510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K200230

B Applicant

Inova Diagnostics, Inc.

C Proprietary and Established Names

Aptiva Celiac Disease IgG Reagent

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MVM	Class II	21 CFR 866.5660 - Multiple Autoantibodies Immunological Test System	IM - Immunology
MST	Class II	21 CFR 866.5750 - Radioallergosorbent (RAST) immunological test system	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Anti-deaminated gliadin peptide (DGP) IgG antibodies
Anti-human tissue transglutaminase (h-tTG) IgG antibodies

C Type of Test:

Semi-quantitative particle-based multi-analyte technology (PMAT)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below

B Indication(s) for Use:

The Aptiva Celiac Disease IgG Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-tissue transglutaminase IgG autoantibodies and anti-deamidated gliadin peptide IgG autoantibodies in human serum. The presence of these antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of celiac disease and dermatitis herpetiformis, particularly in patients with selective IgA deficiency.

The Aptiva Celiac Disease IgG Reagent is intended for use with the Inova Diagnostics Aptiva System.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Inova Diagnostics Aptiva System

IV Device/System Characteristics:

A Device Description:

The Aptiva Celiac Disease IgG Reagent is an immunoassay utilizing particle-based multi-analyte technology. The kit contains one Aptiva Celiac Disease IgG Reagent Cartridge with the following reagents for 250 determinations:

- tTG, DGP, and Control paramagnetic particles, preserved
- Assay buffer – containing protein stabilizers and preservatives
- PE Tracer IgG – PE labeled anti-human IgG antibody, containing buffer, protein stabilizers and preservative
- Rehydration Buffer – containing protein stabilizers and preservatives

The Aptiva Celiac Disease IgG Calibrators and Aptiva Celiac Disease IgG Controls are required but sold separately:

- The Aptiva Celiac Disease IgG Calibrators include three Calibrators: Calibrator 1, Calibrator 2, and Calibrator 3. Each Calibrator is provided with two barcode labeled tubes each containing 0.3 mL pre-diluted and ready to use reagent. Calibrators contain human antibodies to DGP and tTG in stabilizers and preservatives.
- The Aptiva Celiac Disease IgG Controls include two controls: Control 1 and Control 2. Each Control is provided with two barcode labeled tubes each containing 0.5 mL, ready to use reagent. Controls contain human antibodies to DGP and tTG in stabilizers and preservatives.

B Principle of Operation:

Aptiva Celiac Disease IgG Reagent kit contains two different populations of particles; one particle coated with recombinant tissue transglutaminase antigen and one particle coated with a synthetic deamidated gliadin peptide and an additional third particle coated with goat anti-human IgG antibody as control verification.

The Aptiva System dilutes the patient sample 1:23 then combines an aliquot of diluted patient sample, and reagent into a cuvette. The mixture is incubated at 37°C. After a wash cycle, conjugated anti-human IgG antibody is added to the particles and this mixture is incubated at 37°C. Excess conjugate is removed in another wash cycle, and the particles are re-suspended in system fluid.

The system generates multiple images in order to identify and count the two unique analyte particles, as well as determine the amount of conjugate on each particle. A third particle, coated with goat anti-human IgG antibodies, is present in the reagent as a control to flag low concentrations of IgG in the sample as an assay verification step. The median fluorescent intensity (MFI) for each analyte is proportional to the concentration of conjugate bound to human IgG, which is proportional to the concentration of IgG antibodies bound to the corresponding particle region. The system uses the MFI from at least 50 particles of each region. The identity of the particles is determined by the unique signature of the particles.
Each analyte in the Aptiva Celiac Disease IgG Reagent is assigned a predefined lot specific Master Curve. The analyte specific Master Curve is stored on the reagent cartridge RFID label (Radio-frequency identification). Based on results obtained by running calibrators (supplied separately), the system creates instrument specific Working Curves. Working curves are used by the software to calculate Fluorescent Light Units (FLU) for each analyte from the MFI values obtained for each sample.

Based on the defined cut-off for each analyte, the test results are reported for each sample as "positive" or "negative" with test value in FLU for each assay, i.e., DGP IgG and tTG IgG.

V Substantial Equivalence Information:

A Predicate Device Name(s):

- QUANTA Flash DGP IgA, QUANTA Flash DGP IgG, QUANTA Flash DGP IgA Calibrators, QUANTA Flash DGP IgG Calibrators
- QUANTA Flash h-tTG, QUANTA Flash h-tTG IgG Calibrators, QUANTA Flash h-tTG IgG Controls

B Predicate 510(k) Number(s):

K113863
K101644

C Comparison with Predicate(s):

Comparison with DGP IgG:

Device & Predicate Device(s):	K200230 Device	K113863 Predicate
Device Trade Name	Aptiva Celiac Disease IgG Reagent (DGP IgG)	QUANTA Flash DGP IgG
General Device Characteristic Similarities		
Assay Methodology	Solid phase (heterogenous) immunoassay	Same
Sample Matrix	Serum	Same
Solid Phase	Paramagnetic particles	Same
General Device Characteristic Differences		
Intended Use / Indications for Use	The Aptiva Celiac Disease IgG Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-tissue transglutaminase IgG autoantibodies and anti-deamidated gliadin peptide IgG autoantibodies in human serum. The presence of these antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of celiac disease and dermatitis herpetiformis, particularly in patients with selective IgA deficiency. The Aptiva Celiac Disease IgG Reagent is intended for use with the Inova Diagnostics Aptiva System.	The QUANTA Flash DGP IgG is a chemiluminescent immunoassay for the semi-quantitative detection of IgG antibodies to synthetic, deamidated gliadin peptides in human serum. The presence of IgG deamidated gliadin peptide antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of celiac disease in both IgA sufficient and IgA deficient subjects, as well as dermatitis herpetiformis.
Assay Technology	Fluorescent immunoassay	Chemiluminescent immunoassay
Antigen	DGP and recombinant tTG	DGP
Detection Antibody (Conjugate)	Phycoerythrin conjugated polyclonal goat anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent Light Units (FLU)	Chemiluminescent Units (CU)
Cut-off	5 FLU	20 CU
Analytical Measuring Range	DGP IgG: 0.56–250.00 FLU	DGP IgG: 2.8–1936.7 CU

Controls	2 Controls (DGP IgG) with lot specific values assigned: <u>Control 1:</u> 6.72 FLU <u>Control 2:</u> 14.12 FLU	2 Controls (DGP IgG) with lot specific values assigned. <u>Negative Control:</u> 10.9 CU (6.5–15.3) <u>Positive Control:</u> 52.4 CU (31.4–73.4)
Calibration	Lot Specific Master Curve + 3 Calibrators (sold separately)	Lot Specific Master Curve + 2 Calibrators (sold separately)

<u>Comparison with tTG IgG:</u>

Device & Predicate Device(s):	K200230 Device	K101644 Predicate
Device Trade Name	Aptiva Celiac Disease IgG Reagent (tTG IgG)	QUANTA Flash h-tTG IgG
General Device Characteristic Similarities		
Assay Methodology	Solid phase (heterogenous) immunoassay	Same
Antigen	Recombinant tissue transglutaminase	Same
Sample Matrix	Serum	Same
Solid Phase	Paramagnetic particles	Same
General Device Characteristic Differences		
Intended Use/ Indications for Use	The Aptiva Celiac Disease IgG Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-tissue transglutaminase IgG autoantibodies and anti-deamidated gliadin peptide IgG autoantibodies in human serum. The presence of these antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of celiac disease and dermatitis herpetiformis, particularly in patients with selective IgA deficiency. The Aptiva Celiac Disease IgG Reagent is intended for use with the Inova Diagnostics Aptiva System.	The QUANTA Flash™ h-tTG IgG is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgG anti-human tissue transglutaminase (h-tTG) antibodies in human serum. The presence of IgG anti-h-tTG antibodies, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of the gluten sensitive enteropathy celiac disease, particularly in celiac patients with selective IgA deficiency.
Assay Technology	Fluorescent immunoassay	Chemiluminescent immunoassay

Detection Antibody (Conjugate)	Phycoerythrin conjugated polyclonal goat anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent Light Units (FLU)	Chemiluminescent Units (CU)
Cut-off	5 FLU	20 CU
Analytical Measuring Range	tTG IgG: 0.82–250.00 FLU	tTG IgG: 3.75–2560.0 CU
Controls	2 Controls (tTG IgG) with lot specific values assigned: <u>Control 1:</u> 8.18 FLU <u>Control 2:</u> 16.39 FLU	2 Controls (h-tTG IgG) with lot specific values assigned: <u>Negative Control:</u> 11.4 CU (6.8–16.0) <u>Positive Control:</u> 50.4 CU (30.2–70.6)
Calibration	Lot Specific Curve + 3 Calibrators (sold separately)	Lot Specific Curve + 2 Calibrators (sold separately)

VI Standards/Guidance Documents Referenced:

- EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, Third Edition
- EP06-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline, Second Edition
- EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition
- EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition
- EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Addition

VII Performance Characteristics (if/when applicable):

A Analytical Performance: All results presented below met the manufacturer's pre-determined acceptance criteria

1. <u>Precision/Reproducibility:</u>

Within-Laboratory Precision:

The precision of the Aptiva Celiac Disease IgG reagent was evaluated in accordance with CLSI EP05-A3 using eight samples for DGP IgG and tTG IgG. Samples containing various concentrations of antibodies were run in duplicate, twice a day, for 20 days (total of 80 replicates per sample). The standard deviation (SD) and %CV of the within-run (repeatability), between-run, between-day and total within-laboratory precision were calculated for each sample and the results are summarized in the following tables:

Aptiva DGP IgG Precision		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**Mean (FLU)**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**
1	1.73	0.10	6.1%	0.07	4.0%	0.09	5.1%	0.15	8.9%
2	4.29	0.17	3.9%	0.07	1.6%	0.19	4.5%	0.27	6.2%
3	4.47	0.17	3.7%	0.19	4.3%	0.14	3.1%	0.29	6.5%
4	5.42	0.23	4.3%	0.30	5.5%	0.22	4.0%	0.44	8.1%
5	16.17	0.38	2.3%	0.48	2.9%	0.81	5.0%	1.01	6.3%
6	31.27	0.81	2.6%	1.00	3.2%	0.90	2.9%	1.57	5.0%
7	131.92	3.25	2.5%	6.32	4.8%	0.94	0.7%	7.16	5.4%
8	216.46	9.76	4.5%	7.11	3.3%	12.23	5.6%	17.19	7.9%

Aptiva tTG IgG Precision		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**Mean (FLU)**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**
1	1.88	0.13	7.1%	0.00	7.1%	0.14	3.0%	0.14	7.7%
2	2.43	0.15	6.2%	0.04	1.7%	0.12	5.1%	0.20	8.2%
3	4.53	0.23	5.1%	0.05	1.2%	0.18	3.9%	0.30	6.5%
4	5.29	0.27	5.1%	0.00	0.0%	0.15	2.8%	0.31	5.8%
5	11.49	0.42	3.7%	0.12	1.0%	0.44	3.8%	0.62	5.4%
6	46.27	1.40	3.0%	1.04	2.3%	1.02	2.2%	2.02	4.4%
7	117.59	6.73	5.7%	2.14	1.8%	2.51	2.1%	7.49	6.4%
8	210.59	14.77	7.0%	0.00	0.0%	9.17	4.4%	17.38	8.3%

Lot to Lot Imprecision:

To evaluate lot-to-lot variability, six serum samples for DGP IgG and seven serum samples for tTG IgG were tested according to CLSI EP05-A3 using three different lots. Samples were run in replicates of five, once a day, for five days, to generate 25 data points per sample for each lot (75 data points total for each sample). The results are summarized in the tables below:

Aptiva DGP IgG		Repeatability		Between-Day		Within-Lot		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	2.95	0.13	4.4%	0.30	10.3%	0.33	11.2%	0.00	0.0%	0.33	11.2%
2	5.79	0.25	4.3%	0.45	7.7%	0.51	8.9%	0.40	7.0%	0.65	11.3%
3	8.88	0.41	4.6%	0.80	9.0%	0.89	10.0%	0.26	2.9%	0.93	10.5%
4	24.09	0.80	3.3%	1.36	5.7%	1.58	6.6%	0.00	0.0%	1.58	6.6%
5	93.99	2.60	2.8%	5.68	6.0%	6.25	6.7%	5.13	5.5%	8.09	8.6%
6	122.73	5.36	4.4%	5.23	4.3%	7.49	6.1%	10.70	8.7%	13.06	10.6%

Aptiva tTG IgG		Repeatability		Between-Day		Within-Lot		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	1.87	0.12	6.2%	0.06	3.0%	0.13	6.9%	0.03	1.8%	0.13	7.1%
2	5.17	0.21	4.1%	0.15	2.9%	0.26	5.0%	0.38	7.4%	0.46	8.9%
3	5.68	0.22	3.8%	0.18	3.2%	0.28	5.0%	0.33	5.9%	0.44	7.7%
4	11.01	0.40	3.6%	0.61	5.5%	0.73	6.6%	0.41	3.7%	0.83	7.6%
5	25.83	1.12	4.3%	0.99	3.8%	1.49	5.8%	1.42	5.5%	2.05	8.0%
6	73.07	3.51	4.8%	6.18	8.5%	7.11	9.7%	0.00	0.0%	7.11	9.7%
7	170.49	11.27	6.6%	6.87	4.0%	13.20	7.7%	4.66	2.7%	14.00	8.2%

Site-to-Site Reproducibility:

Seven serum samples for DGP IgG and tTG IgG were tested according to CLSI EP05-A3 at three different sites using the same lot of Aptiva Celiac Disease IgG reagent. Samples were run in replicates of five, once a day, for five days, to generate 25 data points per sample at each site (75 data points per sample for all sites combined). The results are summarized in the following tables:

Aptiva DGP IgG		Repeatability		Between-Day		Within-Site		Between-Site		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	2.26	0.25	11.2%	0.00	0.0%	0.25	11.2%	0.14	6.2%	0.29	12.8%
2	5.41	0.27	5.0%	0.32	5.9%	0.42	7.7%	0.27	5.1%	0.50	9.2%
3	6.42	0.35	5.4%	0.38	5.9%	0.51	8.0%	0.24	3.8%	0.57	8.9%
4	12.40	0.57	4.6%	0.86	7.0%	1.04	8.4%	0.00	0.0%	1.04	8.4%
5	44.17	1.40	3.2%	2.34	5.3%	2.73	6.2%	2.16	4.9%	3.48	7.9%
6	119.06	6.15	5.2%	5.38	4.5%	8.17	6.9%	6.72	5.6%	10.58	8.9%
7	160.22	10.82	6.8%	4.54	2.8%	11.74	7.3%	9.78	6.1%	15.28	9.5%

Aptiva tTG IgG		Repeatability		Between-Day		Within-Site		Between-Site		Reproducibility	
Sample	**Mean (FLU)**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**	**SD (FLU)**	**CV**
1	2.33	0.12	5.0%	0.11	4.5%	0.16	6.7%	0.14	6.2%	0.21	9.1%
2	5.33	0.25	4.5%	0.19	3.4%	0.31	5.7%	0.32	5.8%	0.45	8.1%
3	6.03	0.25	4.1%	0.18	3.0%	0.30	5.0%	0.34	5.6%	0.45	7.5%
4	12.12	0.38	3.1%	0.23	1.9%	0.44	3.7%	0.45	3.7%	0.63	5.2%
5	30.43	1.35	4.4%	0.55	1.8%	1.46	4.8%	1.12	3.7%	1.84	6.0%
6	96.08	5.94	6.2%	5.19	5.4%	7.88	8.2%	4.31	4.5%	8.98	9.4%
7	192.87	13.07	6.8%	4.58	2.4%	13.85	7.2%	8.86	4.6%	16.44	8.5%

2. Linearity:

The linearity across the analytical measuring range (AMR) of each analyte was evaluated in accordance with CLSI EP6-A. The study was done by using four human serum samples with various anti-DGP and anti-tTG antibody concentrations which were combined with another human serum sample containing low levels of antibodies in 10% increments (from 0% to 90% of low sample) to obtain values that cover the entire AMR. Each dilution was tested in duplicate. Percentage recovery of obtained mean results was calculated compared to the expected results. Results were analyzed according to the guideline performing regression analysis and identifying the best fitting polynomial.

The linear regression analysis was performed using the samples falling within the master curve and the results of samples within AMR are summarized in the following tables:

DGP IgG Sample	Test Range (FLU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	54.85–274.25	1.04 (1.00–0.09)	-8.73 (-16.69– -0.77)	0.99	93.4–103.4%
2	7.13–71.33	1.04 (1.02–1.06)	-2.29 (-3.27– -1.31)	1.00	80.0–101.5%
3	4.82–48.20	1.04 (1.00–1.08)	-0.16 (-1.26–0.94)	0.99	86.7–106.4%
4	0.52–5.19	1.02 (0.99–1.05)	-0.15 (-0.24– -0.05)	1.00	86.5–100.3%
Combined	0.52–274.25	1.00 (0.99–1.01)	-0.50 (-1.49–0.50)	1.00	80.0–106.4%

For the anti-DGP IgG assay, the data support linearity for the claimed AMR from 0.56 FLU to 250.00 FLU.

tTG IgG Sample	Test Range (FLU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	32.78–327.80	0.93 (0.88–0.99)	13.20 (1.59–24.82)	0.99	93.7–113.2%
2	7.48–74.77	1.00 (0.96–1.05)	1.40 (-0.72–3.53)	0.99	100–113.2%
3	0.99–9.91	1.03 (1.00–1.06)	-0.22 (-0.42– -0.02)	1.00	80.2–102.4%
Combined	0.99–327.80	0.98 (0.96–1.00)	2.32 (-0.08–4.73)	0.99	80.2–113.2%

For the anti-tTG IgG assay, the data support linearity for the claimed AMR from 0.82 FLU to 250.00 FLU

Auto-rerun:

To validate the auto-rerun function with 1:10 dilutions, two positive specimens with anti-DGP IgG (599.54 and 1085.45 FLU) and two positive specimens with anti-tTG IgG (1266.11 and 1705.55 FLU) concentrations well above the assay measuring range were run with the auto-rerun function enabled on the Aptiva instrument. The same set of samples were manually diluted 1:10, tested and used as reference values. Comparing the values obtained by the auto-rerun to the manual dilution, the recovery values ranged from 91.4% to 94.1% for DGP IgG, and 89.4% to 99.9% for tTG IgG.

Hook Effect:

The possibility of hook effect for Aptiva Celiac Disease IgG Reagent on the Aptiva System was evaluated with testing three anti-DGP IgG high positive samples (378.94, 379.85 and 723.10 FLU) and three high positive samples with anti-tTG IgG (264.21, 405.53 and 465.52 FLU). No antigen excess hook effect was observed up to 723.10 FLU for DGP IgG and up to 465.52 FLU for tTG IgG.

3. Analytical Specificity/Interference:

An interference study was performed according to CLSI EP07-A2 using a set of three human serum specimens for DGP IgG: one positive (12.64 FLU), one near the cut-off (5.31 FLU) and one negative (1.14 FLU), and three serum specimens for tTG IgG: one positive (46.65 FLU); one near the cut-off (5.22 FLU); and one negative (2.29 FLU). Each sample was tested in triplicate with the spiked interfering substances in the table below. The percent (%) recovery for the sample spiked with the potential interfering substance was calculated by comparing its result to that of the corresponding control sample without the interfering substance. For both assays, no significant interference (defined as % recovery between 90–110%) was observed in: 1 mg/mL for bilirubin, 2 mg/mL for hemoglobin, 1000 mg/dL for triglycerides, 332.5 mg/dL for cholesterol, and 250 IU/mL for rheumatoid factor (IgM). In the DGP assay, human IgG at a concentration of 35 mg/mL showed % recovery of 114.1% in the sample around the cut-off.

4. Assay Reportable Range:

   DGP IgG assay: 0.56 FLU–250.00 FLU
   tTG IgG assay: 0.82 FLU–250.00 FLU

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability:

   There is no international reference material for anti-DGP or anti-tTG IgG antibodies that allows for the standardization of anti-DGP or anti-tTG IgG antibodies detection assays. Calibrator and control values are directly traceable to in-house standards that are used to create the master curves for the Aptiva Celiac Disease IgG Reagent.

   Stability:

   Kit stability (unopened): An accelerated stability study the Aptiva Celiac Disease IgG Reagent was performed using three lots stored at 37°C±3°C for 5 weeks where one week is equal to 6 months at 5±3°C. Seven samples were run in duplicate for DGP IgG, and 12 samples run in duplicate for tTG IgG. The accelerated stability study supports a claim for a 24-month shelf-life.

   Real time: Real-time stability study was performed at 3–6-month intervals on the Aptiva Celiac Disease IgG reagents stored at 2–8°C. At each timepoint, a low positive sample, a moderately high sample and a high positive patient sample (DGP IgG: 5.83, 12.58 and 47.14 FLU; and tTG IgG: 8.09, 15.03 and 51.23 FLU) were tested at time zero, 7 months, 12 months, 20 months, 24 months, and 25 months. The two lots support a 24-month stability claim.

   On-board (In-use) stability: An on-board stability study was performed for the reagent cartridge. One lot of the reagent cartridge was tested using a set of human serum samples: seven DGP IgG; 12 tTG IgG. In addition, 14 samples of IgG Control bead were tested. The specimens were tested periodically for 31 days at each timepoint with a total of 12 timepoints for the DGP IgG, tTG IgG reagent cartridges and IgG Control beads. At day 15, the reagent cartridge was recalibrated. The results support an on-board stability claim of 28 days.

   Sample stability: Six samples ranging from 4.1 FLU to 103.7 FLU for DGP IgG and seven samples ranging from 2.7 FLU to 110.4 FLU for tTG IgG were tested. All samples were tested in duplicate for up to 21 days while stored at 2–8°C, up to 48 hours while stored at room temperature (RT), and after repeated freeze/thaw cycles up to six cycles. Results were compared to those obtained on control samples (time zero / zero cycles). The results support sample stability for 48 hours at RT, 14 days at 2–8°C, and up to five freeze/thaw cycles when samples are stored at or below -20°C.

6. Detection Limit:

The Limit of Blank (LoB) was determined by assaying eight blank samples in five replicates per sample over three days with two reagent lots. One hundred twenty data points were generated for each lot. LoB was calculated as the 95th percentile using the non-parametric method for each lot as the dataset showed non-normal distribution. The highest LoB was determined to be 0.02 FLU for both DGP IgG and tTG IgG.

The Limit of Detection (LoD) was determined by using four samples with low level of anti-DGP IgG and anti-tTG IgG antibodies. Each sample was tested in five replicates over three days with two reagent lots. LoD was calculated as the LoB+1.652 x SD of the replicates for the samples. The LoD was determined to be 0.15 FLU for DGP IgG and 0.13 FLU for tTG IgG.

The Limit of Quantitation (LoQ) was determined by assaying four low level samples of anti-DGP IgG and anti-tTG IgG with two reagent lots. Each sample was run in replicates of five, twice per day, for three days, with a total of 120 data points generated on each assay, on each reagent lot. The LoQ was defined to be the lowest concentration level that meets the within-laboratory imprecision of < 20% for each lot. The LoQ for each assay was determined as the highest LoQ across the two lots and set as the lower limit of the AMR. The claimed LoQ is 0.56 FLU for the DGP IgG assay and 0.82 FLU for the tTG IgG assay.

7. Assay Cut-Off:

The Aptiva Celiac Disease IgG Reagent cut-off was established by testing 11 celiac disease samples and 192 presumably negative autoimmune disease samples [30 infectious disease, 20 systemic sclerosis, 13 systemic lupus erythematosus, 15 Crohn's disease, 29 autoimmune thyroid disease, 30 primary biliary cholangitis, 40 rheumatoid arthritis, and 15 ulcerative colitis samples]. The assay cut-off was established as 5.00 FLU (109 MFI for DGP IgG and 175 MFI for tTG IgG) to ensure optimal differentiation between DGP IgG and tTG IgG positive and negative samples.

For both DGP IgG and tTG IgG, an interpretation of "positive" is for samples ≥ 5.00 FLU and an interpretation of "negative" is for samples with FLU < 5.00 FLU.

B Comparison Studies:

1. Method Comparison with Predicate Device:

All samples (n=515) from the clinical validation study (described in Section C.1. below) were tested on the Aptiva Celiac Disease IgG Reagent and on their predicates, the QUANTA Flash DGP IgG and tTG IgG assays. Among them, 218 samples have anti-DGP IgG levels within the AMR for DGP IgG of the Aptiva Celiac Disease IgG Reagent and the QUANTA Flash DGP IgG, and 265 samples have anti-tTG IgG levels within the AMR for tTG IgG of the Aptiva Celiac Disease IgG Reagent and the QUANTA Flash tTG IgG. The positive percent agreement (PPA), negative percent agreement (NPA) and total percent agreement (TPA) were calculated by comparing the Aptiva Celiac Disease IgG Reagent with the predicate devices on samples within the AMR. The results are summarized in the following tables for each analyte.

DGP IgG Method Comparison (N=218)		QUANTA Flash DGP IgG		
		Negative	**Positive**	**Total**
Aptiva DGP IgG	Negative	48	4	52
	Positive	25*	141	166
	Total	73	145	218
NPA: 65.8% (48/73) (95% CI: 54.3–75.6%) PPA: 97.2% (141/145) (95% CI: 93.1–98.9%) TPA: 86.7% [(48+141)/218] (95% CI: 81.5–90.6%)				

** 18 out of 25 samples tested as negative by the predicate Quanta Flash DGP IgG, but positive by the Aptiva DGP IgG were celiac disease samples; seven (7) of the discordant samples (28%) were within+25% of the Aptiva DGP assay cut-off.*

tTG IgG Method Comparison (N=265)		QUANTA Flash tTG IgG		
		Negative	**Positive**	**Total**
Aptiva tTG IgG	Negative	139	8	147
	Positive	27**	91	118
	Total	166	99	265
NPA: 83.7% (139/166) (95% CI: 77.4–88.6%) PPA: 91.9% (91/99) (95% CI: 84.9–95.8%) TPA: 86.8% [(139+91)/265] (95% CI: 82.2–90.3%)				

***Among 27 samples tested as negative by the predicate Quanta Flash tTG IgG, but positive by the Aptiva tTG IgG Assay, 24 were celiac disease samples (six of 27 were IgA deficient celiac disease samples) and three (3) were dermatitis herpetiformis samples); eleven (11) of the discordant samples (41%) were within +25% of the Aptiva tTg assay cut-off.*

2. Matrix Comparison:

Not Applicable

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity:

A cohort of 515 characterized samples (none of which were used to establish the reference range), were used to validate the clinical performance of the Aptiva Celiac Disease IgG Reagent. This validation set included 171 samples from celiac disease (CD) patients, 20 samples from patients with IgA deficient celiac disease, 34 samples from dermatitis herpetiformis (DH) patients, and 290 samples from patients with various types of

autoimmune and infectious diseases. The distribution of the cohort and the DGP and tTG positivity rate are shown in the Table below:

Patient Group	N	DGP IgG N Positive	DGP IgG % Positive	tTG IgG N Positive	tTG IgG % Positive
Celiac Disease	171	142	83.0%	100	58.5%
IgA Deficient Celiac Disease	20	15	75.0%	16	80.0%
Dermatitis Herpetiformis	34	24	70.6%	9	26.5%
Total	**225**	-	-	-	-
Rheumatoid Arthritis	69	4	5.8%	0	0.0%
Ulcerative Colitis	31	0	0.0%	0	0.0%
Crohn's Disease	31	0	0.0%	0	0.0%
Hepatitis C Virus	28	0	0.0%	0	0.0%
Hepatitis B Virus	25	0	0.0%	0	0.0%
Syphilis	21	1	4.8%	0	0.0%
Sjögren's Syndrome	20	0	0.0%	0	0.0%
Systemic Sclerosis	19	0	0.0%	0	0.0%
Autoimmune Gastritis	15	0	0.0%	0	0.0%
Human Immunodeficiency Virus	13	0	0.0%	0	0.0%
Systemic Lupus Erythematosus	12	0	0.0%	0	0.0%
Epstein-Barr Virus	6	1	16.7%	0	0.0%
Total Controls	**290**	**6**	**2.1%**	**0**	**0.0%**

Clinical sensitivity and specificity for the Aptiva DGP IgG and Aptiva tTG IgG in diagnosis of celiac disease (CD) were analyzed and results are shown in the following tables:

		Diagnosis		
		CD*	**Non-CD†**	**Total**
Aptiva DGP IgG	**Positive**	157	6	163
	Negative	34	284	318
	Total	191*	290	481
Sensitivity: 82.2% (157/191) (95% CI:76.2–87.0%) Specificity: 97.9% (284/290) (95% CI: 95.6–99.0%)				

***** *The 191 CD patient samples in the study above include 20 samples from CD patients with selective IgA deficiency: 15 out of 20 tested positive on DGP IgG for a sensitivity of 75% (53.1–88.1%).*

† *Non-CD (290 samples) does not include the dermatitis herpetiformis (DH) samples*

		Diagnosis		
		CD*	**Non-CD†**	**Total**
Aptiva tTG IgG	**Positive**	116	0	116
	Negative	75	290	365
	Total	191**	290	481
Sensitivity: 60.7% (116/191) 95% CI: 53.7–67.4% Specificity: 100% (290/290) 95% CI: 98.7–100%				

*** The 191 CD patient samples in the study above include 20 samples from CD patients with selective IgA deficiency: 16 out of 20 tested positive on tTG IgG for a sensitivity of 80.0% (58.4–91.9%).*

† Non-CD (290 samples) does not include the DH samples

Clinical sensitivity and specificity for the Aptiva DGP IgG and Aptiva tTG IgG in diagnosis of dermatitis herpetiformis (DH) were analyzed and results are shown in the following tables:

		Diagnosis		
		DH	**Non-DH**	**Total**
Aptiva DGP IgG	**Positive**	24	6	30
	Negative	10	284	294
	Total	34	290	324
Sensitivity:70.6% (24/34) 95% CI: 53.8–83.2% Specificity: 97.9% (284/290) 95% CI: 95.6–99.0%				

		Diagnosis		
		DH	**Non-DH**	**Total**
Aptiva tTG IgG	**Positive**	9	0	9
	Negative	25	290	315
	Total	34	290	324
Sensitivity: 26.5% (9/34) 95% CI: 14.6–43.1% Specificity: 100% (290/290) 95% CI: 98.7–100%				

D Clinical Cut-Off:

Same as assay cut-off

E Expected Values/Reference Range:

A panel of 120 apparently healthy blood donors (64 females/56 males, ages 17 to 57 years, with an average and median age of 32 years) were tested on the Aptiva Celiac Disease IgG Reagent.

For DGP IgG, with a cut-off of 5.00 FLU, the cohort ranged from 0.03 to 8.06 FLU; three samples were positive.

For tTG IgG, with a cut-off of 5.00 FLU, the cohort ranged from 0.05 to 3.76 FLU; no samples were positive.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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