510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K193604

B Applicant

Inova Diagnostics, Inc.

C Proprietary and Established Names

Aptiva Celiac Disease IgA Reagent

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MST	Class II	21 CFR 866.5750 - Radioallergosorbent (RAST) Immunological Test System	IM - Immunology
MVM	Class II	21 CFR 866.5660 - Multiple autoantibodies immunological test system	IM - Immunology
NSU	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device and new instrument

B Measurand:

Anti-deaminated gliadin peptide (DGP) IgA antibodies
Anti-human tissue transglutaminase (h-tTG) IgA 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 IgA Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-tissue transglutaminase IgA autoantibodies and anti-deamidated gliadin peptide IgA antibodies 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.

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

Aptiva System is an automated particle-based multi-analyte analyzer for in vitro diagnostic testing of clinical specimens. The system is based on digital capture of high-resolution images of the paramagnetic particles to determine the analytes in samples.

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 IgA Reagent is an immunoassay utilizing particle-based multi-analyte technology. The kit contains one Aptiva Celiac Disease IgA 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 IgA – PE labeled anti-human IgA antibody, containing buffer, protein stabilizers and preservative
- Rehydration Buffer – containing protein stabilizers and preservatives

The Aptiva Celiac Disease IgA Calibrators and Aptiva Celiac Disease IgA Controls are sold separately:

- The Aptiva Celiac Disease IgA 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 IgA 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 IgA Reagent contains two (2) different populations of particles; one particle coated with recombinant tissue transglutaminase antigen and one particle coated with synthetic deamidated gliadin peptide.

The Aptiva System dilutes the patient sample 1:46, 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 IgA 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.

Multiple images are generated by the system in order to identify and count the two (2) unique analyte particles, as well as determine the amount of conjugate on each particle. A third particle, coated with goat anti-human IgA antibodies, is present in the reagent as a control to flag low concentrations of IgA 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 IgA, which is proportional to the concentration of IgA 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 IgA Reagent is assigned a predefined lot specific Master Curve. The analyte specific Master Curve is stored on the reagent cartridge RFID label.

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 IgA and tTG IgA.

Instrument Description Information: The Aptiva instrument is a fully automated, random access analyzer. This platform is a closed system with continuous load and random-access capabilities that processes the samples, runs the reagent and reports results. It includes liquid handling hardware, optical module (OM), and integrated computer with proprietary software and touch screen user interface.

1. Instrument Name:

Inova Diagnostics Aptiva System

2. Specimen Identification:

   A barcode scanner is part of the instrument system and is used to identify specimens.

3. Specimen Sampling and Handling:

   The instrument system has automated specimen sampling and handling.

4. Calibration:

   In-house standards are used to create the master curves for the lot specific Aptiva Celiac Disease IgA Reagent. The lot specific master curve is stored in the reagent cartridge RFID label. The three calibrators (sold separately) produce an instrument specific working curve from the parameters of each master curve with a 21-day recalibration.

5. Quality Control:

   Each new lot of reagent cartridge must be calibrated prior to first time use. The software will not allow a new lot to be used until it is calibrated. Aptiva Celiac Disease IgA Controls (sold separately) are recommended to be run once every day that the reagent is used; however, users should also consider national/local regulatory requirements. Quality control (QC) rules allow the laboratory to define the acceptance criteria for any reagent control materials being run. To set QC acceptance criteria, the user must have Manager-level permissions.

   The user can configure days and time to automatically run the Initialize and Prime maintenance operation. It is recommended to set the instrument to prime daily, prior to the start of use. Results are automatically archived daily. It is recommended to set the daily archive at a time when the instrument will not be in use.

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 IgA

B Predicate 510(k) Number(s):

K113863, K094060

C Comparison with Predicate(s):

DGP IgA:

Device & Predicate Device(s):	K193604	K113863
Device Trade Name	Aptiva Celiac Disease IgA	QUANTA Flash DGP IgA
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 IgA Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-tissue transglutaminase IgA autoantibodies and anti-deamidated gliadin peptide IgA antibodies 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. The Aptiva Celiac IgA Reagent is intended for use with the Inova Diagnostics Aptiva System.	The QUANTA Flash DGP IgA is a chemiluminescent immunoassay (CIA) for the semi-quantitative determination of IgA anti-deamidated gliadin peptide (DGP) antibodies in human serum. The presence of IgA anti-DGP antibodies, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of the gluten sensitive enteropathies: celiac disease and dermatitis herpetiformis.
Assay Technology	Fluorescent immunoassay	Chemiluminescent immunoassay
Antigen	DGP and recombinant tTG	DGP
Detection Antibody (Conjugate)	Phycoerythrin conjugated polyclonal goat anti-human IgA antibody	Isoluminol conjugated monoclonal anti-human IgA antibody
Units	Fluorescent Light Units (FLU)	Chemiluminescent Units (CU)
Cut-off	5 FLU	20 CU
Analytical Measuring Range (AMR)	DGP IgA: 0.72 – 250 FLU tTG IgA: 1.02 – 600 FLU	DGP IgA: 5.2 – 2367.3 CU

Controls	2 Controls (DGP IgA) with lot specific values assigned: Control 1: 6.65 FLU (4.65–8.64) Control 2: 13.65 FLU (9.56–17.75)	2 Controls (DGP IgA) with lot specific values assigned. Negative Control: 9.7 CU (5.8– 13.6) Positive Control: 50.9 CU (30.5–71.3)
Calibration	Lot Specific Master Curve + 3 Calibrators (sold separately)	Lot Specific Master Curve + 2 Calibrators (sold separately)

tTG IgA

Device & Predicate Device(s):	K193604	K094060
Device Trade Name	Aptiva Celiac Disease IgA	QUANTA Flash h-tTG IgA
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 IgA Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-tissue transglutaminase IgA autoantibodies and anti-deamidated gliadin peptide IgA antibodies 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. The Aptiva Celiac IgA Reagent is intended for use with the Inova Diagnostics Aptiva System.	The QUANTA Flash h-tTG IgA is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgA anti-human tissue transglutaminase (h-tTG) antibodies in human serum. The presence of IgA anti-h-tTG antibodies, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of the gluten sensitive enteropathies celiac disease (CD) and dermatitis herpetiformis (DH).
Assay Technology	Fluorescent immunoassay	Chemiluminescent immunoassay
Antigen	DGP and recombinant tTG	Recombinant tTG

Detection Antibody (Conjugate)	Phycoerythrin conjugated polyclonal goat anti-human IgA antibody	Isoluminol conjugated monoclonal anti-human IgA antibody
Units	Fluorescent Light Units (FLU)	Chemiluminescent Units (CU)
Cut-off	5 FLU	20 CU
Analytical Measuring Range (AMR)	DGP IgA: 0.72 – 250 FLU tTG IgA: 1.02 – 600 FLU	tTG IgA: 1.9 – 4965.5 CU
Controls	2 Controls (tTG IgA) with lot specific values assigned: <u>Control 1:</u> 10.00 FLU <u>Control 2:</u> 50.00 FLU	2 Controls (h-tTG IgA) with lot specific values assigned: <u>Negative Control:</u> 10.2 CU (6.1–14.3) <u>Positive Control:</u> 62.5 CU (37.5–87.5)
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, Interference Testing in Clinical Chemistry, Approved Guideline, Third 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>

<u>Within-Laboratory Precision:</u>

The precision of the Aptiva Celiac Disease IgA reagent was evaluated in accordance with CLSI EP05-A3 using nine serum samples for DGP IgA and ten samples for tTG IgA. Samples contained various concentrations of antibodies were run in duplicates, 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 imprecision were calculated for each sample and the results are summarized in the following table.

Aptiva DGP IgA Precision		Repeatability		Between-Run		Between-Day		Within Laboratory	
Sample	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	2.02	0.08	3.8%	0.03	1.5%	0.08	3.9%	0.11	5.7%
2	3.75	0.09	2.3%	0.22	5.9%	0.20	5.2%	0.31	8.2%
3	4.53	0.16	3.4%	0.20	4.4%	0.20	4.4%	0.32	7.1%
4	5.41	0.23	4.2%	0.24	4.4%	0.20	3.7%	0.38	7.1%
5	6.53	0.15	2.3%	0.34	5.2%	0.36	5.5%	0.52	7.9%
6	12.42	0.34	2.7%	0.33	2.7%	0.77	6.2%	0.90	7.3%
7	34.03	0.98	2.9%	2.54	7.5%	1.77	5.2%	3.25	9.5%
8	153.51	3.70	2.4%	5.40	3.5%	7.75	5.0%	10.14	6.6%
9	203.78	4.31	2.1%	2.85	1.4%	6.21	3.0%	8.08	4.0%

Aptiva tTG IgA Precision		Repeatability		Between-Run		Between-Day		Within Laboratory	
Sample	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	1.94	0.07	3.8%	17.20	1.8%	0.02	1.2%	0.08	4.4%
2	4.12	0.24	5.8%	0.14	3.3%	0.00	0.0%	0.27	6.6%
3	5.55	0.27	5.0%	0.22	4.0%	0.28	5.1%	0.45	8.1%
4	6.74	0.33	4.9%	0.20	2.9%	0.32	4.7%	0.50	7.3%
5	17.72	0.83	4.7%	0.63	3.5%	0.44	2.5%	1.13	6.4%
6	81.80	2.51	3.1%	2.49	3.0%	2.14	2.6%	4.14	5.1%
7	165.45	7.34	4.4%	5.78	3.5%	8.49	5.1%	12.62	7.6%
8	274.22	13.55	4.9%	5.86	2.1%	7.17	2.6%	16.41	6.0%
9	398.64	17.28	4.3%	10.29	2.6%	11.73	2.9%	23.28	5.8%
10	491.04	25.97	5.3%	19.47	4.0%	14.88	3.0%	35.71	7.3%

Site-to-Site Reproducibility:

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

DGP IgA Reproducibility		Repeatability		Between-Day		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	1.75	0.08	4.3%	0.12	7.1%	0.06	3.2%	0.16	8.9%
2	4.39	0.14	3.2%	0.28	6.3%	0.09	2.1%	0.32	7.4%
3	5.30	0.15	2.8%	0.14	2.7%	0.00	0.0%	0.21	3.9%
4	6.43	0.19	3.0%	0.66	10.2%	0.20	3.1%	0.71	11.1%
5	38.10	1.05	2.7%	2.47	6.5%	1.83	4.8%	3.25	8.5%
6	91.85	2.58	2.8%	8.95	9.7%	0.00	0.0%	9.31	10.1%
7	167.90	3.01	1.8%	10.14	6.1%	11.55	6.9%	15.66	9.4%

tTG IgA Reproducibility		Repeatability		Between-Day		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	2.44	0.07	2.8%	0.23	9.4%	0.05	2.0%	0.24	10.0%
2	4.95	0.13	2.7%	0.22	4.5%	0.00	0.0%	0.26	5.3%
3	6.73	0.25	3.7%	0.53	7.9%	0.24	3.5%	0.63	9.4%
4	77.16	1.58	2.0%	1.50	1.9%	2.30	3.0%	3.17	4.1%
5	140.93	3.98	2.8%	2.77	2.0%	5.53	3.9%	7.36	5.2%
6	219.93	6.65	3.0%	10.00	4.5%	9.08	4.1%	15.05	6.8%

Lot-to-Lot Imprecision:

To evaluate lot-to-lot variability, six serum samples for DGP IgA and six serum samples for tTG IgA 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 for each sample). The results are summarized below.

Aptiva DGP IgA		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	2.48	0.09	3.8%	0.07	2.9%	0.12	4.7%	0.21	8.4%	0.24	9.7%
2	5.27	0.17	3.2%	0.19	3.6%	0.25	4.8%	0.46	8.7%	0.52	9.9%
3	5.45	0.16	3.0%	0.16	2.9%	0.23	4.2%	0.48	8.8%	0.53	9.8%
4	38.52	0.94	2.4%	0.54	1.4%	1.08	2.8%	2.34	6.1%	2.58	6.7%
5	100.94	2.19	2.2%	2.30	2.3%	3.17	3.1%	0.82	0.8%	3.28	3.2%
6	177.97	3.96	2.2%	6.35	3.6%	7.48	4.2%	2.12	1.2%	7.78	4.4%

Aptiva tTG IgA		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	2.26	0.10	4.2%	0.09	3.9%	0.13	5.8%	0.22	9.6%	0.25	11.2%
2	4.04	0.16	3.9%	0.11	2.7%	0.19	4.7%	0.28	6.9%	0.34	8.3%
3	5.20	0.14	2.8%	0.22	4.2%	0.26	5.0%	0.50	9.5%	0.56	10.8%
4	56.36	1.83	3.2%	1.93	3.4%	2.66	4.7%	0.20	0.3%	2.67	4.7%
5	164.52	5.31	3.2%	5.02	3.1%	7.30	4.4%	5.03	3.1%	8.87	5.4%
6	373.22	20.79	5.6%	16.24	4.3%	26.38	7.1%	36.02	9.7%	44.65	12.0%

2. Linearity:

The linearity across the analytical measuring range (AMR) of each analyte, i.e., 0.72 – 250.00 FLU for anti-DGP IgA and 1.02 – 600.00 FLU for anti-tTG IgA, 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 as follows:

DGP IgA Sample	Range (FLU)	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	59.39–296.96	0.98 (0.94–1.02)	10.65 (2.20–19.10)	0.99	99.1 – 116.7%
2	19.28–192.82	0.98 (0.96–1.00)	2.72 (0.44–5.00)	1.00	97.6 – 105.2%
3	3.27–29.02	1.00 (0.96–1.04)	0.61 (-0.12–1.34)	0.99	91.9 – 114.5%
4	0.48–4.79	0.94 (0.88–0.99)	0.11 (-0.05–0.26)	0.99	90.2 – 112.8%
Combined	0.48–296.96	1.02 (1.01–1.03)	0.61 (-0.69–1.92)	1.00	91.9 – 116.7

tTg IgA Sample	Range (FLU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	69.43–694.33	1.04 (1.00–1.08)	-17.18 (-34.97–0.60)	0.99	85.1 – 106.1%
2	10.28–102.79	1.01 (0.97–1.04)	1.12 (-1.06–3.30)	1.00	99.4 – 108.2%
3	1.98–19.80	0.94 (0.87–1.01)	-0.58 (-1.38–0.23)	0.98	80.7 – 100.0%
4	0.78–7.76	1.06 (0.98–1.14)	0.27 (-0.10–0.65)	0.98	100.0 – 118.8%
Combined	0.78–694.33	1.01 (1.00–1.02)	-1.13 (-3.58–1.32)	1.00	80.7 – 118.8%

The data support the linearity for the claimed AMR from 0.72 FLU to 250.00 FLU for the anti-DGP IgA assay and the claimed AMR from 1.02 FLU to 600.00 FLU for the anti-tTG IgA assay, both as part of the Aptiva Celiac Disease IgA Reagent.

Auto-rerun:
To validate the auto-rerun function with 1:10 dilutions, two positive specimens with anti-DGP IgA (2401.17 and 4910.42 FLU) and two positive specimens with anti-tTG IgA (267.21 and 562.56 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 93.8% to 97.7% for DGP IgA, 89.2% to 99.8% for tTG IgA.

Hook Effect
The possibility of hook effect for Aptiva Celiac Disease IgA Reagent on the Aptiva System was evaluated with testing four anti-DGP IgA high positive samples (404.25, 1026.68, 1180.36 and 1229.19 FLU) and seven high positive samples with anti-tTG IgA (690.85, 708.21, 793.38, 897.82, 1609.51, 1624.62 and 1746.20 FLU). No antigen excess hook effect was observed up to 1229.19 FLU for DGP IgA and up to 1746.20 FLU for tTG IgA.

3. Analytical Specificity/Interference:

An interference study was performed according to CLSI EP07-A2 using a set of three human serum specimens for DGP IgA and three serum specimens for tTG IgA: one positive – DGP IgA (98.8 FLU) and tTG IgA (40.42 FLU); one near the cutoff – DGP IgA (5.10 FLU) and tTG IgA (6.68 FLU); and one negative serum sample – DGP IgA (1.62 FLU) and tTG IgA (1.66 FLU) – each tested in triplicates with the spiked interfering substances in the table below. The % recovery for each sample spiked with the potential interfering substance was calculated by comparing its result to that of the corresponding control sample without the interfering substance. No significant interferences were observed in the study up to the interferant's levels tested as shown in the table below.

Potential Interfering Substance	No interference up to Concentration
Bilirubin, Conjugated	1 mg/mL
Hemoglobin	2 mg/mL
Triglycerides	1000 mg/dL
Cholesterol	332.5 mg/dL
Rheumatoid factor IgM	250 IU/mL
Human IgG	70 mg/mL

4. Assay Reportable Range:

   DGP IgA assay: 0.72 FLU – 250.00 FLU
   tTG IgA assay: 1.02 FLU – 600.00 FLU

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

   Traceability:
   There is no international reference material for anti-DGP or anti-tTG antibodies that allows for the standardization of anti-DGP or anti-tTG 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 IgA Reagent.

   Stability:
   Kit stability (unopened): An accelerated stability study the Aptiva Celiac Disease IgA Reagent was performed using three lots stored at 37°C for five weeks. Nine samples were run in duplicate for DGP IgA and seven samples run in duplicate for tTG IgA. The accelerated stability study supports a claim for a 24-month shelf-life.

   Real-time stability study was performed at 8-month intervals on the Aptiva Celiac Disease IgA reagents stored at 2–8°C. At each timepoint, a negative sample (Negative Control), a low positive sample (Positive Control) and a high positive patient sample (DGP IgA: 51.10 FLU and tTG IgA: 1113.90 FLU) were tested at time zero, 12 months, 20 months, 24 months and 25 months. The first lot supports the 24-month stability claim. The second and third lots are on-going.

   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 human serum samples: nine DGP IgA; 12 tTG IgA. In addition, 14 samples of IgA Control bead were also tested. The specimens were tested periodically for 45 days at each timepoints with a total of 15 timepoints for the DPP IgA, tTG IgA reagent cartridges and IgA Control beads. At day 21 the reagent cartridge was recalibrated. The results support an on-board stability claim of 42 days with a 21-day recalibration.

   Sample stability: Eight samples ranging from 2.20 FLU to 177.16 FLU for DGP IgA and six samples ranging from 3.35 FLU to 337.81 FLU for tTG IgA were tested. All samples were tested in duplicates 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 five 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 0.00 FLU DGP IgA and 0.01 FLU for tTG IgA.

The Limit of Detection (LoD) was determined by using four samples with low level of anti-DGP and anti-tTG 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 0.65 FLU for DGP IgA and 0.37 FLU for tTG IgA.

The Limit of Quantitation (LoQ) was determined by assaying four low level samples of anti-DGP IgA and anti-tTG IgA 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 greatest LoQ across the two lots and set as the lower limit of the AMR. The claimed LoQ is 0.72 FLU for the DGP IgA assay and 1.02 FLU for the tTG IgA assay.

7. Assay Cut-Off:

The Aptiva Celiac Disease IgA Reagent cut-off was established by testing 12 celiac disease samples and 200 presumably negative autoimmune disease samples [30 infectious disease, 20 systemic sclerosis, 20 systemic lupus erythematosus, 15 Crohn's disease, 30 autoimmune thyroid, 30 primary biliary cholangitis, 40 rheumatoid arthritis, and 15 ulcerative colitis samples]. The assay cut-off was established as 5.00 FLU (78 MFI for DGP IgA and 190 MFI for tTG IgA) to ensure optimal differentiation between DGP IgA and tTG IgA positive and negative samples.

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

8. Accuracy (Instrument):

Analytical and clinical studies results support the accuracy of the instrument.

9. Carry-Over:

Samples from celiac disease patients were used to study potential carry-over of the Aptiva Celiac Disease IgA assay. All samples were selected based on high positivity for either one of the markers (tTG, DGP) or for both. All samples were tested 10 times alternating with a reagent blank (no serum pipetted). Results confirmed that no reagent blank result is above the

lower limit of the AMRs for both DGP IgA and tTG IgA when tested after positive samples that are above the AMRs.

B Comparison Studies:

1. Method Comparison with Predicate Device:

All samples (n=495) from the clinical validation study (described in Section C.1. below) were tested on the Aptiva Celiac Disease IgA Reagent and on their predicates, the QUANTA Flash DGP IgA and tTG assays. Among them, 200 samples have anti-DGP IgA levels within the AMR for DGP IgA of the Aptiva Celiac Disease IgA Reagent and the QUANTA Flash DGP IgA, and 197 samples have anti-tTG IgA levels within the AMR for tTG IgA of the Aptiva Celiac Disease IgA Reagent and the QUANTA Flash tTG IgA. The method comparison analysis comparing the Aptiva Celiac Disease IgA Reagent with the predicate devices on samples within the AMR is summarized in the following tables for each analyte.

DGP IgA Method Comparison (N=200)		**QUANTA Flash DGP IgA**			**Percent Agreement (95% CI)**
		Negative	**Positive**	**Total**	
Aptiva DGP IgA	Negative	63	2	65	NPA: 96.9% (89.5–99.2%)
	Positive	20	115	135	PPA: 85.2% (78.2–90.2%)
	Total	83	117	200	TPA: 89.0% (83.9–92.6%)

NPA: Negative Percent Agreement; PPA: Positive Percent Agreement; TPA: Total Percent Agreement; CI: Confidence Interval

tTG IgA Method Comparison (N=197)		**QUANTA Flash tTG IgA**			**Percent Agreement (95% CI)**
		Negative	**Positive**	**Total**	
Aptiva tTG IgA	Negative	31	2	32	NPA: 96.9% (84.3–99.4%)
	Positive	1	163	165	PPA: 98.8% (95.7–99.7%)
	Total	32	164	197	TPA: 98.5% (95.6–99.5%)

NPA: Negative Percent Agreement; PPA: Positive Percent Agreement; TPA: Total Percent Agreement; CI: Confidence Interval

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

A cohort of 495 characterized samples, none of which were used for establishing the reference range, were used to validate the clinical performance of the Aptiva Celiac Disease IgA Reagent. This validation set included 171 samples from celiac disease patients, 34

samples from dermatitis herpetiformis 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 IgA N Positive	DGP IgA % Positive	tTG IgA N Positive	tTG IgA % Positive
Celiac Disease	171	101	59.1%	159	93.0%
Dermatitis Herpetiformis	34	22	64.7%	31	91.2%
Total	**205**	**-**	**-**	**-**	**-**
Rheumatoid Arthritis	69	1	1.4%	2	2.9%
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	0	0.0%	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**	**2**	**0.7%**	**2**	**0.7%**

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

		Diagnosis			Clinical Analysis
		CD	Non-CD	Total	
Aptiva DGP IgA	**Positive**	101	2	103	Sensitivity (95% CI): 59.1% (51.6–66.2%) Specificity (95% CI): 99.3% (97.5–99.8%)
	Negative	70	288	358	
	Total	171	290	461	

		Diagnosis			Clinical Analysis
		CD	Non-CD	Total	
Aptiva tTG IgA	**Positive**	159	2	161	Sensitivity (95% CI): 93.0% (88.1–95.9%) Specificity (95% CI): 99.3% (97.5–99.8%)
	Negative	12	288	300	
	Total	171	290	461	

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

		Diagnosis			Clinical Analysis
		DH	Non-DH	Total	
Aptiva DGP IgA	Positive	22	2	24	Sensitivity (95% CI): 64.7% (47.9–78.5%)
	Negative	12	288	291	
	Total	34	290	324	Specificity (95% CI): 99.3% (97.5–99.8%)

		Diagnosis			Clinical Analysis
		DH	Non-DH	Total	
Aptiva tTG IgA	Positive	31	2	33	Sensitivity (95% CI): 91.2% (77.0–97.0%)
	Negative	3	288	291	
	Total	34	291	324	Specificity (95% CI): 99.3% (97.5–99.8%)

2. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

Same as assay cut-off

E Expected Values/Reference Range:

A panel of 120 apparently healthy blood donors (70 females/50 males, ages 17 to 57 years, with an average and median age of 32 and 31 years, respectively) were tested on the Aptiva Celiac Disease IgA Reagent.

For DGP IgA, with a cut-off of 5.00 FLU, no samples were positive, with a mean concentration of 1.08 FLU, and values ranging from 0.22 to 4.86 FLU.

For tTG IgA, with a cut-off of 5.00 FLU, one sample (0.8%) was positive, with a mean concentration of 0.66 FLU, and values ranging from 0.06 to 11.88 FLU.

F Other Supportive Instrument Performance Characteristics Data

Not applicable

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.