

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

A. 510(k) Number:

k130053

B. Purpose for Submission:

New Assay

C. Measurand:

IgA and IgG anti-tissue Transglutaminase (tTG) antibodies
IgA and IgG anti-deamidated gliadin peptide (DGP) antibodies

D. Type of Test:

Semi-quantitative, flow cytometry-based, multiplex immunoassay

E. Applicant:

Bio-Rad Laboratories, Inc.

F. Proprietary and Established Names:

BioPlex® 2200 Celiac IgA kit
BioPlex® 2200 Celiac IgG kit
BioPlex® 2200 Celiac IgA Calibrator Set
BioPlex® 2200 Celiac IgA Control Set
BioPlex® 2200 Celiac IgG Calibrator Set
BioPlex® 2200 Celiac IgG Control Set

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5750 – Radioallergosorbent (RAST) Immunological Test System
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 (Assay and calibrator)
Class I (Control)

3. Product code:

MST – Antibodies, Gliadin
MVM – Autoantibodies, Endomysial (Tissue Transglutaminase)
JIX – Calibrator, Multi-Analyte Mixture
JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

Immunology (82)
Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

BioPlex 2200 Celiac IgA Kit

The BioPlex 2200 Celiac IgA kit is an *in vitro* multiplex flow immunoassay intended for the semi-quantitative detection of IgA autoantibodies to deamidated gliadin peptide (DGP) and tissue Transglutaminase (tTG) in human serum. In conjunction with clinical findings and other diagnostic tests, the test system is used as an aid in the diagnosis of Celiac Disease (gluten-sensitive enteropathy).

The BioPlex 2200 Celiac IgA kit is intended for use with the Bio-Rad BioPlex 2200 System.

BioPlex 2200 Celiac IgG Kit

The BioPlex 2200 Celiac IgG kit is an *in vitro* multiplex flow immunoassay intended for the semi-quantitative detection of IgG autoantibodies to deamidated gliadin peptide (DGP) and tissue Transglutaminase (tTG) in human serum. In conjunction with clinical findings and other diagnostic tests, the test system is used as an aid in the diagnosis of Celiac Disease (gluten-sensitive enteropathy).

The BioPlex 2200 Celiac IgG kit is intended for use with the Bio-Rad BioPlex 2200 System.

BioPlex 2200 Celiac IgA Control Set

The BioPlex 2200 Celiac IgA Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and the BioPlex 2200 Celiac IgA Reagent Pack in the clinical laboratory. The performance of the BioPlex 2200 Celiac IgA Control Set has not been established with any other anti-tissue Transglutaminase (tTG) and anti-deamidated gliadin peptide IgA assays.

BioPlex 2200 Celiac IgG Control Set

The BioPlex 2200 Celiac IgG Control Set is intended for use as an assayed quality control to monitor the overall performance of the BioPlex 2200 Instrument and the BioPlex 2200 Celiac IgG Reagent Pack in the clinical laboratory. The performance of the BioPlex 2200 Celiac IgG Control Set has not been established with any other anti-tissue Transglutaminase (tTG) and anti-deamidated gliadin peptide IgG assays.

2. Indication(s) for use:

Same as above

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Bio-Rad BioPlex® 2200 System, software version 4.1

I. Device Description:

BioPlex® 2200 Celiac IgA and IgG kits include the following components:

1. One 10 mL vial of Bead Set, containing dyed beads coated with recombinant antigens; an Internal Standard bead (ISB), a Serum Verification bead (SVB) and IgA Verification Bead (AVB) (in Celiac IgA only), in MOPS (3-[N-Morpholino] propanesulfonic acid) buffer supplemented with Glycerol and protein stabilizer (bovine and porcine). ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) are added as preservatives.
2. One 5 mL vial of Conjugate, containing phycoerythrin conjugated murine monoclonal anti-human IgA or IgG and phycoerythrin conjugated sheep anti-human Factor XIII in MOPS (3-[N-Morpholino] propanesulfonic acid) buffer supplemented with bovine protein stabilizers. ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) are added as preservatives.
3. One 10 mL vial of Sample Diluent, containing bovine and murine protein stabilizers in triethanolamine buffer. ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) are added as preservatives.

BioPlex 2200 Celiac IgA and IgG Calibrator Sets contain nine (9) 0.5 mL vials of human antibodies to tTG and DGP in a buffer supplemented with protein stabilizer (porcine for IgA and porcine/human for IgG) with ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) as preservatives.

BioPlex 2200 Celiac IgA and IgG Control Sets contain four (4) 1.5 mL vials of Positive Controls of human antibodies to tTG or DGP and two vials of Negative Controls in a human serum matrix made from defibrinated plasma; and, in a human serum matrix made from defibrinated plasma with ProClin 300 ($\leq 0.3\%$), sodium benzoate ($\leq 0.1\%$) and sodium azide ($< 0.1\%$) as preservatives.

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

J. Substantial Equivalence Information:

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

Quanta Lite™ h-tTG IgA, k011566
 Quanta Lite™ Gliadin IgA II, k052143
 Quanta Lite™ h-tTG IgG, k011570
 Quanta Lite™ Gliadin IgG II, k052142

2. Comparison with predicate:

Similarities			
Item	Device: BioPlex 2200 Celiac IgA and IgG Kits	Predicate Quanta Lite h-tTG IgA and IgG Kits	Predicate Quanta Lite Gliadin IgA II and IgG II Kits
Intended Use	Semi-quantitative detection of IgA and IgG autoantibodies to deamidated gliadin peptide (DGP) and tissue Transglutaminase (tTG) in human serum. In conjunction with clinical findings and other diagnostic tests, the test system is used as an aid in the diagnosis of Celiac Disease (gluten-sensitive enteropathy).	Semi-quantitative detection of IgA and IgG autoantibodies to tissue Transglutaminase (endomysium) in human serum. Detection of these antibodies in an aid in diagnosis of certain gluten sensitive enteropathies such as celiac disease and dermatitis herpetiformis.	Semi-quantitative detection of gliadin IgA and IgG antibodies in human serum. The presence of gliadin antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of celiac disease.
Assay Type	Semi-quantitative	Same	Same
Specimen Type	Serum	Same	Same
Analyte Detected	Human IgA or IgG antibodies to human tissue transglutaminase	Same	Not Applicable
	Human IgA or IgG antibodies to deamidated gliadin peptide	Not Applicable	Same

Differences			
Item	Device BioPlex 2200 Celiac IgA and IgG Kits	Predicate INOVA QUANTA Lite h-tTG IgA and IgG Kits	Predicate INOVA QUANTA Lite Gliadin IgA II and IgG II Kits
Assay Technology	Automated multiplex flow immunoassay	Manual, microtiter plate format, Enzyme-linked Immunosorbent assay (ELISA)	Manual, microtiter plate format, Enzyme-linked Immunosorbent assay (ELISA)
Capture Antigen	Recombinant tTG protein and DGP antigen	Native human tissue transglutaminase (h-tTG)	Synthetic, deamidated peptide

Differences			
Item	Device BioPlex 2200 Celiac IgA and IgG Kits	Predicate INOVA QUANTA Lite h-tTG IgA and IgG Kits	Predicate INOVA QUANTA Lite Gliadin IgA II and IgG II Kits
Conjugate Antibody	Phycoerythrin conjugated murine monoclonal anti- human IgA or IgG	Goat anti-human IgG or IgA HRP- conjugated antibody solution	Goat anti-human IgG or IgA HRP- conjugated antibody solution
Substrate	None	Tetramethylbenzidine (TMB) Chromogen	Tetramethylbenzidine (TMB) Chromogen
Signal Detection	Fluorescence	Color, read at 450nm	Color, read at 450nm
Solid Phase	Antigen-coated paramagnetic microbead reagent	Antigen-coated solid phase microtiter wells	Antigen-coated solid phase microtiter wells
Calibrator(s)	5 levels of Calibrator for both IgA and IgG	None. Used 1 level of Low Positive control to extrapolate results	None. Used 1 level of Low Positive control to extrapolate results
Control	One Negative and one Positive Controls	One Negative, one low Positive, and one high Positive Controls	One Negative, one low Positive, and one high Positive Controls
Assay range	Anti-tTG_IgA: 0.5 to 250 U/mL Anti-tTG_IgG: 0.8 to 250 U/mL Anti-DGP IgA: 0.2 to 250 U/mL Anti-DGP IgG: 0.4 to 250 U/mL	Not Applicable	Not Applicable
Calibrators and Controls	Sold Separately	Kit components	Kit components
Quantitation	Results are determined from a standard calibration curve utilizing a 4- PL (4-Parameter Logistic) curve fitting.	One point calculation from the OD of the low positive control.	One point calculation from the OD of the low positive control.
Instrumentation	Bio-Rad BioPlex 2200 system	Spectrophotometer	Spectrophotometer

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

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

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

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

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

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

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

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

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

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

L. Test Principle:

The BioPlex 2200 Celiac IgA and IgG kits use a multiplexed micro particle bead-based immunoassay for the semi-quantitative detection of IgA and IgG antibodies to tissue Transglutaminase (tTG) and deamidated gliadin peptide (DGP) in human serum using the Luminex flow cytometry technology.

In the BioPlex Celiac IgA and IgG assays, two (2) different populations of dyed beads are coated with antigens associated with celiac disease (recombinant antigens). The BioPlex 2200 System combines an aliquot of patient sample, sample diluent, and bead set reagent into a reaction vessel. The mixture is incubated at 37°C. After a wash cycle, anti-human IgA or IgG antibody conjugated to phycoerythrin (PE) is added to the dyed 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 detector. The identity of the dyed beads is determined by the fluorescence of the dyes, and the amount of antibody captured by the antigen is determined by the fluorescence of the attached PE. Raw data is calculated in relative fluorescence intensity (RFI).

Two additional dyed beads, Internal Standard Bead (ISB) and Serum Verification Bead (SVB) are present in each reaction mixture to verify detector response and the addition of serum to the reaction vessel. An additional control bead, IgA Verification Bead (AVB) has been included for the Celiac IgA kit to flag results for samples with deficient IgA levels. Refer to the BioPlex 2200 System Operation Manual for more information on internal quality control beads.

The system is calibrated using a set of nine (9) distinct calibrator vials, supplied separately by Bio-Rad Laboratories. For anti-tTG, five (5) vials, representing five (5) different antibody concentrations, are used for semi-quantitative calibration for anti-tTG IgA and IgG. For anti-DGP, five (5) vials, representing five (5) different antibody concentrations, are used for semi-quantitative calibration for anti-DGP IgA and IgG. The result for each of these antibodies is expressed in Units/mL (U/mL).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

- i) *Reproducibility:* BioPlex® 2200 Celiac IgA and IgG assays reproducibility testing was performed in accordance with CLSI EP5-A2 Guideline, using 10 patient serum samples within the assay reportable range in replicates of ten on each of three BioPlex 2200 instruments with three independent reagent lots comprised of a reagent pack, matched calibrator set and control set. Two runs were conducted on each instrument for each of three set reagent. Each lot mean (U/mL) was calculated using data 60 points for each patient sample (ten replicates, three instruments, two runs per instrument). Total coefficient of variation (CV) was calculated based on within-run, between-run, between-instruments/operators and between-reagent-lots precision. The acceptance criteria for total precision are %CV < 25% (for > 15 U/mL Positive) and SD < 3 U/mL (<15 U/mL Negative). Results of the reproducibility study are summarized below.

BioPlex® 2200 Celiac IgA – Anti-tTG IgA

Sample Type	Mean U/mL	Within Run %CV	Between Run %CV	Between Instrument/ Operator %CV	Between Lot %CV	Total %CV
High Negative	7.2	4.8%	7.5%	0.0%	10.5%	13.8%
	7.5	3.9%	6.9%	0.0%	11.6%	14.1%
Near Cutoff	14.6	3.7%	9.0%	0.0%	9.8%	13.8%
	18.3	3.7%	9.0%	0.0%	7.2%	12.1%
Low Positive	39.5	5.8%	6.9%	1.9%	14.3%	17.0%
	39.7	4.2%	6.7%	0.0%	6.4%	10.2%
Mid Positive	86.0	4.6%	6.5%	0.0%	12.3%	14.6%
	107.7	3.7%	8.7%	0.0%	10.5%	14.1%
High Positive	196.6	4.6%	7.8%	0.0%	9.8%	13.3%
	213.3	4.1%	8.7%	0.0%	6.3%	11.5%

BioPlex® 2200 Celiac IgA – Anti-DGP IgA

Sample Type	Mean U/mL	Within Run %CV	Between Run %CV	Between Instrument/ Operator %CV	Between Lot %CV	Total %CV
High Negative	1.5	6.3%	10.3%	0.0%	0.0%	12.1%
	4.6	4.8%	9.2%	1.6%	1.0%	10.6%
Near Cutoff	15.4	4.3%	6.6%	0.0%	3.4%	8.6%
	16.3	3.7%	4.6%	0.0%	2.7%	6.5%
Low Positive	33.3	3.7%	4.6%	0.0%	4.8%	7.6%
	35.7	4.8%	5.6%	0.0%	5.0%	8.9%
Mid Positive	99.3	4.4%	5.9%	0.0%	6.7%	10.0%
	102.0	3.4%	4.9%	0.0%	9.0%	10.8%
High Positive	188.9	3.6%	6.3%	0.0%	8.4%	11.1%
	207.0	3.9%	6.7%	0.0%	6.1%	9.9%

BioPlex® 2200 Celiac IgG – Anti-tTG IgG

Sample Type	Mean U/mL	Within Run %CV	Between Run %CV	Between Instrument/ Operator %CV	Between Lot %CV	Total %CV
High Negative	3.9	6.5%	15.0%	0.0%	20.1%	25.9%
	8.8	6.5%	12.5%	1.2%	0.0%	14.2%
Near Cutoff	15.9	5.4%	6.1%	0.0%	3.9%	9.0%
	17.6	5.7%	13.3%	0.0%	0.0%	14.5%
Low Positive	34.2	5.0%	5.2%	2.4%	0.0%	7.6%
	34.7	5.1%	5.4%	0.0%	9.0%	11.7%
Mid Positive	76.2	5.4%	7.7%	0.0%	8.4%	12.6%
	120.6	5.9%	7.5%	0.0%	11.7%	15.1%
High Positive	178.2	6.2%	10.7%	0.0%	0.0%	12.4%
	180.0	8.3%	6.0%	0.0%	15.0%	18.2%

BioPlex® 2200 Celiac IgG – Anti-DGP IgG

Sample Type	Mean U/mL	Within Run %CV	Between Run %CV	Between Instrument/ Operator %CV	Between Lot %CV	Total %CV
High Negative	2.2	7.1%	16.8%	0.0%	0.0%	18.2%
	10.2	5.7%	5.5%	2.0%	4.1%	9.2%
Near Cutoff	14.8	4.7%	4.9%	0.0%	2.4%	7.2%
	15.5	5.3%	6.0%	0.0%	5.9%	9.9%
Low Positive	31.6	7.2%	5.9%	0.0%	0.0%	9.3%
	32.6	4.5%	3.9%	3.0%	6.1%	3.9%
Mid Positive	87.8	6.9%	8.5%	1.7%	1.0%	11.1%
	92.1	5.2%	6.4%	0.0%	2.3%	8.6%
High Positive	165.1	6.0%	5.1%	0.0%	11.5%	14.0%
	197.7	7.7%	4.6%	0.0%	9.0%	12.7%

- ii) *Within-Laboratory Precision:* The within laboratory precision testing of the BioPlex® 2200 Celiac IgA and IgG kits on the BioPlex® 2200 instrument was performed in accordance with CLSI EP5-A2. Two serum panels consisting of samples spanning the measuring range (Negative (< 8 U/mL), near cut-off (15 U/mL), low positive (30 - 40 U/mL), medium positive (80- 120 U/mL) and high positive (170 - 230 U/mL)) plus controls (one negative and one positive) were included in the study. These samples were assayed in replicate twice daily over 20 days (n=80). The data were analyzed for within-run, between-run, between-day, and total precision and the mean U/mL and percent coefficient of variation (%CV) are summarized below:

BioPlex® 2200 Celiac IgA – Anti-tTG IgA

Sample Type	N	Mean U/mL	Within Run %CV	Between Run %CV	Between Day %CV	Total Precision %CV
High Negative	80	6.3	6.2%	3.3%	6.3%	9.4%
	80	6.2	6.1%	0.0%	5.8%	8.4%
Near Cutoff	80	12.5	6.9%	0.0%	8.4%	10.9%
	80	15.7	5.2%	4.4%	5.0%	8.5%
Low Positive	80	36.5	6.4%	3.4%	7.1%	10.2%
	80	38.4	5.8%	0.0%	7.8%	9.7%
Mid Positive	80	108.4	5.8%	3.1%	7.3%	9.9%
	80	94.8	6.8%	0.0%	5.6%	8.9%
High Positive	80	210.9	5.2%	0.0%	5.6%	7.6%
	80	227.7	3.6%	2.4%	3.9%	5.8%

BioPlex® 2200 Celiac IgA – Anti-DGP IgA

Sample Type	N	Mean U/mL	Within Run %CV	Between Run %CV	Between Day %CV	Total Precision %CV
High Negative	80	8.3	6.4%	0.0%	8.9%	11.0%
	80	4.1	6.7%	6.5%	4.6%	10.4%
Near Cutoff	80	16.1	4.6%	2.0%	4.2%	6.5%
	80	15.3	6.0%	0.0%	3.3%	6.8%
Low Positive	80	34.5	5.1%	0.0%	2.9%	5.9%
	80	33.9	5.8%	1.9%	6.3%	8.8%
Mid Positive	80	96.8	6.3%	0.0%	2.9%	6.9%
	80	98.5	4.7%	0.0%	5.8%	7.5%
High Positive	80	185.5	4.5%	0.0%	6.6%	8.0%
	80	200.3	4.6%	0.0%	5.5%	7.2%

BioPlex® 2200 Celiac IgG – Anti-tTG IgG

Sample Type	N	Mean U/mL	Within Run %CV	Between Run %CV	Between Day %CV	Total Precision %CV
High Negative	80	7.7	6.2%	3.3%	9.4%	11.7%
	80	9.3	5.6%	5.7%	13.2%	15.4%
Near Cutoff	80	14.6	5.8%	2.6%	8.4%	10.5%
	80	15.7	8.1%	0.0%	4.9%	9.5%
Low Positive	80	33.3	4.1%	3.9%	4.5%	7.2%
	80	34.0	3.8%	0.0%	4.4%	5.8%
Mid Positive	80	101.9	3.5%	2.1%	4.3%	5.9%
	80	133.3	3.6%	0.2%	4.8%	6.1%
High Positive	80	172.3	3.7%	0.0%	3.4%	5.0%
	80	207.9	5.3%	0.0%	7.7%	9.4%

BioPlex® 2200 Celiac IgG – Anti-DGP IgG

Sample Type	N	Mean U/mL	Within Run %CV	Between Run %CV	Between Day %CV	Total Precision %CV
High Negative	80	10.1	4.0%	2.2%	3.9%	6.0%
	80	11.6	5.0%	4.8%	6.8%	9.7%
Near Cutoff	80	15.0	2.4%	2.3%	3.9%	5.1%
	80	16.6	4.9%	3.1%	5.3%	7.8%
Low Positive	80	32.7	5.3%	0.0%	7.0%	8.8%
	80	33.2	3.3%	0.0%	2.6%	4.2%
Mid Positive	80	89.7	4.6%	2.9%	6.8%	8.7%
	80	93.9	4.4%	0.0%	4.8%	6.5%
High Positive	80	164.6	2.9%	2.2%	2.9%	4.5%
	80	194.6	4.8%	4.8%	5.5%	7.4%

b. *Linearity/assay reportable range:*

- i) **Linearity:** Three low and 4-5 high Celiac anti-tTG IgA or IgG and anti-DGP IgA or IgG positive patient samples were tested to demonstrate linearity. These samples were diluted with immunodepleted serum according to CLSI EP06-A. Each sample and dilution was evaluated in replicates of four using one Celiac IgA and IgG lot on one instrument. Linear and polynomial regression analysis of Celiac IgA and IgG recovery vs. sample dilution was performed to determine if the dilution curves exhibit statistically significant non-linear regression based on the CLSI guideline EP06-A.

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

Celiac Assays	Sample	Conc. (U/mL)	Slope	Intercept	r^2	Dilution range (U/mL)
Anti-tTG IgA	1	58.4	1.00	-0.002	0.999	0.3 – 58.4
	2	68.7	1.00	0.001	0.999	0.3 – 68.7
	3	61.8	1.00	0.001	0.998	0.3 – 61.8
	4	263.0	1.00	0.002	0.996	0.3 – 263.0
	5	235.1	1.00	0.002	0.999	0.3 – 235.1
	6	254.1	1.00	-0.004	0.998	0.3 – 254.1
	7	299.3	0.999	-0.001	0.999	0.3 – 299.3
	8	294.3	1.00	-0.014	0.997	0.3 – 294.3
Anti-DGP IgA	1	60.9	1.00	0.001	0.999	0.0 – 60.9
	2	55.0	1.00	-0.002	0.998	0.0 – 55.0
	3	72.6	1.00	-0.001	0.996	0.0 – 72.6
	4	223.1	1.00	0.000	0.999	0.0 – 223.1
	5	206.2	1.00	0.002	0.998	0.0 – 206.2
	6	242.6	1.00	0.001	0.999	0.0 – 242.6
	7	290.0	0.998	0.016	0.999	9.0 – 290.0
	8	308.0	1.00	-0.001	0.999	6.5 – 308.0

Celiac Assays	Sample	Conc. (U/mL)	Slope	Intercept	r ²	Dilution range (U/mL)
Anti-tTG IgG	1	67.5	1.00	0.001	0.999	0.5 – 67.5
	2	68.0	1.00	-0.001	0.999	0.5 – 68.0
	3	46.7	1.00	-0.001	0.997	0.5 – 46.7
	4	259.9	1.00	-0.005	0.998	0.3 – 259.9
	5	232.7	1.00	-0.000	0.998	0.3 – 232.7
	6	253.2	1.00	-0.002	0.997	0.3 – 253.2
	7	309.5	1.00	-0.005	0.993	0.0 – 309.5
	8	350.6	1.00	0.006	0.996	0.0 – 350.6
Anti-DGP IgG	1	48.5	1.00	0.002	0.999	0.0 – 48.5
	2	57.9	1.00	-0.001	0.998	0.0 – 57.9
	3	49.2	1.00	0.000	0.998	0.0 – 49.2
	4	192.9	1.00	-0.002	0.998	0.0 – 192.9
	5	183.4	1.00	0.000	0.999	0.0 – 183.4
	6	238.6	1.00	-0.002	0.998	0.0 – 238.6
	7	399.0	1.00	-0.004	0.998	0.0 – 399.0

The claimed reportable Analytical Measuring range is shown in the table below:

BioPlex 2200® Celiac Assay	Assay reportable range
Anti-tTG IgA	0.5 to 250 U/mL
Anti-DGP IgA	0.2 to 250 U/mL
Anti-tTG IgG	0.8 to 250 U/mL
Anti-DGP IgG	0.4 to 250 U/mL

ii) *Dilution Recovery:*

The BioPlex 2200 instrument has the ability to perform 1:10 onboard dilution of samples using wash buffer as the diluent. The BioPlex 2200 instrument has dilution screen for loading and analysis of diluted specimens. If this option is selected, the instrument will automatically run any sample that has a result >250 U/mL by further diluting it by a factor of 10.

To confirm the onboard dilution function, high positive specimens with results above the analytical measuring range were selected for each assay. The samples were run with the onboard dilution function enabled on the BioPlex 2200 instrument. Then the specimens were manually diluted the same way as it happens in the onboard dilution function (10 fold dilution), and tested on the BioPlex 2200 instrument. The results were within the analytical measuring range after onboard dilution or manual dilution for all specimens. The percent-recoveries in onboard dilution compared to manual dilution are tabulated below.

Celiac Assay	# of Samples	Range (U/mL)	Minimum Recovery (%)	Maximum Recovery (%)
Anti-tTG IgA	23	354 - 1791	93%	112%
Anti-DGP IgA	21	277 - 2325	93%	110%
Anti-tTG IgG	21	280- 1963	92%	109%
Anti-DGP IgG	18	277 - 2084	86%	110%

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

- i) Traceability: There is no international or certified reference material available for Celiac anti-tTG and anti-DGP IgA and IgG. The calibrators are assigned relative arbitrary units (U/mL).
- ii) Calibrator and controls: The calibrators are manufactured independently from the controls, and are stabilized with <0.3% ProClin® 300, <0.1% sodium benzoate, and <0.1% sodium azide. Calibrator assignment is established for matched lots of BioPlex® 2200 Celiac IgA or IgG kit and calibrators using a master set of calibrators as reference and replicate analyses on multiple BioPlex® 2200 instruments. The BioPlex® 2200 Celiac IgA or IgG Reagent Kit is calibrated using a set of five (5) distinct calibrators for anti-tTG and anti-DGP IgG or IgA, which are used to establish points of reference for determining the presence of Celiac IgA or IgG in human specimens.

The negative control has been tested to give results with values below the cut-off for each assay. The positive control is prepared by blending human disease state serum with negative serum matrix and is manufactured to give results with values above the assay cut-off. The positive controls are manufactured to give positive results, with values above the cut-off for each specific analyte. The negative control is manufactured to give negative results, with values below the cut-off for each specific analyte.

- iii) Stability: Stability studies have been performed to support the following claims:

Calibrator and Control: BioPlex® 2200 Celiac IgA and IgG Control and Calibrator Sets: Calibrator Open Vial Stability (2 to 8°C), 60 days from first opening; Control Open Vial Stability (2 to 8°C), 60 days from first opening; Calibration Curve On-board Stability, 30 days; Calibrators and Controls Real Time Stability (2 to 8°C), 9 months; labeled as until expiration date; Calibrators and Controls Accelerated Stability (2 to 8°C), 2.5 years predicted. Calibrators and controls were shown to be stable after 5 freeze-thaw cycles at either -20°C or -80°C.

Kit Stability: BioPlex® 2200 Celiac IgA and IgG Kit: Real-time (unopened) Kit Stability is 9 months or until the date of expiration when stored unopened on the instrument or at 2 to 8°C. The Open kit claim is 60 days.

Serum Sample Stability: Sample stability studies were also performed: Sample stability fresh (2 to 8°C), 7 days; Sample stability frozen (-20 or -80°C), 8 months; Sample Freeze-thaw (-20 or -80°C), up to 3-freeze/thaw cycles acceptable.

d. *Detection limit:*

Three (3) serum samples per assay with analyte concentration near cutoff (15 U/mL) were serially diluted in a factor of 2 in negative human serum (IgG depleted) to a concentration level of 0.54 U/mL (anti-DGP IgA), 0.64 U/mL (anti-tTG IgA), 0.73/mL (anti-DGP IgG), 0.61 U/mL (anti-tTG IgG). Each dilution was assayed daily in replicates of 12 for a period of five days (60 data points total per dilution) on one BioPlex 2200 instrument. All assays used a passing on-board calibration and assay controls were run prior to samples.

The BioPlex 2200 Celiac IgA and IgG LoD was calculated based on CLSI EP17-A guideline as “LoD = LoB + c β SDs”. For this calculation, the LoB was calculated at the 95th percentile of negative samples. c β is the 95th percentile of the standard Gaussian distribution with a correction factor applied to account for the biased estimate of the population standard deviation (1.645/(1-1/(4xf))), where f is the degrees of freedom of the estimated standard deviation SDs. LoQ = LoD if the %CV of LoD is less than or equal to 20%; otherwise, LoQ was calculated from the regression line of the LoD standard deviation versus the analyte concentration. The results of LoQ, LoD and LoB are summarized in the table below.

BioPlex® 2200 Celiac Assay	LoQ (U/mL)	LoD (U/mL)	LoB (U/mL)
Anti-tTG IgA	0.5	0.5	0.4
Anti-DGP IgA	0.2	0.1	0.0
Anti-tTG IgG	0.8	0.8	0.6
Anti-DGP IgG	0.4	0.1	0.0

e. *Analytical specificity:*

i) **Interferences:** An interfering substance study was conducted in pooled serum samples at multiple analyte levels including below cut-off (<15 U/mL), low positive (15 to 25 U/mL), medium positive (40 to 60 U/mL) and high positive (78 to 160 U/mL), to evaluate the potential interference of specific endogenous and exogenous substances with the BioPlex® 2200 Celiac IgA and IgG kits according to CLSI EP7-A2. Bias exceeding 20% was considered interference.

Results showed no interference from the endogenous substances tested, except anti-tTG IgA and IgG at hemolysate level above hemoglobin concentration of 63 mg/dL. 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
Triglycerides	≤3300 mg/dL
Total Protein	≤12 g/dL
Cholesterol	≤500 mg/dL
Red Blood Cells	≤0.4% (v/v)
Gamma-Globulin	≤6 g/dL
Beta-Carotene	≤0.6 mg/dL
Ascorbic Acid	≤3 mg/dL
EDTA	≤800 mg/dL

*for anti-DGP IgA and IgG only

- ii) Cross-Reactivity (Clinical): A cross-reactivity study was performed to determine if samples from individuals with various disease states and other potentially interfering factors interfere with test results from the BioPlex® 2200 Celiac IgA or IgG kit. The table below shows the number (N) of samples containing potential cross reactants as disease state evaluated by the BioPlex® Celiac IgA and IgG kits. The cross reactivity was obtained as the positivity rate from the ratio of the number of samples scored positive by the BioPlex® Celiac IgA and IgG assays to the total number of cross reactant samples evaluated.

Cross Reactive Disease State	N	Anti-tTG IgA		Anti-DGP IgA		Anti-tTG IgG		Anti-DGP IgG	
		(+)	%	(+)	%	(+)	%	(+)	%
Chronic Active Hepatitis	20	0	0%	0	0%	5	25%	0	0%
Crohn's Disease	23	1	4%	1	4%	0	0%	0	0%
Diabetes Mellitus Type 1	20	0	0%	0	0%	0	0%	0	0%
Gastritis	20	0	0%	0	0%	0	0%	0	0%
Graves'/Hashimoto's Disease	23	0	0%	1	4%	0	0%	1	4%
Irritable Bowel Syndrome	15	0	0%	0	0%	0	0%	0	0%
Pernicious Anemia	6	0	0%	0	0%	0	0%	0	0%
Primary Biliary Cirrhosis	19	0	0%	0	0%	0	0%	2	11%
Rheumatoid Arthritis	20	1	5%	2	10%	1	5%	1	5%
Scleroderma	21	0	0%	1	5%	0	0%	1	5%
Sjögren's Syndrome	24	0	0%	1	4%	1	4%	1	4%
Syphilis	10	0	0%	1	10%	0	0%	0	0%
Systemic Lupus Erythematosus	30*	0	0%	0	0%	0	0%	0	0%
Ulcerative Colitis	22	0	0%	0	0%	0	0%	0	0%

* 1 sample exhibited repeated instrument error flags and was excluded from the anti-tTG IgA and IgG data analysis.

- iii) Cross-Reactivity in the multiplex (Analytical):

The BioPlex 2200 Celiac IgA and IgG kits are immunoassays and detect

autoantibodies to DGP and tTG in a multiplex format. Five to eight patient samples (representing from low to high) were tested in presence of increasing concentrations of DGP or tTG antigens. Inhibition titers were evaluated for the five microspheres (i.e., DGP beads, tTG beads, Internal Standard bead (ISB), Serum Verification bead (SVB) and IgA Verification Bead (AVB)). The BioPlex 2200 Celiac IgA Kit was evaluated for DGP IgA and tTG IgA concentrations, SVB values, ISB signal and AVB values and recoveries in patient samples in the presence of (a) added DGP antigen and (b) added tTG Antigen. Similarly, BioPlex 2200 Celiac IgG Kit was evaluated for DGP IgG and tTG IgG concentrations, SVB values, and ISB signal and recoveries in patient samples in the presence of (a) added DGP antigen and (b) added tTG antigen. The data from these studies demonstrate that recombinant DGP and tTG coupled microspheres in the BioPlex 2200 Celiac IgA or IgG devices specifically detect antibodies to DGP or tTG, respectively.

In addition, phycoerythrin (PE) conjugated murine monoclonal anti-human IgA or IgG and PE conjugated sheep anti-human Factor XIII were tested for cross-reactivity. The signal intensity (RFI) and specificity of each conjugate was evaluated by mixing/incubating the conjugates with microspheres covalently coupled with immunoglobulin A, immunoglobulin G and Factor XIII, and subsequently analyzing on the BioPlex 2200. The results of these tests demonstrate that each PE conjugate is specific for their intended antibody target while showing no cross reactivity to the other antibodies in the multiplex mixture.

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 the clinical diagnosis as the standard. The study to determine the Celiac IgA or IgG assay cutoff is comprised of two sample groups – one clinical cohort has 123 samples from patients diagnosed as celiac disease and 112 from non-celiac or other rheumatic disease control donors. It was later confirmed by testing 298 samples from apparently healthy donors.

The cut-off was established to achieve an optimal clinical specificity while accepting the resultant clinical sensitivity. For anti-tTG IgA or IgG and for anti-DGP IgA or IgG, the cut-off is 15.0 U/mL.

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the BioPlex® 2200 Celiac IgA and IgG kits was evaluated in retrospectively collected /repository samples, including 156 samples from patients diagnosed with celiac disease 163 from patients with other rheumatic or non-CD disease control, celiac IgA deficient patients, and dermatitis herpetiformis (DH) patients. Results from values in the measuring range of both the new and the predicate immunoassays are compared. Results are summarized in the tables below:

BioPlex 2200 Celiac IgA

		QUANTA Lite IgA EIA Kit		
		Positive	Negative	Total
anti-tTG IgA (0.5 – 250 U/mL)	Positive	97	0	97
	Negative	6 ¹	108	114
	Total	103	108	211
anti-DGP IgA (0.2 – 250 U/mL)	Positive	129	13 ²	142
	Negative	4 ³	164	168
	Total	133	177	310

¹Of 6 discrepant results, all 3 non-CD samples were scored negative by the BioPlex anti-DGP IgA and positive by the QUANTA Lite IgA. The 3 celiac disease (CD) samples were scored negative by the BioPlex anti-DGP IgA, but positive by the QUANTA Lite IgA.

²Of 13 discrepant results, all 9 celiac disease (CD) samples were scored positive by the BioPlex anti-DGP IgA but negative by the QUANTA Lite IgA. The 4 non-CD samples were scored positive by the BioPlex anti-DGP IgA and negative by the QUANTA Lite IgA.

³Of 4 discrepant results, all 3 non-CD samples were scored negative by the BioPlex anti-DGP IgA but positive by the QUANTA Lite IgA. The 1CD sample was scored negative by the BioPlex anti-DGP IgA and positive by the QUANTA Lite IgA.

Anti-tTG IgA

Positive Agreement (97/103) = 94.2% (95% CI = 87.9 – 97.3%)

Negative Agreement (108/108) = 100% (95% CI = 96.6 - 100%)

Total Agreement (205/211) = 97.2% (95% CI = 93.9 – 98.7%)

Anti-DGP IgA

Positive Agreement (129/133) = 97.0% (95% CI = 92.5 – 98.8%)

Negative Agreement (164/177) = 92.7% (95% CI = 87.8 – 95.7%)

Total Agreement (293/310) = 94.5% (95% CI = 91.4 – 96.5%)

BioPlex 2200 Celiac IgG

		QUANTA Lite IgG EIA Kit		
		Positive	Negative	Total
anti-tTG IgG (0.8 – 250 U/mL)	Positive	54	36 ¹	90
	Negative	4 ²	194	198
	Total	58	230	288
anti-DGP IgG (0.4 – 250 U/mL)	Positive	140	17 ³	157
	Negative	9 ⁴	82	91
	Total	149	99	248

¹Of the 36 discrepant results, all 30 CD samples were positive by the BioPlex anti-tTG IgG but negative by the QUANTA Lite IgG. The 6 non-CD samples were scored positive by the BioPlex anti-tTG IgG and negative by the QUANTA Lite IgG.

²Of the 4 discrepant results, all 4 CD samples were negative by the BioPlex anti-tTG IgG but positive by the QUANTA Lite IgG.

³Of the 17 discrepant results, all 13 CD samples were scored positive by the BioPlex anti-DGP IgG but negative by the QUANTA Lite IgG. The 4 non-CD samples were scored positive by the BioPlex anti-DGP IgG and negative by the QUANTA Lite IgG.

⁴Of the 9 discrepant results, all 9 CD sample were scored negative by the BioPlex anti-DGP IgG and positive by the QUANTA Lite IgG.

Anti-tTG IgG

Positive Agreement (54/58) = 93.1% (95% CI = 83.6 – 97.3%)
 Negative Agreement (194/230) = 84.3% (95% CI = 79.1 – 88.5%)
 Total Agreement (248/288) = 86.1% (95% CI = 81.6 – 89.6%)

Anti-DGP IgG

Positive Agreement (140/149) = 94.0% (95% CI = 88.9 – 96.8%)
 Negative Agreement (82/99) = 82.8% (95% CI = 74.2 – 89.0%)
 Total Agreement (222/248) = 89.5% (95% CI = 85.1 – 92.7%)

b. *Matrix comparison:*

Serum is the only sample matrix.

3. Clinical studies:

a. *Clinical Sensitivity:*

The clinical studies involved testing 319 specimens including 163 non-Celiac disease control patients and 156 diagnosed (biopsy confirmed) Celiac disease (CD) patients. The BioPlex® 2200 Celiac IgA and IgG sensitivity and specificity are shown below:

BioPlex 2200 Celiac IgA

		Clinical Diagnosis		
		CD	Not CD	Total
anti-tTG IgA	Positive	148	2	150
	Negative	8	160	168
	Total	156	162	318*
anti-DGP IgA	Positive	136	5	141
	Negative	20	158	178
	Total	156	163	319

* 1 sample exhibited repeated instrument error flags and was excluded from the data analysis

Anti-tTG IgA

Sensitivity (148/156) = 94.9% (95% CI = 90.2 – 97.4%)
 Specificity (160/162) = 98.8% (95% CI = 95.6 – 99.7%)

Anti-DGP IgA

Sensitivity (136/156) = 87.2% (95% CI = 81.0 – 91.5%)
 Specificity (158/163) = 96.9% (95% CI = 93.0 – 98.7%)

BioPlex 2200 Celiac IgG

		Clinical Diagnosis		
		CD	Not CD	Total
anti-tTG IgG	Positive	69	7	76
	Negative	87	155	242
	Total	156	162	318*
anti-DGP IgG	Positive	132	5	137
	Negative	24	158	182
	Total	156	163	319

* 1 sample exhibited repeated instrument error flags and was excluded from the data analysis

Note: The clinical sensitivity of the BioPlex 2200 Celiac anti-tTG IgG is within the range published in peer reviewed literature.

Anti-tTG IgG

Sensitivity (69/156) = 44.2% (95% CI = 36.7 – 52.1%)

Specificity (155/162) = 95.7% (95% CI = 91.4 – 97.9%)

Anti-DGP IgG

Sensitivity (132/156) = 84.6% (95% CI = 78.1 – 89.4%)

Specificity (158/163) = 96.9% (95% CI = 93.0 – 98.7%)

Samples from patients previously diagnosed with celiac disease and IgA deficiency (N=11) were run on the BioPlex 2200 Celiac IgG kits and the # positive samples is shown in the table below:

Patient Group	# Positive Anti-tTG IgG	# Positive Anti-DGP IgG
Celiac Disease with IgA deficiency	9 (81.8%) (95% CI = 52.3 - 94.9%)	8 (72.7%) (95% CI = 43.4 - 90.3%)

b. Clinical specificity:

The positive rates of the BioPlex APLS IgG and IgA in each of disease category are shown below.

Disease Category	Number Enrolled	Anti-tTG IgA	Anti-DGP IgA	Anti-tTG IgG	Anti-DGP IgG
Celiac Disease	156	148 (94.9%)	136 (87.2%)	69	132 (84.6%)
Apparently Healthy Subject	300	0 (0.0%)	5 (1.7%)	2 (0.7%)	5 (1.7%)
IgA Deficiency	11	0 (0.0%)	0 (0.0%)	9 (81.8%)	8 (72.7%)
Dermatitis Herpetiformis	16	13 (81.3%)	16 (100%)	7 (43.8%)	12 (75.0%)
Chronic Active Hepatitis	10	0 (0%)	0 (0%)	5 (50%)	0 (0%)
Crohn's Disease	13	1 (7.7%)	0 (0%)	0 (0%)	0 (0%)
Diabetes Mellitus Type 1	10	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Disease Category	Number Enrolled	Anti-tTG IgA	Anti-DGP IgA	Anti-tTG IgG	Anti-DGP IgG
Gastritis	10	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Graves'/Hashimoto's Disease	13	0 (0%)	1 (7.7%)	0 (0%)	0 (0%)
Irritable Bowel Syndrome	15	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pernicious Anemia	6	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Primary Biliary Cirrhosis	9	0 (0%)	0 (0%)	0 (0%)	2 (22.2%)
Rheumatoid Arthritis	10	1 (10%)	1 (10%)	1 (10%)	1 (10%)
Scleroderma	11	0 (0%)	1 (9.1%)	0 (0%)	1 (9.1%)
Sjögren's Syndrome	14	0 (0%)	1 (7.1%)	1 (7.1%)	1 (7.1%)
Systemic Lupus Erythematosus	20*	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ulcerative Colitis	12	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Syphilis	10	0 (0%)	1 (10%)	0 (0%)	0 (0%)

* 1 sample exhibited repeated instrument error flags and was excluded from the anti-tTG IgG and IgA data analysis

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

See Assay Cutoff

5. Expected values/Reference range:

Three hundred samples from apparently healthy donors including 139 males ranging in age from <1 to 94 and 161 females ranging in age from 5 to 101 were tested with BioPlex® 2200 Celiac IgA and IgG kits. The number of positive and mean value of the BioPlex® Celiac IgA and IgG results are shown below.

Assay	N (%Positive)	Mean (U/mL)
anti-tTG IgA	0 (0.0%)	0.6
anti-DGP IgA	5 (1.7%)	2.2
anti-tTG IgG	2 (0.7%)	2.3
anti-DGP IgG	5 (1.7%)	1.7

Results of <15.0 U/mL for anti-tTG IgA or IgG and for anti-DGP IgA or IgG are reported as negative and results >15.0 U/mL for anti-tTG IgA or IgG and for anti-DGP IgA or IgG are reported as positive.

N. Proposed Labeling:

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

O. Conclusion:

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