August 26, 2021

Inova Diagnostics, Inc.
Andrea Seaman
Manager, Research and Development
9900 Old Grove Road
San Diego, California 92131

Re: K200230
Trade/Device Name: Aptiva Celiac Disease IgG Reagent
Regulation Number: 21 CFR 866.5660
Regulation Name: Multiple Autoantibodies Immunological Test System
Regulatory Class: Class II
Product Code: MVM, MST
Dated: January 27, 2020
Received: January 30, 2020

Dear Andrea Seaman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part

801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

For

Ying Mao
Chief
Division of Immunology
and Hematology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K200230

Device Name
Aptiva Celiac Disease IgG Reagents

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Table of Contents

This summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.
The assigned 510K number is: K200230

Administrative data

Submitter:	Inova Diagnostics, Inc 9900 Old Grove Road, San Diego, CA, 92131
Purpose of submission:	New device
Device in the submission:	Aptiva Celiac Disease IgG Reagent
Revision Date:	August 23, 2021
Scientific contact:	Andrea Seaman, Manager, Research and Development Inova Diagnostics, Inc. 9900 Old Grove Road, San Diego, CA, 92131 Phone: 858-586-9900 x1395 Fax: 858-863-0025 Email: aseaman@inovadx.com
Quality Systems contact:	Ronda Elliott, VP, Quality Systems and RA Inova Diagnostics, Inc 9900 Old Grove Road, San Diego, CA, 92131 Phone: 858-586-9900 x1381 Fax: 858-863-0025 Email: relliott@inovadx.com

Device name (kit):	Proprietary name:	Aptiva Celiac Disease IgG Reagent
	Common name:	anti-deamidated gliadin peptide antibody immunoassay, anti-tissue transglutaminase antibody immunoassay
	Classification name:	DGP IgG: Radioallergosorbent (RAST) immunological test system tTG IgG: Multiple autoantibodies immunological test system
Regulation Medical Specialty	Immunology	
Review Panel	Immunology	
Product Code	DGP IgG: MST	

	tTG IgG: MVM
Regulation Number	866.5750, 866.5660
Device Class	2

Predicate device

QUANTA Flash® DGP IgG, 510(k) number: k113863. Date declared: October 23, 2012.
QUANTA Flash® h-tTG IgG, 510(k) number: k101644. Date declared: March 23, 2011.

Device description

The Aptiva Celiac Disease IgG reagent utilizes particle based multi-analyte technology (PMAT) in a cartridge format. Each analyte (tissue transglutaminase [tTG] and deamidated gliadin peptide [DGP]) in the Aptiva Celiac Disease IgG reagent is a solid phase immunoassay utilizing fluorescent microparticles. This technology allows each of the two analytes, along with a human IgG capture antibody (IgG Control Microparticle), to be coated onto three uniquely recognizable paramagnetic microparticles, which are combined into one tube.

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.

The two analyte microparticles, along with the control microparticle, are stored in the reagent cartridge under conditions that preserve the proteins in their reactive states. When the assay cartridge is ready to be used for the first time, the reagent tube seals are pierced using the cartridge lid. The reagent cartridge is then loaded onto the Aptiva instrument, where the microparticles are automatically rehydrated using buffer located within the cartridge.

A patient's serum is diluted 1:23 with Aptiva system rinse by the instrument in a disposable cuvette. A small amount of the diluted sample is combined with assay buffer and the microparticle suspension in a second cuvette, and mixed (final serum dilution: 1:230). This reaction cuvette is incubated for 9 ½ minutes at 37°C. The cuvette is then exposed to a small magnet that holds the microparticles in place. The liquid is aspirated, and the microparticles are resuspended as system rinse is added to the cuvette and the magnet is removed. This wash cycle is repeated one more time. During the third wash, no system rinse is added after the aspiration step. After the third wash, phycoerythrin conjugated polyclonal anti-human IgG (known as PE Tracer IgG) is added to the microparticles in the cuvette, and mixed. Again, the cuvette is incubated for 9 ½ minutes at 37°C. Three wash steps, as described above, are performed on the microparticles. Following the wash steps, the microparticles are transferred to the optical module of the instrument, where a charge coupled device (CCD) camera takes multiple images in order to identify and count the three unique microparticle regions, as well as determine the amount of conjugate on the microparticles. The control microparticle, a third particle, coated with goat anti-human IgG, is included in the reagent in as a control to flag low concentrations of IgG the patient serum sample as an assay verification step. The median fluorescent intensity (MFI) is proportional to the amount of PE Tracer that is bound to the human IgG, which is proportional to the amount of IgG antibodies bound to the corresponding microparticle regions.

For quantitation, the DGP IgG and tTG IgG assays (together as part of the Aptiva Celiac Disease IgG Reagent) each utilizes a predefined lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge RFID tag. Every new lot of reagent cartridge must be calibrated before first use with the reagent specific calibrators. Based on the results obtained with the calibrators included in the Aptiva Celiac Disease IgG Calibrator kit (sold separately), an instrument specific Working Curve is created for each assay, which is used to calculate reported fluorescent light units (FLU) from the median fluorescent intensity (MFI) instrument signal obtained for each sample, on each of the two assays within the reagent.

Aptiva Celiac Disease IgG Calibrators and Aptiva Celiac Disease IgG Controls are sold separately.

The Aptiva Celiac Disease IgG Reagent kit contains the following materials:

One (1) Aptiva Celiac Disease IgG Reagent Cartridge, containing the following reagents for 200 determinations:

a. Aptiva Celiac Disease IgG microparticle containing 3 unique microparticle regions coated with recombinant tissue transglutaminase, deamidated gliadin peptide, or goat anti-human IgG antibody.
b. Assay buffer – colored pink, containing protein stabilizers and preservatives.
c. PE Tracer IgG – phycoerythrin (PE) labeled anti-human IgG antibody, containing buffer, protein stabilizers and preservative.
d. Rehydration Buffer - containing protein stabilizers and preservatives.

Intended use(s)

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.

Indications for use

Same as intended use.

Substantial equivalence

The Aptiva Celiac Disease IgG Reagent has the same intended use and assay principle as the predicate devices.

Comparison to predicate device

Aptiva Celiac Disease IgG Reagent – DGP IgG Assay

Similarities		
Item	**Aptiva Celiac Disease IgG Reagent (DGP IgG)**	**QUANTA Flash DGP IgG**
Intended 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 (CIA) for the semi-quantitative determination of IgG anti-deamidated gliadin peptide (DGP) antibodies in human serum. The presence of IgG anti-DGP antibodies, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of celiac disease in IgA sufficient and IgA deficient patients, as well as dermatitis herpetiformis.
Assay Methodology	solid phase (heterogeneous) immunoassay	solid phase (heterogeneous) immunoassay
Antigen	deamidated gliadin peptide	deamidated gliadin peptide
Sample Type	human serum	human serum
Solid Phase	paramagnetic microparticles	paramagnetic microparticles
Differences		
Item	**Aptiva Celiac Disease IgG Reagent (DGP IgG)**	**QUANTA Flash DGP IgG**
Detection/Operating Principle	fluorescent immunoassay	chemiluminescent immunoassay
Conjugate	phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	fluorescent light units (FLU)	chemiluminescent units (CU)
Cut-off	5.00 FLU	20.0 CU

Analytical Measuring Range	0.56 FLU – 250.00 FLU	2.8 CU – 1936.7 CU
Control	Controls have lot specific values assigned. Control 1 DGP IgG – 6.72 FLU Control 2 DGP IgG – 14.12 FLU (8.47 – 19.77)	Controls have lot specific values assigned. Negative Control DGP IgG – 10.9 CU (6.5 – 15.3) Positive Control DGP IgG – 52.4 CU (31.4 – 73.4)
Calibration	Lot specific Master Curve + 3 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Aptiva Celiac Disease IgG Reagent – tTG IgG Assay

Similarities		
Item	**Aptiva Celiac Disease IgG Reagent (tTG IgG)**	**QUANTA Flash tTG IgG**
Intended 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 patients with selective IgA deficiency.
Assay Methodology	solid phase (heterogeneous) immunoassay	solid phase (heterogeneous) immunoassay
Antigen	recombinant tissue transglutaminase	recombinant tissue transglutaminase
Sample Type	human serum	human serum
Solid Phase	paramagnetic microparticles	paramagnetic microparticles
Differences		

Item	Aptiva Celiac Disease IgG Reagent (tTG IgG)	QUANTA Flash tTG IgG
Detection/Operating Principle	fluorescent immunoassay	chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	fluorescent light units (FLU)	chemiluminescent units (CU)
Cut-off	5.00 FLU	20.0 CU
Analytical Measuring Range	0.82 FLU – 250.00 FLU	3.75 CU – 2560.0 CU
Control	Controls have lot specific values assigned. Control 1 tTG IgA – 8.18 FLU Control 2 tTG IgA – 16.39 FLU (9.83 – 22.95)	Controls have lot specific values assigned. Negative Control h-tTG IgA – 11.4 CU (6.8 – 16.0) Positive Control h-tTG IgA – 50.4 CU (30.2 – 70.6)
Calibration	Lot specific Master Curve + 3 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Analytical performance characteristics

Quantitation and units of measure

For quantitation, the Aptiva Celiac Disease IgG reagent utilizes predefined lot specific Master Curves, one for tTG IgG and one for DGP IgG that is uploaded onto the instrument through the reagent cartridge RFID. The analyte specific Master Curves are generated at Inova for each reagent lot, where in-house Master Curve Standards with assigned FLU values are run multiple times. The resulting MFI values generated are used to create a unique 4 parameter logistic (4PL) curve for each of the two analytes. The IgG control bead is present in the reagent as a control to flag low concentration IgG in a patient serum sample as an assay verification step. This microparticle also has an in-house standard which is run each time a new reagent lot is manufactured. The MFI produced by this standard is used as the cut-off threshold for the IgG control microparticle for that reagent lot. These four parameters of the analyte curves, as well as the MFI cut-off for the IgG control microparticle are embedded in the reagent cartridge RFID.

List of Aptiva Celiac Disease IgG Master Curve Standards – DGP IgG:

Material	Assigned Value (FLU)
Celiac IgG Master Curve Standard 1	0.00
Celiac IgG Master Curve Standard 2	3.81
Celiac IgG Master Curve Standard 3	8.97
Celiac IgG Master Curve Standard 4	47.37
Celiac IgG Master Curve Standard 5	250.07

List of Aptiva Celiac Disease IgG Master Curve Standards – tTG IgG

Material	Assigned Value (FLU)
Celiac IgG Master Curve Standard 1	0.00
Celiac IgG Master Curve Standard 2	2.62
Celiac IgG Master Curve Standard 3	31.14
Celiac IgG Master Curve Standard 4	73.23
Celiac IgG Master Curve Standard 5	386.85

Precision

The precision of the Aptiva Celiac Disease IgG reagent was evaluated on 8 samples for DGP IgG and tTG IgG, containing various concentrations of antibodies in accordance with CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline. Samples were run in duplicates, twice a day, for 20 days.

Data were analyzed with the Analyse-it for Excel method evaluation software, and repeatability (within-run), between run, between day and within-laboratory precision (total precision) were calculated. Results are summarized in the two tables below.

Acceptance criteria: Total %CV: < 12% or SD < 0.6 FLU

DGP IgG Precision			Repeatability		Between Run		Between Day		Within Laboratory	
Sample	Replicates (N)	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	80	1.73	0.1	6.1%	0.07	4.0%	0.09	5.1%	0.15	8.9%
2	80	4.29	0.17	3.9%	0.07	1.6%	0.19	4.5%	0.27	6.2%
3	80	4.47	0.17	3.7%	0.19	4.3%	0.14	3.1%	0.29	6.5%
4	80	5.42	0.23	4.3%	0.30	5.5%	0.22	4.0%	0.44	8.1%
5	80	16.17	0.38	2.3%	0.48	2.9%	0.81	5.0%	1.01	6.3%
6	80	31.27	0.81	2.6%	1.00	3.2%	0.90	2.9%	1.57	5.0%
7	80	131.92	3.25	2.5%	6.32	4.8%	0.94	0.7%	7.16	5.4%
8	80	216.46	9.76	4.5%	7.11	3.3%	12.23	5.6%	17.19	7.9%

tTG IgG Precision			Repeatability		Between Run		Between Day		Within Laboratory	
Sample	Replicates (N)	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	80	1.88	0.13	7.1%	0.00	7.1%	0.14	3.0%	0.14	7.7%
2	80	2.43	0.15	6.2%	0.04	1.7%	0.12	5.1%	0.20	8.2%
3	80	4.53	0.23	5.1%	0.05	1.2%	0.18	3.9%	0.30	6.5%
4	80	5.29	0.27	5.1%	0.00	0.0%	0.15	2.8%	0.31	5.8%
5	80	11.49	0.42	3.7%	0.12	1.0%	0.44	3.8%	0.62	5.4%
6	80	46.27	1.40	3.0%	1.04	2.3%	1.02	2.2%	2.02	4.4%
7	80	117.59	6.73	5.7%	2.14	1.8%	2.51	2.1%	7.49	6.4%
8	80	210.59	14.77	7.0%	0.00	0.0%	9.17	4.4%	17.38	8.3%

Reproducibility Studies

Reproducibility between sites (instruments)

Seven samples for DGP IgG and tTG IgG were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, at three different sites. Samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per site. Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between site precision.
Acceptance criteria: Reproducibility Between-Site %CV: < 12% or SD < 0.6 FLU
Results are summarized in the tables below.

Aptiva DGP IgG			Repeatability		Between-Day		Within-Site		Between-Site/Instrument		Reproducibility	
Sample	N	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	75	2.26	0.25	11.2%	0.00	0.0%	0.25	11.2%	0.14	6.2%	0.29	12.8%
2	75	5.41	0.27	5.0%	0.32	5.9%	0.42	7.7%	0.27	5.1%	0.50	9.2%
3	75	6.42	0.35	5.4%	0.38	5.9%	0.51	8.0%	0.24	3.8%	0.57	8.9%
4	75	12.40	0.57	4.6%	0.86	7.0%	1.04	8.4%	0.00	0.0%	1.04	8.4%
5	75	44.17	1.40	3.2%	2.34	5.3%	2.73	6.2%	2.16	4.9%	3.48	7.9%
6	75	119.06	6.15	5.2%	5.38	4.5%	8.17	6.9%	6.72	5.6%	10.58	8.9%
7	75	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/Instrument		Reproducibility	
Sample	N	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	75	2.33	0.12	5.0%	0.11	4.5%	0.16	6.7%	0.14	6.2%	0.21	9.1%
2	75	5.33	0.25	4.5%	0.19	3.4%	0.31	5.7%	0.32	5.8%	0.45	8.1%
3	75	6.03	0.25	4.1%	0.18	3.0%	0.30	5.0%	0.34	5.6%	0.45	7.5%
4	75	12.12	0.38	3.1%	0.23	1.9%	0.44	3.7%	0.45	3.7%	0.63	5.2%
5	75	30.43	1.35	4.4%	0.55	1.8%	1.46	4.8%	1.12	3.7%	1.84	6.0%
6	75	96.08	5.94	6.2%	5.19	5.4%	7.88	8.2%	4.31	4.5%	8.98	9.4%
7	75	192.87	13.07	6.8%	4.58	2.4%	13.85	7.2%	8.86	4.6%	16.44	8.5%

Reproducibility between lots

Six samples for DGP IgG and seven samples for tTG IgG were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline, using three different lots. Samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per lot, 75 data points total for each sample. Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between lot precision.
Acceptance criteria: Reproducibility Between-Lot %CV: < 12% or SD < 0.6 FLU

Results are summarized in the tables below.

Aptiva DGP IgG			Repeatability		Between-Day		Within-Lot		Between-Lot		Reproducibility	
Sample	N	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	75	2.95	0.13	4.4%	0.30	10.3%	0.33	11.2%	0.00	0.0%	0.33	11.2%
2	75	5.79	0.25	4.3%	0.45	7.7%	0.51	8.9%	0.40	7.0%	0.65	11.3%
3	75	8.88	0.41	4.6%	0.80	9.0%	0.89	10.0%	0.26	2.9%	0.93	10.5%
4	75	24.09	0.80	3.3%	1.36	5.7%	1.58	6.6%	0.00	0.0%	1.58	6.6%
5	75	93.99	2.60	2.8%	5.68	6.0%	6.25	6.7%	5.13	5.5%	8.09	8.6%
6	75	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		Reproducibility	
Sample	N	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	75	1.87	0.12	6.2%	0.06	3.0%	0.13	6.9%	0.03	1.8%	0.13	7.1%
2	75	5.17	0.21	4.1%	0.15	2.9%	0.26	5.0%	0.38	7.4%	0.46	8.9%
3	75	5.68	0.22	3.8%	0.18	3.2%	0.28	5.0%	0.33	5.9%	0.44	7.7%
4	75	11.01	0.40	3.6%	0.61	5.5%	0.73	6.6%	0.41	3.7%	0.83	7.6%
5	75	25.83	1.12	4.3%	0.99	3.8%	1.49	5.8%	1.42	5.5%	2.05	8.0%
6	75	73.07	3.51	4.8%	6.18	8.5%	7.11	9.7%	0.00	0.0%	7.11	9.7%
7	75	170.49	11.27	6.6%	6.87	4.0%	13.20	7.7%	4.66	2.7%	14.00	8.2%

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ)

The LoB, LoD, and LoQ of the DGP IgG and tTG IgG assays in the Aptiva Celiac Disease IgG Reagent were calculated separately by a study according to CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline- Second Edition.

Study protocol for LoB:
Eight blank samples (Aptiva System Rinse) from two different lots were run in replicates of five on two reagent lots, once per day, for 3 days, with 120 data points generated on each lot. The LoB was determined for each assay, on each reagent lot separately with the Analyse-it for Excel software's Reference Interval function, at the 95th percentile, using the non-parametric method for all 4 analyses (DGP IgG on two reagent lots, and tTG IgG on two reagent lots; all having a p-value = <0.0001)

The DGP IgG LoB for one reagent lot was determined as 0.01 FLU (41 MFI), and for the second reagent lot as 0.02 FLU (60 MFI). The final LoB value for DGP IgG is 0.02 FLU.

The tTG IgG LoB for one reagent lot was determined as 0.00 FLU (37 MFI), and for the second reagent lot as 0.02 FLU (34 MFI). The final LoB value for tTG IgG is 0.02 FLU.

Study protocol for LoD:
Four low level samples for each DGP IgG and tTG IgG assay (prepared by mixing human serum samples with high and low levels of antibodies) were run in replicates of five on two reagent lots, twice per day, for 3 days, with 120 data points generated on each assay, on each reagent lot. The LoD was determined separately for each assay, on each reagent lot.

The DGP IgG limit of detection for one reagent lot was determined as 0.15 FLU, and for the second reagent lot as 0.13 FLU. The final LoD value is 0.15 FLU.

The tTG IgG limit of detection for one reagent lot was determined as 0.13 FLU, and for the second reagent lot as 0.12 FLU. The final LoD value is 0.13 FLU.

Study protocol for LoQ:
Four low level samples for each DGP IgG and tTG IgG assay (prepared by mixing human serum samples with high and low levels of antibodies) were run in replicates of five on two reagent lots, twice per day, for 3 days, with 120 data points generated on each assay, on each reagent lot. The LoQ was determined separately for each assay, on each reagent lot. The LoQ was determined in each case by calculating the total imprecision of each sample (acceptance criteria: total imprecision <20%).

The DGP IgG limit of quantitation for one reagent lot was determined as 0.53 FLU, and for the second reagent lot as 0.56 FLU. The final LoQ value is 0.56 FLU, which has been set as the lower limit of the analytical measuring range of the DGP IgG assay.

The tTG IgG limit of quantitation for one reagent lot was determined as 0.80 FLU, and for the second reagent lot as 0.82 FLU. The final LoQ value is 0.82 FLU, which has been set as the lower limit of the analytical measuring range of the tTG IgG assay.

Analytical Measuring Range (AMR)

Within the Aptiva Celiac Disease IgG Reagent:

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

Auto-rerun function and reportable results

The Aptiva software has an auto-rerun option available. If this option is selected, the instrument will automatically rerun any sample that has a result >250.00 FLU for DGP IgG or tTG IgG after performing an additional 10-fold dilution, thereby bringing the measured value within the AMR. The reported result will be calculated by the software factoring the additional dilution. As the highest value that can be measured is 2500.00 FLU for DGP IgG or tTG IgG.

High concentration hook effect

To assess hook effect, 3 samples for DGP IgG and 3 samples for tTG IgG were tested at two increasing 2-fold serial dilutions from the standard 1:23 dilution used by the Aptiva Celiac Disease IgG Reagent. All FLU values above the analytical measuring ranges of the two assays are theoretical and were mathematically calculated using the 4 parameters of their respective calibration curves. All samples showed increase in FLU values as dilution factor became more concentrated, thereby confirming that high positive specimens above the AMR do not show hook effect up to 723.10 FLU for the DGP IgG assay and 465.52 FLU for the tTG IgG assay (theoretical values calculated) in the Aptiva Celiac Disease IgG Reagent.

Linearity

The Linearity of the AMR was calculated separately for DGP IgG and tTG IgG as part of the Aptiva Celiac Disease IgG Reagent.

The linearity of the AMR of DGP IgG and tTG IgG was evaluated by a study according to CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Four human serum samples for DGP IgG and three human serum samples for tTG IgG with various antibody concentrations were serially diluted to obtain values that cover the entire AMR. The dilutions were assayed in duplicates. Results were analyzed according to the guideline performing regression analysis and identifying the best fitting polynomial.

Acceptance criteria:

- Best fitting polynomial is a linear one, otherwise, the difference between the best-fitting nonlinear and linear polynomial is less than 15% or ±0.75 FLU for low level samples (allowable nonlinearity).

For DGP IgG, the best fitting polynomial found for samples 1, 2 and 4 was a linear one, while third order polynomial was found to provide the best fit for sample 3. All acceptance criteria were fulfilled.

Sample	Test Range (FLU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	54.85 - 274.25	1.04 (1.00 to 1.09)	-8.73 (-16.69 to -0.77)	0.99	98.0%
2	7.13 - 71.33	1.04 (1.02 to 1.06)	-2.29 (-3.27 to -1.31)	1.00	95.1%
3	4.82 - 48.20	1.04 (1.00 to 1.08)	-0.16 (-1.26 to 0.94)	0.99	101.8%
4	0.52 - 5.19	1.02 (0.99 to 1.05)	-0.15 (-0.24 to -0.05)	1.00	95.4%
Combined	0.52 - 274.25	1.00 (0.99 to 1.01)	-0.50 (-1.49 to 0.50)	1.00	97.6%

For tTG IgG, the best fitting polynomial found for samples 1, 2 and 3 was a linear one. All acceptance criteria were fulfilled.

Sample	Test Range (FLU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	32.78 - 327.80	0.93 (0.88 to 0.99)	13.20 (1.59 to 24.82)	0.99	103.5%
2	7.48 - 74.77	1.00 (0.96 to 1.05)	1.40 (-0.72 to 3.53)	0.99	104.8%
3	0.99 - 9.91	1.03 (1.00 to 1.06)	-0.22 (-0.42 to -0.02)	1.00	96.3%
Combined	0.99 - 327.80	0.98 (0.96 to 1.00)	2.32 (-0.08 to 4.73)	0.99	101.6%

These data demonstrate the linearity of the analytical measuring range (0.56 FLU – 250.00 FLU) of the DGP IgG assay and the analytical measuring range 0.82 FLU – 250.00 FLU) of the tTG IgG assay, both as part of the Aptiva Celiac Disease IgG Reagent.

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. A set of three human serum specimens, one positive, one near the cutoff and one negative sample were tested using the following interfering substances (bilirubin, hemoglobin, triglycerides, cholesterol, rheumatoid factor IgM and human IgG). All interferents were spiked into every serum specimen and the resulting samples were assessed in triplicates with the Aptiva Celiac Disease IgG assays. Recovery of the unit values was calculated compared to control samples. Acceptance criteria for the interference studies were 85% - 115% recovery, or ± 20% of the cut-off (±1.0 FLU) difference, whichever is greater.

Less than 15% of interference was observed for DGP or tTG IgG with the following interferents: bilirubin up to 1 mg/mL (recovery: from 96.0%-101.3% and from 97.7% to 102.5% for DGP IgG and tTG IgG respectively), hemoglobin up to 2 mg/mL (recovery: from 97.6% to 104.2% and from 96.9% to 100.8% for DGP IgG and tTG IgG respectively), triglycerides up to 1000 mg/dL (recovery: from 93.8% to 102.5% and from 94.5% to 100.8% for DGP IgG and tTG IgG respectively), cholesterol up to 332.5 mg/dL (recovery: from 85.4% to 98.7% and from 89.2% to 100.4% for DGP IgG and tTG IgG respectively), RF IgM up to 250 IU/mL (recovery: 95.4% to 97.6 and 92.1% to 97.3% for DGP IgG and tTG IgG, respectively) and human IgG up to 35 mg/mL (recovery: from 105.0% to 114.1% or -0.80 FLU and from 101.7 to 110.8% or 0.47 FLU for DGP IgG and tTG IgG, respectively).

Sample Stability and Handling

For the DGP IgG assay, six test samples were tested and for tTG IgG assay seven test samples were tested. The samples used for this study were achieved by combining high and low antibody level to yield their desired reactivity. Test samples covered the analytical measuring ranges of each analyte. 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, and after repeated freeze/thaw cycles up to 6 cycles. Results were compared to those obtained on control samples (time zero / zero cycles).
Acceptance criteria: percent recovery is between 85-115% for positive samples, and between 80-120% for negative samples (<5.00 FLU).
All samples fulfilled the acceptance criteria at each time point for each condition. Based on these results, we recommend that samples may be stored up to 48 hours at room temperature, up to 14 days at 2-8°C and can be subjected to up to 5 freeze/thaw cycles.

Reagent Stability

Shelf life

To establish the initial claim for shelf life, accelerated stability studies were performed for 5 weeks at 37°C ± 3°C, where one week is equal to six months at 5 ± 3°C.

Accelerated stability testing was performed on each of the following sealed components to establish initial stability claim:

- Aptiva Celiac Disease IgG microparticle (bead) – 3 lots
- PE Tracer IgG – 3 lots

Each week a new sealed component was placed in the incubator, and all components were tested at the end of the experiment together with the one that was stored at 5 ± 3°C. The recovery of the measured values was calculated for each time point (compared to those obtained with 5 ± 3°C stored reagent). All calculations were performed by comparing results of sealed components stored at 5 ± 3°C (control) to those stored at 37 ± 3°C (test) for 1, 2, 3, 4, and 5 weeks, where one week is equal to six months at 5 ± 3°C. Linear regression analysis was performed between recovery values and the number of days. For each component tested, linear regression analysis was performed separately on each bead in the Aptiva Celiac Disease IgG Reagent (DGP IgG, tTG IgG, IgG Control Bead).

Acceptance criteria for two-year preliminary expiration dating: With regression analysis, the lower and upper 95% Cl interval of the regression line is between 80% and 120% recovery at day 28 (week 4).

All components tested fulfilled the acceptance criteria above, therefore, two-year expiration dating was assigned to each component.

In-use (onboard) stability

Reagent Cartridge

To establish the in-use stability of the Aptiva Celiac Disease IgG reagent cartridges, one lot of reagent cartridge was tested using up to 12 human serum samples (with different reactivity levels). The specimens were tested periodically for 31 days. At day 15 the reagent cartridge was recalibrated, and a cartridge specific Working Curve was generated. Percent recoveries were calculated compared to the day zero

average values, and linear regression analysis was performed by plotting percent recovery against the number of days. The claim was established using the following criteria (using the one that is fulfilled first):
- The stability claim is established at the actual measurement day proceeding the day when the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
- At the actual measurement day preceding the day when ≥2% of the recovery data, (3 data points) is ≤75% or ≥125% recovery.

The onboard stability results for the Aptiva Celiac Disease IgG is as follows:
Lot 100017: 31 days

Using these criteria, the in-use (onboard) stability of the Aptiva Celiac Disease IgG reagent cartridge was set at 28 days.

Real time stability

Real time stability testing has been scheduled to be performed every three or six months on the Aptiva Celiac Disease IgG Reagents kit, to verify the two-year expiration that was assigned based on accelerated stability studies.

A negative sample, a low positive sample, and a high positive sample will be tested at each time point.
- Acceptance criteria: results should fall within their respective ranges.

The data from two different lots of Aptiva Celiac Disease IgG assay is available up to 25 months of real time stability.

For lot 100015, the percent recovery for all samples tested for DGP IgG ranges from 88.0% to 108.0%, and for tTG IgG ranges from 100.5% to 107.8%.

For lot 100017, the percent recovery for all samples tested for DGP IgG ranges from 88.6% to 91.9%, and for tTG IgG ranges from 97.5% to 98.1%.

Cut-off, reference range

The following cut-off is used for both the DGP IgG and tTG IgG assays in the Aptiva Celiac Disease IgG Reagent:

Negative	<5.00 FLU
Positive	≥5.00 FLU

The reference population for establishing the reference interval for the DGP IgG and tTG IgG assays, within the Aptiva Celiac Disease IgG Reagent, consisted of 192 subjects:

Sample Group	N
Crohn's Disease	15
Autoimmune Thyroid Disease	29
Infectious Disease	30
Primary Biliary Cholangitis	30
Rheumatoid Arthritis	40

Systemic Lupus Erythematosus	13
Systemic Sclerosis	20
Ulcerative Colitis	15

Additionally, 11 diagnosed celiac disease (CD) patient specimens were assayed to aid in the determination of the cutoff values.

All specimens were the same matrix (human serum) as specified in the Intended Use. All specimens were unaltered. The cut-off values were established in accordance to CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. The Analyse-it for Excel software was used to make the calculations. The distribution of the results was non-normal (Shapiro-Wilk $p<0.0001$), therefore the non-parametric percentile method was used.

The cut-off was established based on greater than the 99th percentile of the results obtained on the reference subjects, along with the results of 11 samples from patients with celiac disease.

A cutoff of 5.00 FLU (109 MFI and 175 MFI for DGP IgG and tTG IgG, respectively) has been set to ensure optimal differentiation between negatives and positives samples.

Clinical performance characteristics

Clinical sensitivity, specificity

A cohort of characterized samples, none of which were used for establishing the reference range, was used to validate the clinical performance of the Aptiva Celiac Disease IgG Reagent. A total of 515 characterized samples were included in this Validation Set, including 171 samples from celiac disease patients, 20 samples from patients with IgA deficient celiac disease, 34 dermatitis herpetiformis patients and 290 control samples from patients with various types of autoimmune and infectious diseases. All samples were run on the Aptiva Celiac Disease IgG Reagent. The distribution of the cohort and the DGP and tTG positivity rate is in the Table below:

Patient Group	N	DGP IgG N Positive	DGP IgG % Positive	tTG IgG N Positive	tTG IgG % Positive
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%**
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	**515**	**-**	**-**	**-**	**-**

Clinical sensitivity and specificity for the Aptiva DGP IgG were analyzed in the table below:

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

* The study above includes 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 does not include the DH samples

Clinical sensitivity and specificity for the Aptiva tTG IgG were analyzed in the table below:

Clinical Analysis (N=481)		Diagnosis			Analysis
		CD**	Non-CD	Total	
Aptiva tTG IgG	Positive	116	0	116	Sensitivity: 60.7% (116/191) 95% CI: 53.7 – 67.4%
	Negative	75	290	365	Specificity: 100.0% (290/290) 95% CI: 98.7– 100.0%
	Total	191	290	481	

**The study above includes 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%).

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:

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

Clinical Analysis (N=324)		Diagnosis			Analysis
		DH	Non-DH	Total	
Aptiva tTG IgG	Positive	9	0	9	Sensitivity: 26.5% (9/34) 95% CI: 14.6 – 43.1%
	Negative	25	290	315	Specificity: 100.0% (290/290) 95% CI: 98.7– 100.0%
	Total	34	290	324	

Expected values

The expected value in the normal population is “negative”. A panel of 120 apparently healthy blood donors (64 females/56 males, ages 17 to 57 years, with an average age of 32 years) were tested on the Aptiva Celiac Disease IgG Reagent. For DGP IgG, with a cut-off of 5.00 FLU, three samples (2.5%) were positive, with a mean concentration of 0.56 FLU, and values ranging from 0.03 to 8.06 FLU. For tTG IgG, with a cut-off of 5.00 FLU, no samples were positive, with a mean concentration of 1.26 FLU, and values ranging from 0.05 to 3.76 FLU.

Comparison with predicate device

Samples for method comparison analysis included all samples (n=515) from the clinical validation study. These samples were tested on both the Aptiva Celiac Disease IgG Reagent and on their predicate QUANTA Flash DGP IgG and tTG IgG assays.

Method comparison of the Aptiva DGP IgG with the predicate device. Samples within AMR.

Method Comparison (N=218)		QUANTA Flash DGP IgG			Percent Agreement
		Negative	Positive	Total	
Aptiva DGP IgG	Negative	48	4	52	NPA: 65.8% (48/73) 95% CI: 54.3–75.6%
	Positive	25*	141	187	PPA: 97.2% (141/145) 95% CI: 93.1–98.9%
	Total	73	145	218	TPA: 86.7% (48+141/218) 95% CI: 81.5–90.6%

NPA: Negative Percent Agreement; PPA: Positive Percent Agreement; TPA: Total Percent Agreement

** 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.*

Method comparison of the Aptiva tTG IgG with the predicate device. Samples within AMR.

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

NPA: Negative Percent Agreement; PPA: Positive Percent Agreement; TPA: Total Percent Agreement

*** 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 sample); eleven (11) of the discordant samples (41%) were within +25% of the Aptiva tTg assay cut-off.*