calculated LoD for the anti-CCP IgG assay is 0.2 U/mL by using the equation LoD = LoB + cBSDs. The LoB (0.0000) was calculated at the 95th percentile of 50 negative samples. cB 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.

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 Anti-CCP assay according to CLSI EP7-A2. Samples were prepared by blending a pool of negative human serum with samples positive for CCP IgG to achieve low positive values of 5.0 to 15.0 U/mL. Test and control samples were evaluated in alternating order in replicates of five each. This sequence was repeated twice for a total of ten replicates per interferent. 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

Substance	Concentration
Triglycerides	≤ 3300 mg/dL
Protein (total)	≤ 12 g/dL
Rheumatoid Factor	200 IU/mL
Ascorbic Acid	≤ 3 mg/dL
Lithium Heparin	≤ 8000 units/dL
Sodium Heparin	≤ 8000 units/dL
EDTA	≤ 800 mg/dL

Cross-Reactivity:
A cross-reactivity study was performed to determine if samples from various disease states and other potentially interfering factors interfere with test results from the BioPlex 2200 Anti-CCP kit. Samples known to be positive for one of the potential cross reactants listed in the table below were evaluated with the BioPlex 2200 Anti-CCP assay.

A total of 163 ANA samples and 72 other samples containing potential cross reactants were evaluated by the BioPlex Anti-CCP. The following table summarizes the potential percent cross reactivity rate of the BioPlex 2200 Anti-CCP kit. Of the 163 ANA samples, some samples contained more than one analyte such as dsDNA, Chromatin, Scl-70, Sm, Centromere B, SmRNP, RiboP, SS-B, SS-A, RNP, and Jo-1.

Possible cross reactivity at 11% was observed with ANA samples, inclusive of all analytes. More specifically, Centromere B (23%) and SS-A (12%) appear to have the highest potential cross reactivity rates. Samples containing Myeloma IgG may also cross react with the BioPlex 2200 Anti-CCP kit (30%).

Potential Cross Reactant	N	% Cross Reactivity
ANA	163	18/163 (11%)
dsDNA		2/27 (7%)
Chromatin		5/45 (11%)
Scl-70		2/24 (8%)
Sm		2/19 (11%)
Centromere B*		7/23 (23%)
SmRNP		4/37 (11%)
Ribo P		0/9 (0%)
SS-B		0/18 (0%)
SS-A*		8/66 (12%)
RNP		3/34 (9%)
Jo-1		0/8 (0%)
TPO IgG	13	0/13 (0%)
VCA IgG	17	0/17 (0%)
T. gondii IgG	10	1/10 (10%)

Potential Cross Reactant	N	% Cross Reactivity
CMV IgG	12	0/12 (0%)
Myeloma IgG	10	3/10 (30%)
Lyme IgG	10	0/10 (0%)

* Anti-CCP antibodies have been documented in patients with primary Sjögren's Syndrome (See references below).

Reference:

1. Atzeni, F., *et al*., Anti-cyclic citrullinated peptide antibodies in primary Sjögren's Syndrome may be associated with non-erosive synovitis. Arthritis Research & Therapy 2008. 10(3):R51
2. Zendman, A.J.W., *et al*., Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. Rheumatology 2006. 45:20-25. (review article)
3. Nakamura H., *et al*., Anti-centromere antibody-seropositive Sjögren's Syndrome differs from conventional subgroup in clinical and pathological study. BMC Musculoskeletal Disorders, 2010. 11:140.

Hook effect:
Not applicable

f. Assay cut-off:
The cutoff value and assignment of the calibrators are determined by performing concordance testing and Receiver Operator Characteristic (ROC) analysis, using predicate results as the standard.
A total of 1394 patient samples including 177 normal patients, 504 patients with either Rheumatoid Factor (RF) tested or positive, 82 older patients (age >70), 287 Rheumatoid Arthritis (RA) diagnosed patients and 344 non-RA patients were evaluated to determine the anti-CCP IgG assay cutoff. All samples were confirmed positive or negative by the Axis-Shield DIASTAT anti-CCP predicate assay. A cutoff of 3.0 U/mL was obtained to achieve the percent positive and negative agreement at 92.9% and 98.2%, respectively. These patient samples were not reused for any other study.

2. Comparison studies:

a. Method comparison with predicate device:
Performance of the BioPlex 2200 Anti-CCP kit was evaluated against predicate device, Axis-Shield DIASTAT Anti-CCP immunoassay. A total of 997 speciments: 300 apparently healthy blood donors, 496 patients previously diagnosed with Rheumatoid Arthritis (RA), and 201 patients with other rheumatic disease were tested at one clinical site.
Patients diagnosed with other rheumatic or inflammatory diseases include 3 Atherosclerotic disease, 2 CREST Syndrome, 18 Crohn's disease, 15 Fibromyalgia, 19 Gout, 16 Inflammatory Arthritis, 8 Osteoarthritis, 18

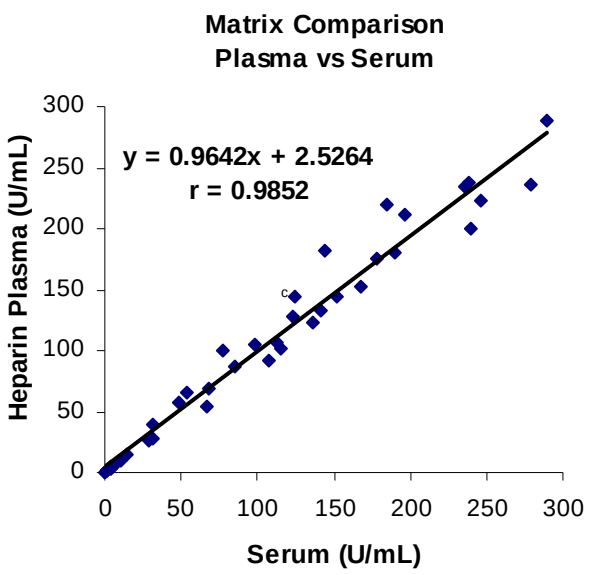
Scleroderma, 21 Sjogrens Syndrome, 31 Systemic Lupus Erythematosus, 17 Ulcerative Colitis and 2 Wegener's Granulomatosis.
A total of 822 samples within the detection range were evaluated and results are presented in the table below:

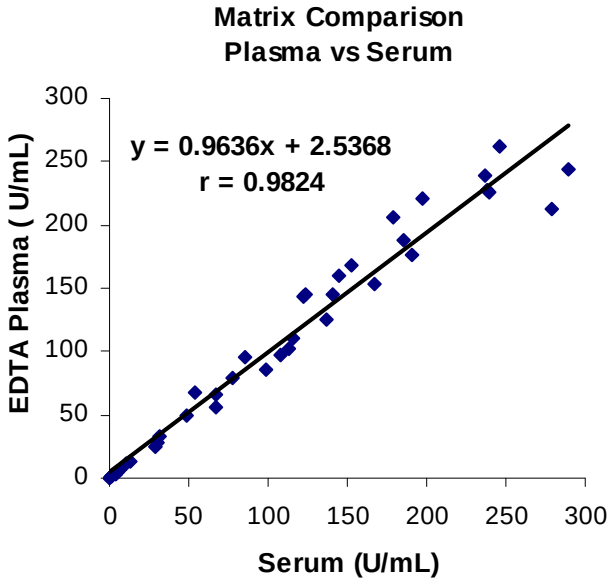
Healthy, Diagnosed RA and Non-RA Patients		BioPlex 2200 Anti-CCP				
		Positive	Negative	Total	% Positive Agreement (95% CI)	% Negative Agreement (95% CI)
Predicate Immunoassay	Positive	358	9	367	97.5%(358/367) 95% CI 95.4 - 98.7%	91.4% (416/455) 95% CI 88.5 – 93.7%
	Negative	39	416	455		
	Total	397	425	822		

Positive Agreement (95% CI) = 97.5% (358/367) (95.7 – 98.7%)
Negative Agreement (95% CI) = 91.4% (416/455) (88.5 – 93.7%)

b. Matrix comparison:
Testing for matrix effects was conducted in accordance with CLSI EP9-A2. Forty-one matched sets of serum and plasma (EDTA and heparin) samples drawn from the same donor were acquired from commercial sources. The matched sets were spiked with high positive anti-CCP IgG serum in order to cover the measuring range of the assay from 0.5 to 300 U/mL. All samples were evaluated in replicates of two. Plasma U/mL values were compared to matched serum U/mL values. Scatter plots comparing the performance of EDTA and heparin plasma samples against serum samples along with the corresponding slopes of regression and coefficient of determination (r^2) are shown below comparing the first replicate values only.

Matrix Comparison	N	Slope (95% CI)	Intercept (95% CI)	Correlation (r) (95% CI)
EDTA vs. Serum	41	0.9636 (0.8753, 1.0519)	2.5368 (-2.5799, 7.6536)	0.9824 (0.9670, 0.9906)
Heparin vs. Serum	41	0.9642 (0.8995, 1.0289)	2.5264 (-1.4891, 6.5419)	09852 (0.9723, 0.9921)





3. Clinical studies:
   a. *Clinical sensitivity and specificity*:
   The clinical studies involved testing 997 specimens including 300 apparently healthy blood donors, 496 diagnosed RA patients, and 201 other rheumatic disease patients. The BioPlex 2200 Anti-CCP Sensitivity and Specificity are shown below:

Anti-CCP Clinical Sensitivity and Specificity	**BioPlex 2200 Anti-CCP**				
	Positive	Negative	Total	**% Sensitivity (95% CI)**	**% Specificity (95% CI)**
Previously Diagnosed Rheumatoid	412	84	496	83.1%(412/496) 95% CI 79.5 – 86.1%	97.8% (490/501) 95% CI 96.1 – 98.8%
Healthy Blood Donors and Patients with Other Rheumatic Diseases	11	490	501		
Total	423	574	997		

   b. *Other clinical supportive data (when a. is not applicable):*
   Not applicable
4. Clinical cut-off:
   Not applicable

5. Expected values/Reference range:
   Three hundred samples from apparently healthy donors including 114 males ranging in age from 4 to 84y and 186 females ranging in age from 4 to 88y were tested with BioPlex 2200 Anti-CCP assay. The Anti-CCP results range from <0.5 to 1.5 U/mL as shown below. Results of <3.0 U/mL are reported as negative and results ≥ 3.0 U/mL are reported as positive.

BioPlex 2200 Anti-CCP (U/mL)	Gender	Minimum	Maximum	N
Apparently healthy donors	Male	<0.5	1.5	114
	Female	<0.5	1.2	186

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.