June 16, 2021

Inova Diagnostics, Inc.
Ronda Elliott
VP, Quality Systems and Regulatory Affairs
9900 Old Grove Road
San Diego, California 92131

Re: K193604
Trade/Device Name: Aptiva Celiac Disease IgA Reagent
Regulation Number: 21 CFR 866.5750
Regulation Name: Radioallergosorbent (RAST) immunological test system
Regulatory Class: Class II
Product Code: MST, MVM, NSU
Dated: October 20, 2020
Received: October 21, 2020

Dear Ronda Elliott:

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)*
K193604

Device Name
Aptiva Celiac Disease IgA Reagent

Indications for Use *(Describe)*
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 autoantibodies in human serum. The presence of these autoantibodies, 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.

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.

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 IgA Reagent

Revision Date: June 4, 2021

Scientific contact:
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Quality Systems contact:
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Email: relliott@inovadx.com

Device name (kit):

Proprietary name:	Aptiva Celiac Disease IgA Reagent
Common name:	anti-deamidated gliadin peptide (DGP) antibody immunoassay, anti-tissue transglutaminase antibody immunoassay
Classification name:	DGP IgA: Radioallergosorbent (RAST) immunological test system tTG IgA: Multiple autoantibodies immunological test system

Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	DGP IgA: MST tTG IgA: MVM Aptiva instrument: NSU
Regulation Number	866.5750, 866.5660, Aptiva instrument: 862.2570
Device Class	2

Predicate device

QUANTA Flash® DGP IgA, 510(k) number: k113863. Date of clearance: September 20, 2012.
QUANTA Flash® h-tTG IgA, 510(k) number: k094060. Date of clearance: October 13, 2010.

Device description

The Aptiva Celiac Disease IgA 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 IgA reagent is a solid phase immunoassay utilizing fluorescent microparticles. This technology allows each of the two analytes, along with a human IgA capture antibody (IgA 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 1:46 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 IgA (known as PE Tracer IgA) 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. 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) is proportional to the amount of PE Tracer that is bound to the human IgA, which is proportional to the amount of IgA antibodies bound to the corresponding microparticle regions.

For quantitation, the DGP IgA and tTG IgA assays (together as part of the Aptiva Celiac Disease IgA 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 IgA 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 IgA Calibrators and Aptiva Celiac Disease IgA Controls are sold separately.

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

One (1) Aptiva Celiac Disease IgA Reagent Cartridge, containing the following reagents for 250 determinations:

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

Intended use(s)

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 autoantibodies in human serum. The presence of these autoantibodies, 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.

Indications for use

Same as intended use.

Substantial equivalence

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

Comparison to predicate device

Aptiva Celiac Disease IgA Reagent – DGP IgA Assay

Similarities		
Item	**Aptiva Celiac Disease IgA Reagent (DGP IgA)**	**QUANTA Flash DGP IgA**
Intended 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 autoantibodies in human serum. The presence of these autoantibodies, 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	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 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 IgA Reagent (DGP IgA)**	**QUANTA Flash DGP IgA**
Detection/Operating Principle	fluorescent immunoassay	chemiluminescent immunoassay
Conjugate	phycoerythrin conjugated polyclonal 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

Similarities		
Item	**Aptiva Celiac Disease IgA Reagent (DGP IgA)**	**QUANTA Flash DGP IgA**
Analytical Measuring Range	0.72 FLU – 250.00 FLU	5.2 CU - 2367.3 CU
Control	Controls have lot specific values assigned. Control 1 DGP IgA – 6.65 FLU (4.65 – 8.64) tTG IgA - 10.00 FLU (5.00-100.00) Control 2 DGP IgA – 13.65 FLU (9.56 – 17.75) tTG IgA – 50.00 FLU (25.00 – 250.00)	Controls have lot specific values assigned. Negative Control DGP IgA – 9.7 CU (5.8 – 13.6) h-tTG IgA – 10.2 CU (6.1 – 14.3) Positive Control DGP IgA – 50.9 CU (30.5 – 71.3) h-tTG IgA – 62.5 CU (37.5 – 87.5)
Calibration	Lot specific Master Curve + 3 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Aptiva Celiac Disease IgA Reagent – tTG IgA Assay

Similarities		
Item	**Aptiva Celiac Disease IgA Reagent (tTG IgA)**	**QUANTA Flash tTG IgA**
Intended 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 autoantibodies in human serum. The presence of these autoantibodies, 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	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 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 IgA Reagent (tTG IgA)**	**QUANTA Flash tTG IgA**
Detection/Operating Principle	fluorescent immunoassay	chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal 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	1.02 FLU – 600.00 FLU	1.9 CU - 4965.5 CU
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 IgA reagent utilizes predefined lot specific Master Curves, one for tTG IgA and one for DGP IgA 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 IgA control bead is present in the reagent as a control to flag low concentrations of IgA in the 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 IgA control microparticle for that reagent lot. These four parameters of the analyte curves, as well as the MFI cut-off for the IgA control microparticle are embedded in the reagent cartridge RFID.

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

Material	**Assigned Value (FLU)**
Celiac IgA Master Curve Standard 1	0.00
Celiac IgA Master Curve Standard 2	4.81
Celiac IgA Master Curve Standard 3	16.83
Celiac IgA Master Curve Standard 4	58.89
Celiac IgA Master Curve Standard 5	206.13
Celiac IgA Master Curve Standard 6	721.45

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

Material	Assigned Value (FLU)
Celiac IgA Master Curve Standard 1	0.00
Celiac IgA Master Curve Standard 2	4.55
Celiac IgA Master Curve Standard 3	15.93
Celiac IgA Master Curve Standard 4	55.76
Celiac IgA Master Curve Standard 5	194.90
Celiac IgA Master Curve Standard 6	685.57

IgA Control Microparticle Standard: 1 mg/dL human IgA

Precision

The precision of the Aptiva Celiac Disease IgA reagent was evaluated on 9 samples for DGP IgA and 10 samples for tTG IgA, 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%

Aptiva DGP IgA 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	2.02	0.08	3.8%	0.03	1.5%	0.08	3.9%	0.11	5.7%
2	80	3.75	0.09	2.3%	0.22	5.9%	0.20	5.2%	0.31	8.2%
3	80	4.53	0.16	3.4%	0.20	4.4%	0.20	4.4%	0.32	7.1%
4	80	5.41	0.23	4.2%	0.24	4.4%	0.20	3.7%	0.38	7.1%
5	80	6.53	0.15	2.3%	0.34	5.2%	0.36	5.5%	0.52	7.9%
6	80	12.42	0.34	2.7%	0.33	2.7%	0.77	6.2%	0.90	7.3%
7	80	34.03	0.98	2.9%	2.54	7.5%	1.77	5.2%	3.25	9.5%
8	80	153.51	3.70	2.4%	5.40	3.5%	7.75	5.0%	10.14	6.6%
9	80	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	Replicates (N)	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	80	1.94	0.07	3.8%	17.20	1.8%	0.02	1.2%	0.08	4.4%
2	80	4.12	0.24	5.8%	0.14	3.3%	0.00	0.0%	0.27	6.6%
3	80	5.55	0.27	5.0%	0.22	4.0%	0.28	5.1%	0.45	8.1%
4	80	6.74	0.33	4.9%	0.20	2.9%	0.32	4.7%	0.50	7.3%
5	80	17.72	0.83	4.7%	0.63	3.5%	0.44	2.5%	1.13	6.4%
6	80	81.80	2.51	3.1%	2.49	3.0%	2.14	2.6%	4.14	5.1%
7	80	165.45	7.34	4.4%	5.78	3.5%	8.49	5.1%	12.62	7.6%
8	80	274.22	13.55	4.9%	5.86	2.1%	7.17	2.6%	16.41	6.0%
9	80	398.64	17.28	4.3%	10.29	2.6%	11.73	2.9%	23.28	5.8%
10	80	491.04	25.97	5.3%	29.7%	4.0%	14.88	3.0%	35.71	7.3%

Reproducibility Studies

Reproducibility between sites (instruments)

Seven samples for DGP IgA and six samples for tTG IgA 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%

Results are summarized in the tables below.

Aptiva DGP IgA			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	1.75	0.08	4.3%	0.12	7.1%	0.15	8.3%	0.06	3.2%	0.16	8.9%
2	75	4.39	0.14	3.2%	0.28	6.3%	0.31	7.1%	0.09	2.1%	0.32	7.4%
3	75	5.30	0.15	2.8%	0.14	2.7%	0.21	3.9%	0.00	0.0%	0.21	3.9%
4	75	6.43	0.19	3.0%	0.66	10.2%	0.69	10.7%	0.20	3.1%	0.71	11.1%
5	75	38.10	1.05	2.7%	2.47	6.5%	2.68	7.0%	1.83	4.8%	3.25	8.5%
6	75	91.85	2.57	2.8%	8.95	9.7%	9.31	10.1%	0.00	0.0%	9.31	10.1%
7	75	167.90	3.01	1.8%	10.14	6.1%	10.58	6.3%	11.55	6.9%	15.66	9.4%

Aptiva tTG IgA			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	2.44	0.07	2.8%	0.23	9.4%	0.24	9.8%	0.05	2.0%	0.24	10.0%
2	75	4.95	0.13	2.7%	0.22	4.5%	0.26	5.3%	0.00	0.0%	0.26	5.3%
3	75	6.73	0.25	3.7%	0.53	7.9%	0.59	8.7%	0.24	3.5%	0.63	9.4%
4	75	77.16	1.58	2.0%	1.50	1.9%	2.17	2.8%	2.30	3.0%	3.17	4.1%
5	75	140.93	3.98	2.8%	2.77	2.0%	4.85	3.4%	5.53	3.9%	7.36	5.2%
6	75	219.93	6.65	3.0%	10.00	4.5%	12.01	5.5%	9.08	4.1%	15.05	6.8%

Reproducibility between lots

Six samples for DGP IgA and six samples for tTG IgA 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%

Results are summarized in the tables below.

Aptiva DGP IgA			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	2.48	0.09	3.8%	0.07	2.9%	0.12	4.7%	0.21	8.4%	0.24	9.7%
2	75	5.27	0.17	3.2%	0.19	3.6%	0.25	4.8%	0.46	8.7%	0.52	9.9%
3	75	5.45	0.16	3.0%	0.16	2.9%	0.23	4.2%	0.48	8.8%	0.53	9.8%
4	75	38.52	0.94	2.4%	0.54	1.4%	1.08	2.8%	2.34	6.1%	2.58	6.7%
5	75	100.94	2.19	2.2%	2.30	2.3%	3.17	3.1%	0.82	0.8%	3.28	3.2%
6	75	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		Reproducibility	
Sample	N	Mean (FLU)	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV	SD (FLU)	CV
1	75	2.26	0.10	4.2%	0.09	3.9%	0.13	5.8%	0.22	9.6%	0.25	11.2%
2	75	4.04	0.16	3.9%	0.11	2.7%	0.19	4.7%	0.28	6.9%	0.34	8.3%
3	75	5.20	0.14	2.8%	0.22	4.2%	0.26	5.0%	0.50	9.5%	0.56	10.8%
4	75	56.36	1.83	3.2%	1.93	3.4%	2.66	4.7%	0.20	0.3%	2.67	4.7%
5	75	164.52	5.31	3.2%	5.02	3.1%	7.30	4.4%	5.03	3.1%	8.87	5.4%
6	75	373.22	20.79	5.6%	16.24	4.3%	26.38	7.1%	36.02	9.7%	44.65	12.0%

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

The LoB, LoD, and LoQ of the DGP IgA and tTG IgA assays in the Aptiva Celiac Disease IgA 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 IgA on two reagent lots, and tTG IgA on two reagent lots; all having a p-value = <0.0001)

The DGP IgA LoB for both reagent lots were determined as 0.00 FLU. The final LoB value for DGP IgA is 0.00 FLU.

The tTG IgA LoB for both reagent lots were determined as 0.01 FLU. The final LoB value for tTG IgA is 0.01 FLU.

Study protocol for LoD:
Four low level samples for each DGP IgA and tTG IgA 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 IgA limit of detection for one reagent lot was determined as 0.65 FLU, and for the second reagent lot as 0.39 FLU. The final LoD value is 0.65 FLU.

The tTG IgA limit of detection for one reagent lot was determined as 0.15 FLU, and for the second reagent lot as 0.37 FLU. The final LoD value is 0.37 FLU.

Study protocol for LoQ:
Four low level samples for each DGP IgA and tTG IgA 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 IgA limit of quantitation for one reagent lot was determined as 0.67 FLU, and for the second reagent lot as 0.72 FLU. The final LoQ value is 0.72 FLU, which has been set as the lower limit of the analytical measuring range of the DGP IgA assay.

The tTG IgA limit of quantitation for one reagent lot was determined as 0.81 FLU, and for the second reagent lot as 1.02 FLU. The final LoQ value is 1.02 FLU, which has been set as the lower limit of the analytical measuring range of the tTG IgA assay.

Analytical Measuring Range (AMR)

Within the Aptiva Celiac Disease IgA Reagent:

DGP IgA:	0.72 FLU – 250.00 FLU
tTG IgA:	1.02 FLU – 600.00 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 IgA or a result >600.00 FLU for tTG IgA 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 or 6000.00 FLU for DGP IgA or tTG IgA, respectively.

High concentration hook effect

To assess hook effect, 4 samples for DGP IgA and 7 samples for tTG IgA were tested at three increasing 2-fold serial dilutions from the standard 1:46 dilution used by the Aptiva Celiac Disease IgA 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 1229.19 FLU for the DGP IgA assay and 1746.20 FLU for the tTG IgA assay (theoretical values calculated) in the Aptiva Celiac Disease IgA Reagent.

Linearity

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

The linearity of the AMR of DGP IgA and tTG IgA 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 each assay 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 IgA, the best fitting polynomial found for samples 1 and 4 was a linear one, while third order polynomial was found for sample 2 and second order polynomial for sample 3. All acceptance criteria were fulfilled.

Sample	Test Range (FLU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	59.39 - 296.96	0.98 (0.94 to 1.02)	10.65 (2.20 to 19.10)	0.99	105.9%
2	19.28 - 192.82	0.98 (0.96 to 1.00)	2.72 (0.44 to 5.00)	1.00	101.5%
3	3.27 - 29.02	1.00 (0.96 to 1.04)	0.61 (-0.12 to 1.34)	0.99	104.1%
4	0.48 - 4.79	0.94 (0.88 to 0.99)	0.11 (-0.05 to 0.26)	0.99	100.0%
Combined	0.48 - 296.96	1.02 (1.01 to 1.03)	0.61 (-0.69 to 1.92)	1.00	102.9%

For tTG IgA, the best fitting polynomial found for all samples 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	69.43 - 694.33	1.04 (1.00 to 1.08)	-17.18 (-34.97 to 0.60)	0.99	96.7%
2	10.28 - 102.79	1.01 (0.97 to 1.04)	1.12 (-1.06 to 3.30)	1.00	102.9%
3	1.98 - 19.80	0.94 (0.87 to 1.01)	-0.58 (-1.38 to 0.23)	0.98	87.2%
4	0.78 - 7.76	1.06 (0.98 to 1.14)	0.27 (-0.10 to 0.65)	0.98	113.2%
Combined	0.78 - 694.33	1.01 (1.00 to 1.02)	-1.13 (-3.58 to 1.32)	1.00	100.0%

These data demonstrate the linearity of the analytical measuring range (0.72 FLU – 250.00 FLU) of the DGP IgA assay and the analytical measuring range (1.02 FLU – 600.00 FLU) of the tTG IgA assay, both as part of the Aptiva Celiac Disease IgA 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 IgA assays. Recovery of the unit values was calculated compared to control samples.

Acceptance criteria for the interference studies were 85% - 115% recovery, or ± 15% of the cut-off (±0.75 FLU) difference, whichever is greater.
No interference was detected for DGP or tTG IgA with bilirubin up to 1 mg/mL (recovery: from 96.4%-101.5% and from 96.2% to 99.7% for DGP IgA and tTG IgA respectively), hemoglobin up to 2 mg/mL (recovery: from 102.7% to 108.4% and from 100.4% to 111.8% for DGP IgA and tTG IgA respectively), triglycerides up to 1000 mg/dL (recovery: from 102.6% to 107.7% and from 98.2% to 110.3% for DGP IgA and tTG IgA respectively), cholesterol up to 332.5 mg/dL (recovery: from 102.4% to 103.9% or -0.43 FLU and from 99.6% to 109.5% or -0.44 FLU for DGP IgA and tTG IgA respectively), RF IgM up to 250 IU/mL (recovery: 93.1% to 99.2% or 0.30 FLU and 98.8% to 102.6% or 0.35 FLU for DGP IgA and tTG IgA, respectively) and human IgG up to 70 mg/mL (recovery: from 99.8% to 102.3% or -0.36 FLU and from 99.5 to 102.4% or 0.32 FLU for DGP IgA and tTG IgA, respectively).

Sample Stability and Handling

For the DGP IgA assay, eight test samples were tested and for tTG IgA assay six 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 5 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 IgA microparticle – 3 lots
- PE Tracer IgA – 3 lots
- Rehydration Buffer – 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 IgA Reagent (DGP IgA, tTG IgA, IgA 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 IgA reagent cartridges, one lot of reagent cartridge was tested using up to 14 human serum samples (with different reactivity levels). The specimens were tested periodically for 45 days. At day 21 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 IgA is as follows:
Lot 100014: 45 days

Using these criteria, the in-use (onboard) stability of the Aptiva Celiac Disease IgA reagent cartridge was set at 42 days.

Real time stability

Real time stability testing has been scheduled to be performed every three or six months on the Aptiva Celiac Disease IgA Reagents kit, to verify the two-year expiration that was assigned based on accelerated stability studies. Results for the first time point at 6 months will be available in January 2020.

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.

Cut-off, reference range

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

Negative	<5.00 FLU
Positive	≥5.00 FLU

The reference population for establishing the reference interval for the DGP IgA and tTG assays, within the Aptiva Celiac Disease IgA Reagent, consisted of 200 subjects:

Sample Group	N
Crohn's Disease	15
Autoimmune Thyroid Disease	30
Infectious Disease	30
Primary Biliary Cholangitis	30
Rheumatoid Arthritis	40
Systemic Lupus Erythematosus	20
Systemic Sclerosis	20
Ulcerative Colitis	15

Additionally, 12 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 95th percentile of the results obtained on the reference subjects, along with the results of 12 samples from patients with celiac disease.

A cutoff of 5.00 FLU (78 MFI and 190 MFI for DGP IgA and tTG IgA, 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 IgA Reagent. A total of 495 characterized samples were included in this Validation Set, including 171 samples from celiac disease patients, 34 dermatitis herpetiformis and 290 control samples from patients with various types of autoimmune and infectious diseases. All samples were run on the Aptiva Celiac Disease IgA Reagent. The distribution of the cohort and the DGP and tTG positivity rate is in the Table below:

Patient Group	**N**	**DGP IgA N Positive**	**DGP IgA % Positive**	**tTG IgA N Positive**	**tTG IgA % Positive**
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%**
Celiac Disease	171	101	59.1%	159	93.0%
Dermatitis Herpetiformis	34	22	64.7%	31	91.2%
Total	**495**	**-**	**-**	**-**	**-**

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

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

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

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

In addition to the clinical validation cohort, thirty-four samples from dermatitis herpetiformis patients were tested for the Aptiva Celiac Disease IgA assay. The sensitivity of DGP IgA in dermatitis herpetiformis patients is 64.7% (47.9 – 78.5%) and the specificity of DGP IgA is 99.3% (97.5-99.8%). The sensitivity of tTG IgA in dermatitis herpetiformis patients is 91.2% (77.0 – 97.0%) and the specificity of tTG IgA is 99.3% (97.5-99.8%).

Expected values

The expected value in the normal population is "negative". 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.

Comparison with predicate device

Samples for method comparison analysis included all samples (n=495) from the clinical validation study. These samples were tested on both the Aptiva Celiac Disease IgA Reagent and on their predicate QUANTA Flash DGP IgA and tTG assays.

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

Method Comparison (N=200)		QUANTA Flash DGP IgA			Percent Agreement
		Negative	Positive	Total	(95% Confidence)
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

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

Method Comparison (N=197)		QUANTA Flash tTG IgA			Percent Agreement
		Negative	Positive	Total	(95% Confidence)
Aptiva tTG IgA	Negative	31	1	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