510(k) Summary Report
BioPlex™ 2200 Anti-CCP
Bio-Rad Laboratories, Inc.

K093954

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1. Applicant/Sponsor

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2. Device Name

Proprietary Name: BioPlex™ 2200 Anti-CCP Kit
BioPlex™ 2200 Anti-CCP Calibrator Set
BioPlex™ 2200 Anti-CCP Control Set

Common/Usual Name: Multi-Analyte Detection System: Anti-CCP IgG

Classification Name: Antibodies, Anti-Cyclic Citrullinated Peptide (CCP)
Calibrator, multi-analyte mixture
Single-analyte controls, all kinds (assayed and unassayed)

3. Regulatory Information

Product Code	Classification	Regulation Section	Panel
Antibodies, Anti-Cyclic Citrullinated Peptide (CCP) (NHX)	Class II	21 CFR § 866.5775, Rheumatoid factor immunological test system	Immunology
Calibrator, multi-analyte mixture (JIX)	Class II	21 CFR § 862.1150 - Calibrator	Clinical Chemistry
single (specified) analyte controls (assayed and unassayed) (JJX)	Class I	21 CFR § 862.1660 – Quality control Material (Assayed and Unassayed)	Clinical Chemistry

4. Predicate Devices

DIASTAT™ Anti-CCP K023285

5. Device Description

The BioPlex™ 2200 Anti-CCP IgG kit uses multiplex flow immunoassay, a methodology that greatly resembles traditional EIA, but permits simultaneous detection and identification of many antibodies in a single tube. "CCP IgG" is an acronym for the detection of IgG antibodies to Cyclic Citrullinated Peptide.

One (1) population of fluorescent beads is coated with antigens associated with cyclic citrullinated peptide (CCP). Three populations of fluorescent beads function as assay controls (see below). The BioPlex™ 2200 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, anti-human IgG conjugated to phycoerythrin 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. Raw data are calculated in relative fluorescence intensity (RFI).

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. Refer to the BioPlex™ 2200 System Operation Manual for more information.

The instrument is calibrated using a set of six distinct calibrator vials, supplied separately by Bio-Rad Laboratories. The six vials representing six different analyte concentrations are used for calibration. The cut-off value and assignment of the calibrators are determined by performing concordance and Receiver Operator Characteristic (ROC) analysis using Axis-Shield DIAStat Anti-CCP predicate results as the reference. The result for anti-CCP is expressed as arbitrary units (U/mL). Results of <3 U/mL are negative and results of ≥ 3 U/mL as positive.

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 2200 Anti-CCP Reagent Pack in the clinical laboratory. The Control Set includes a negative and a positive control. The BioPlex Anti-CCP Positive Control is manufactured to give positive results, with values above the cut-off for the analyte. The BioPlex Anti-CCP Negative Control is manufactured to give negative results, with values below the cut-off for the analyte. The recommended frequency for performing quality control is once every 24-hour testing period. Performing quality control is also necessary after each new assay calibration and certain service procedures.

6. BioPlex™ 2200 Anti-CCP Kit Components

The BioPlex 2200 Anti-CCP kit (665-3250) contains supplies sufficient for 100 tests.

Vial	Description
Bead Set	One (1) 10 mL vial, containing dyed beads coated with Cyclic Citrullinated Peptide, plus Internal Standard beads (ISB), Serum Verification beads (SVB), and Reagent Blank beads (RBB) in buffer with glycerol and protein stabilizers (bovine). ProClin 300 (0.3%), sodium benzoate (0.1%) and sodium azide (<0.1%) as preservatives.
Conjugate	One (1) 5 mL vial, containing phycoerythrin conjugated murine monoclonal anti-human IgG antibody and phycoerythrin conjugated murine monoclonal anti-human FXIII antibody, in buffer with protein stabilizers (bovine). ProClin 300 (0.3%), sodium benzoate (0.1%) and sodium azide (< 0.1%) as preservatives
Sample Diluent	One (1) 10 mL vial, containing buffer with protein stabilizers (bovine and murine). ProClin 300 (0.3%), sodium benzoate (0.1%) and sodium azide (< 0.1%) as preservatives.

Additional Required Items, Available from Bio-Rad:

Catalog #	Description
663-3200	BioPlex 2200 Anti-CCP Calibrator Set: Six 0.5 mL vials, each containing human antibodies to Cyclic Citrullinated Peptide, in a human serum matrix made from defibrinated plasma, with protein stabilizers (bovine). All antibodies are derived from human disease state plasma. All calibrators contain ProClin 300 (≤ 0.3%), sodium benzoate (0.1%) and sodium azide (< 0.1%) as preservatives

Catalog #	Description
663-3230	BioPlex 2200 Anti-CCP Control Set: Two 1.5 mL Positive Control serum vials containing human antibodies to Cyclic Citrullinated Peptide, in a human serum matrix made from defibrinated plasma. Two 1.5 mL Negative Control serum vials, in a human serum matrix made from defibrinated plasma. All antibodies are derived from human disease state 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%) 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%) as preservatives.
660-0000	BioPlex 2200 Instrument and Software System.

7. Intended Use

BioPlex™ 2200 Anti-CCP Kit

1. 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 or 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.

BioPlex™ 2200 Anti-CCP Calibrator Set

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

BioPlex™ 2200 Anti-CCP Control Set

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.

8. Technological Characteristics and Substantial Equivalence

The following tables summarize the similarities and differences between the BioPlex 2200 Anti-CCP kit and the predicate devices used in comparative studies with the BioPlex 2200 Anti-CCP kit.

BioPlex™ 2200 Anti-CCP Kit vs. Predicate Devices - Similarities

Similarities between Components / Materials	BioPlex™ 2200 Anti-CCP Kit	Axis-Shield DIASTAT™ Anti-CCP (K023285)
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 and EDTA or heparinized plasma. Detection of CCP antibodies may be used as an aid in the diagnosis of rheumatoid arthritis and should be used in conjunction with other clinical information. The BioPlex 2200 Anti-CCP kit is intended for use with the Bio-Rad BioPlex 2200 System.	The DIASTAT™ Anti-CCP test is a semi-quantitative/qualitative enzyme-linked immunosorbent assay (ELISA) for the detection of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum or plasma (EDTA, Lithium heparin or sodium citrate). It is intended to aid in the diagnosis of Rheumatoid Arthritis (RA) and is not definitive in isolation. Autoantibody levels represent one parameter in a multicriterion diagnostic process, encompassing both clinical and laboratory-based assessments.
Capture Antigen	Cyclic citrullinated peptide (CCP), second generation	Same
Assay Type	Semi-Quantitative detection	Same

Similarities between Components / Materials	BioPlex™ 2200 Anti-CCP Kit	Axis-Shield DIASTAT™ Anti-CCP (K023285)
Analyte Detected	Human IgG antibodies to Cyclic Citrullinated Peptide	Same
Specimen Type	Serum and plasma	Same
Controls	Negative and Positive Controls	Same
Calibrator(s)	Multiple Calibrators	Same
Quantitation	Results are determined from a standard calibration curve utilizing a four-parameter logistic (4-PL) curve fit algorithm.	Same

BioPlex 2200 Anti-CCP Kit vs. Predicate Device - Differences

Differences between Components / Materials	BioPlex™ 2200 Anti-CCP Kit	Axis-Shield DIASTAT™ Anti-CCP (K023285)
Assay Technology	Automated Multiplex flow immunoassay	Manual, microtitre 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 550nm

Differences between Components / Materials	BioPlex™ 2200 Anti-CCP Kit	Axis-Shield DIASTAT™ Anti-CCP (K023285)
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 microtitre wells
Calibrator(s)	6 levels of Calibrator	5 levels of Calibrator
Calibrator Range	0 – 300 U/mL	0 – 100 U/mL
Assay Type	Semi-Quantitative assay	Semi-quantitative/qualitative assay
Calibrators and Controls	Sold separately	Kit components

9. Performance Characteristics

A series of studies was conducted to evaluate the performance of the BioPlex™ 2200 Anti-CCP kit. The studies included reproducibility, linearity, limits of detection, interfering substances, cross-reactivity, assay cut-off, expected values and method comparison. The results of all studies demonstrated that the BioPlex 2200 Anti-CCP kit performed according to its specifications.

A. Analytical Performance

i. Precision/Reproducibility

Separate CLSI EP5-A2 and EP15-A2 reproducibility studies were conducted to evaluate the reproducibility of the BioPlex 2200 Anti-CCP kit on the BioPlex 2200 instrument. Reproducibility studies were based on the principles described in Clinical and Laboratory Standards Institute (CLSI) EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods* and EP15-A2, *User Verification of Performance for Precision and Trueness.*

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

CLSI EP5-A2 Reproducibility Study

Three (3) panels made from serum and plasma (EDTA and heparinized). Each panel composed of 10 samples covering the measuring range of the device was assayed two times in two separate daily runs over 20 days (n=80) plus one negative and one positive control according to CLSI EP5-A2 guideline.

The data were analyzed for within-run, between-run, between-day, and total precision and the standard deviation (SD) and percent coefficient of variation (% CV) were calculated. Precision results from EP5-A2 reproducibility study are summarized below for all samples covering the measuring range.

CLSI EP15-A2 Reproducibility Study

The study was performed at one clinical trial site. One lot each of BioPlex 200 Anti-CCP reagent pack, Calibrator Set and Control Set was evaluated. Three panels were made from serum and plasma (EDTA and heparinized) plus two controls (one negative and one positive). Each panel of 10 samples covering the assay measuring range was tested in quadruplicate over five days (4 replicates x 1 run x 5 days x 1 testing site = 20 replicates per panel member) according to CLSI EP15-A2 guideline.

The data were analyzed for within-run, between-day, and total precision and the standard deviation (SD) and percent coefficient of variation (% CV) were calculated.

The within-run precision (%CV) for positive samples near the cut-off (3 U/mL) in all sample matrices ranged from 3.5% to 5.3%. The total precision (%CV) for positive samples near the cut-off (3 U/mL) ranged from 4.0% to 5.3%. Precision results from EP15-A2 Reproducibility study are listed below.

Anti-CCP Serum Panel			Within-Run		Between-Day		Total	
Sample	N	Mean (U/mL)	SD	%CV	SD	%CV	SD	%CV
Negative 1	20	1.7	0.09	4.9	0.07	3.9	0.11	6.2
Negative 2	20	1.7	0.09	5.5	0.04	2.3	0.10	5.9
Near Cutoff 1	20	2.7	0.12	4.3	0.00	0.0	0.12	4.3
Near Cutoff 2	20	3.1	0.12	3.8	0.11	3.4	0.16	5.1
Low positive 1	20	23.2	1.16	5.0	1.22	5.3	1.68	7.2
Low positive 2	20	19.3	1.13	5.8	0.69	3.6	1.32	6.8
High Positive 1	20	113.4	6.85	6.0	0.45	0.4	6.87	6.1
High Positive 2	20	128.3	5.97	4.7	1.86	1.4	6.25	4.9
Very High Positive 1	20	251.0	9.55	3.8	8.19	3.3	12.58	5.0
Very High Positive 2	20	238.4	16.23	6.8	0.00	0.0	16.23	6.8
Control-1	20	0.02	0.04	244.2	0.00	0.0	0.04	244.2
Control -2	20	13.4	0.67	5.0	0.31	2.3	0.74	5.5
Anti-CCP EDTA Panel			**Within-Run**		**Between-Day**		**Total**	
Sample	N	Mean (U/mL)	SD	%CV	SD	%CV	SD	%CV
Negative 1	20	2.1	0.13	6.1	0.06	2.6	0.14	6.6
Negative 2	20	1.8	0.08	4.6	0.08	4.5	0.12	6.4
Near Cutoff 1	20	3.0	0.14	4.6	0.06	2.1	0.15	5.1
Near Cutoff 2	20	3.1	0.13	4.2	0.00	0.0	0.13	4.2
Low positive 1	20	20.2	0.68	3.4	0.42	2.1	0.80	4.0
Low positive 2	20	29.3	1.05	3.6	0.86	2.9	1.36	4.6
High Positive 1	20	124.6	4.68	3.8	0.00	0.0	4.68	3.8
High Positive 2	20	136.3	6.46	4.7	0.00	0.0	6.46	4.7
Very High Positive 1	20	224.2	8.40	3.7	9.61	4.3	12.76	5.7
Very High Positive 2	20	236.6	17.25	7.3	0.00	0.0	17.25	7.3

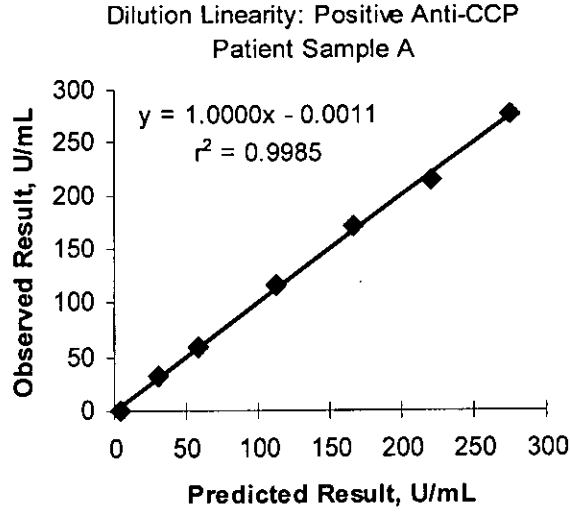
Anti-CCP Heparin Panel			Within-Run		Between-Day		Total	
Sample	N	Mean (U/mL)	SD	%CV	SD	%CV	SD	%CV
Negative 1	20	1.7	0.08	4.7	0.00	0.0	0.08	4.7
Negative 2	20	1.8	0.12	6.5	0.00	0.0	0.12	6.5
Near Cutoff 1	20	2.9	0.10	3.5	0.06	1.9	0.12	4.0
Near Cutoff 2	20	3.0	0.16	5.3	0.00	0.0	0.16	5.3
Low positive 1	20	18.7	0.64	3.4	0.36	1.9	0.73	3.9
Low positive 2	20	20.8	1.46	7.0	1.24	6.0	1.92	9.2
High Positive 1	20	113.3	4.45	3.9	3.69	3.3	5.78	5.1
High Positive 2	20	119.5	4.16	3.5	1.42	1.2	4.40	3.7
Very High Positive 1	20	223.3	13.12	5.9	3.39	1.5	13.55	6.1
Very High Positive 2	20	227.7	11.38	5.0	5.38	2.4	12.59	5.5

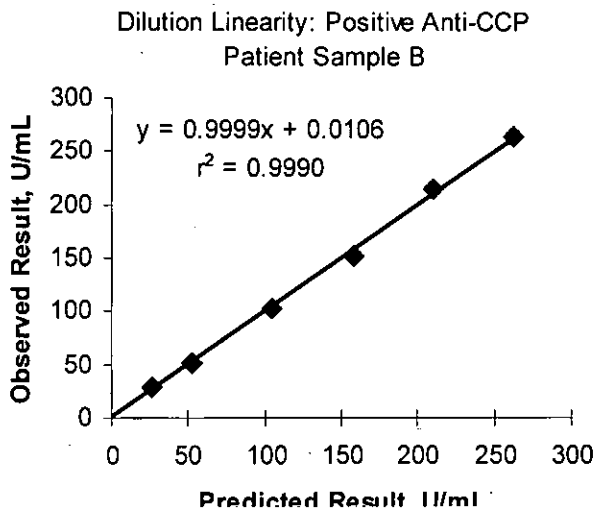
ii. Linearity/Assay Reportable Ranges

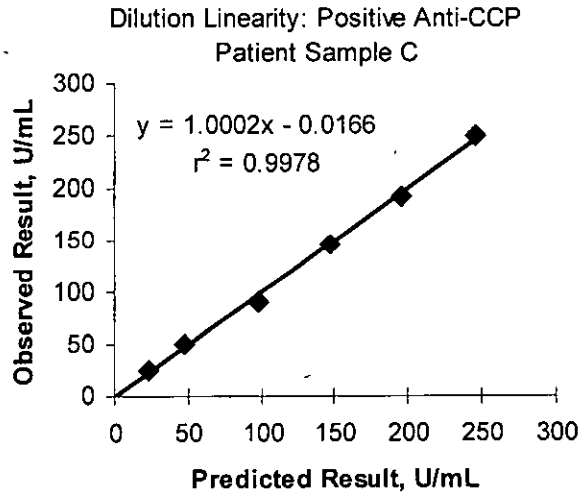
Three (3) high anti-CCP IgG positive patient samples ranging from 241 to 270 U/mL were tested to demonstrate linearity. These samples were diluted with immunodepleted serum according to CLSI EP06-A, *Evaluation of the Linearity of Quantitative Measruement Procedures: A Statistical Approach.*

Each sample and dilution was evaluated in replicates of four using one anti-CCP IgG lot on one instrument. Linear and polynomial regression analysis of anti-CCP IgG recovery U/mL *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. The dilution linearity graphs are also presented below. 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 BioPlex 2200 system also offers an on-board dilution features 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%

iii. Traceability of Calibrators/Controls

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 characterized patient samples with the BioPlex 2200 Anti-CCP IgG assay.

Both Negative and Positive Controls were made in a human serum matrix from defibrinated plasma. All antibodies are derived from human disease state plasma.

iv. Limit of Detection

The Limit of Detection (LoD) of BioPlex 2200 Anti-CCP was determined by assaying low positive, high negative and blank samples in replicates of fifty (50). The LoD was calculated according to CLSI EP17-A, *Protocols for Determination of Limits of Detection and Limits of Quantitation.* The calculated LoD for the anti-CCP IgG assay is 0.2 U/mL.

v. 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. The study was conducted based on the principles described in Clinical and Laboratory Standards Institute (CLSI) EP7-A2, *Interference Testing in Clinical Chemistry*. No interference was observed with any of the substances tested. The substances and the maximum levels tested are shown in the table below.

Interfering Substances

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
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%).

Cross-Reactivity

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%)
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.

vi. 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.

B. Comparative Performance

i. Comparative Testing

Method Comparison

Performance of the BioPlex 2200 Anti-CCP kit was evaluated against predicate device, Axis-Shield DIASTAT Anti-CCP immunoassay.

The performance of the BioPlex 2200 Anti-CCP was evaluated using a total of 997 specimens: 300 apparently healthy blood donors, 496 patients previously diagnosed with Rheumatoid Arthritis (RA), and 201 patients with other rheumatic diseases were tested at one clinical site. Patient specimens were purchased from commercial suppliers and were frozen serum. Each sample was unique and was unlinked to patient identity and not individual identifiable. Eight hundred twenty-two (822) samples within the detection range were evaluated.

Patients previously diagnosed with RA were selected by either physicians' diagnosis or an ICD-9 code 714.0.

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 Sjögren's Syndrome, 31 Systemic Lupus Erythematosus, 17 Ulcerative Colitis and 2 Wegener's Granulomatosis.

The results are presented in the table below.

	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		

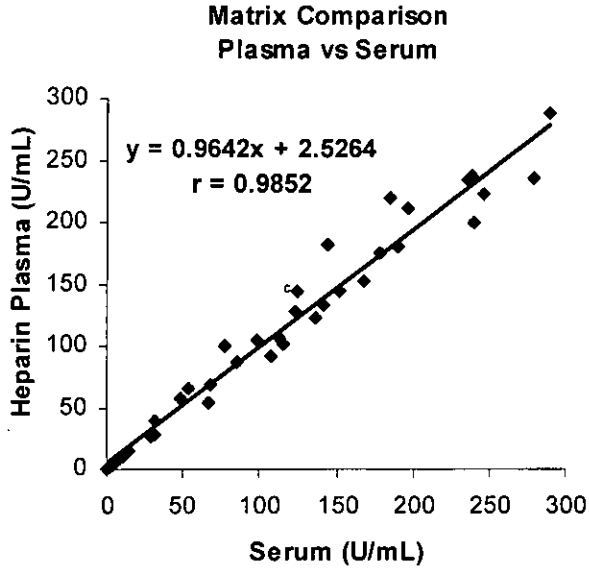
Positive Agreement (95% CI) = 97.5% (358/367) (95.4 – 98.7%)

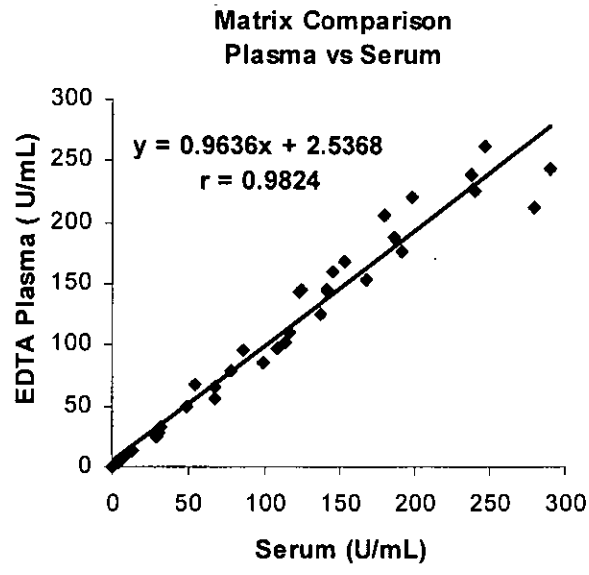
Negative Agreement (95% CI) = 91.4% (416/455) (88.5 – 93.7%)

Matrix Comparison

Testing for matrix effects was conducted in accordance with CLSI EP9-A2 (Vol. 22, No. 19). 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 using the first replicate results to compare the performance of EDTA and heparin plasma samples against serum samples along with the corresponding slopes of regression and correlation coefficient (r) are shown below.

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)


Matrix Comparison
Plasma vs Serum
y = 0.9642x + 2.5264
r = 0.9852
Heparin Plasma (U/mL)
Serum (U/mL)
0
50
100
150
200
250
300


Matrix Comparison
Plasma vs Serum
y = 0.9636x + 2.5368
r = 0.9824
EDTA Plasma (U/mL)
Serum (U/mL)
0
50
100
150
200
250
300

ii. 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 Arthritis	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		

iii. Expected Values

Expected Values/Reference Range:

300 samples from apparently healthy donors including 114 males ranging in age from 4 to 84 and 186 females ranging in age from 4 to 88 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

Note: Each laboratory should establish its own reference range pertinent to their specific patient populations.

Prevalence:

The observed prevalence for the Anti-CCP assay was determined using samples collected from apparently healthy blood donors (N=300) including 114 males ranging in age from 4 to 84 and 186 females ranging in age from 4 to 88 as well as in clinical samples submitted for RF and/or anti-CCP testing (N=300) from 156 males ranging in age from 12 to 92 and 144 females ranging in age from 27 to 93. The results are presented in the tables below.

Note: Each laboratory should establish frequency distributions for their specific patient populations.

Prevalence in Samples from Apparently Healthy Blood Donors
BioPlex2200 Anti-CCP Positive Results by Age and Gender

Age	Gender	BioPlex 2200 Anti-CCP	
		Pos/Total	% Prevalence
0-10	F	0/6	0%
	M	0/3	0%
11-20	F	0/34	0%
	M	0/19	0%
21-30	F	0/26	0%
	M	0/15	0%
31-40	F	0/34	0%
	M	0/10	0%
41-50	F	0/51	0%
	M	0/12	0%
51-60	F	0/24	0%
	M	0/32	0%
61-70	F	0/6	0%

Age	Gender	BioPlex 2200 Anti-CCP	
		Pos/Total	% Prevalence
	M	0/16	0%
71-80	F	0/4	0%
	M	0/5	0%
81+	F	0/1	0%
	M	0/2	0%
Total		0/300	0%

Prevalence in Samples sent to the lab with RF or CCP Test Ordered
BioPlex2200 Anti-CCP Results by Age and Gender

Age	Gender	BioPlex 2200 Anti-CCP	
		Pos/Total	% Prevalence
11-20	F	0/0	0%
	M	0/1	0%
21-30	F	0/3	0%
	M	0/1	0%
31-40	F	1/4	25%
	M	0/3	0%
41-50	F	0/17	0%
	M	0/6	0%
51-60	F	0/37	0%
	M	0/27	0%
61-70	F	1/37	2.7%
	M	0/44	0%

Age	Gender	BioPlex 2200 Anti-CCP	
		Pos/Total	% Prevalence
71-80	F	0/30	0%
	M	0/52	0%
81-90	F	0/13	0%
	M	2/20	10%
91+	F	0/3	0%
	M	0/2	0%
Total		4/300	1.3%

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Mail Center-WO66-G609
Silver Spring, MD 20993-0002

Bio-Rad Laboratories, Inc.
c/o Mr. Juang Wang
Regulatory Affairs Representative
BioPlex Division
5500 East Second Street
Benicia, CA 94510

AUG 2 4 2010

Re: k093954
Trade/Device Name: BioPlex™ 2200 Anti-CCP Kit
BioPlex™ 2200 Anti-CCP Calibrator Set
BioPlex™ 2200 Anti-CCP Control Set
Regulation Number: 21 CFR 866.5575
Regulation Name: Rheumatoid factor immunological test system
Regulatory Class: Class II
Product Code: NHX, JIX, JJY
Dated: August 20, 2010
Received: August 23, 2010

Dear Mr. Wang:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21

CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Maria M. Chan

Maria M. Chan, Ph.D.
Director
Division of Immunology and Hematology Devices
Office of *In Vitro* Diagnostic Device Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indication(s) for use

510(k) Number (if known): ~~Unknown~~ K093954

Device Name: BioPlex 2200 Anti-CCP Kit

AUG 24 2010

Indications for 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 and EDTA or heparinized plasma. Detection of CCP antibodies may be used as an aid in the diagnosis of rheumatoid arthritis and should be used in conjunction with other clinical information.

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 the 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 2200 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 Anti-CCP assay.

Prescription Use X AND/OR Over-the-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(Please do not write below this line-Continue on another page of needed)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

maria m chan

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K093954