

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k072632

B. Purpose for Submission:

New device

C. Measurand:

IgG Anti-human tissue transglutaminase (htTG) antibody

IgG Anti-deaminated gliadin peptide (DGP) antibody

D. Type of Test:

Semi-quantitative ELISA

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Plex™ Celiac IgG Profile

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.5660 Multiple autoantibodies immunological test system

21 § CFR 866.5750 Radioallergosorbent (RAST) immunological test system

2. Classification:

Class II

3. Product codes:

MVM, Autoantibodies, Endomysial (Tissue Transglutaminase)

MST, Antibodies, Gliadin

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

The QUANTA Plex™ Celiac IgG Profile is a fluorescent immunoassay for the semi-quantitative detection of IgG anti-human tissue transglutaminase (htTG) and anti-deaminated gliadin peptide (DGP) antibodies in human serum. The presence of these antibodies in conjunction with other laboratory and clinical findings is an aid in the diagnosis of the gluten sensitive enteropathy celiac disease. This test is intended to provide added sensitivity when testing IgA deficient patients.

2. Indication(s) for use:

Same as Intended use.

3. Special conditions for use statement(s):

For prescription only.

4. Special instrument requirements:

Luminex™ laser flow analyzer (Luminex 100 and 200)

Luminex™ Integrated System (IS) software program

I. Device Description:

Each device contains the following: polystyrene microwell plate, 12 (1x8) microwell strips with holder, each microwell contains 3 different colored beadsets and each

beadset is coated with either purified htTG, DGP, or anti-IgG; positive and negative controls, Celiac IgG calibrators (human serum IgG antibodies to htTG and DGP antigens); HRP sample diluent; fluorescent-labeled IgG conjugate (goat anti-human IgG f_c specific) and conjugate diluent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
QUANTA Lite™ h-tTG IgG
QUANTA Lite™ Gliadin IgG II
2. Predicate K number(s):
k011570 (h-tTG IgG)
k052142 (Gliadin IgG II)
3. Comparison with predicate:

Similarities			
Item	New Device	Predicate Device	
	QUANTA Plex™ Celiac IgG Profile	QUANTA Lite™ h-tTG IgG	QUANTA Lite™ Gliadin IgG II
Antigen	Recombinant htTG and purified synthetic deaminated gliadin peptide	Native h-tTG	Purified gliadin peptides
Measurement	Semi-quantitative	Same	Same
Sample	Serum	Same	Same
Positive and Negative Control	Pre-diluted human serum. Ready to use.	Same	Same
Sample volume required	5 µL	Same	Same
Diluent	HRP sample diluent	Same	Same

Differences			
Item	Device	Predicate	
	QUANTA Plex™ Celiac IgG Profile	QUANTA Lite™ h-tTG IgG	QUANTA Lite™ Gliadin IgG II
Intended use/ Indications for Use	For the semi-quantitative detection of IgG antibodies to anti-htTG and synthetic DGP in human serum as an aid in diagnosis of celiac disease (CD) and IgA deficient CD.	For the semi-quantitative detection of IgG antibodies to tissue transglutaminase (endomysium) in human serum as an aid in diagnosis of CD, dermatitis herpetiformis and IgA deficient CD.	For the semi-quantitative detection of gliadin IgG antibodies in human serum as an aid in diagnosis of CD.

Differences			
Item	Device	Predicate	
Technology	Flow cytometer based	ELISA	ELISA
Assay Format	Multiplexed	Individual analytes	Individual analytes
Assay Platform	96 microtiter wells with four differently color-coded sets of antigen coated microspheres	96 well microtiter plates coated with specific antigen	96 well microtiter plates coated with specific antigen
Conjugate	Fluorescent Goat anti-human IgG (alpha chain specific)	Horseradish Peroxidase, Goat anti-human IgG	Horseradish Peroxidase, Goat anti-human IgG
Assay washing step	None	Two steps	Two steps
Stop solution	None	0.344M Sulfuric Acid	0.344M Sulfuric Acid
Reading	Luminometer	Spectrophotometer	Spectrophotometer
Detection Method	Fluorescence	Colorimetric	Colorimetric
Units of measure	LU (Luminex)	Units (ELISA)	Units (ELISA)
Cut-off	20.0 LU	20.0 units	20.0 units
Dynamic range	20 to >20,000 FU	200 to 300	200 to 300
Result Interpretation	Neg: <20 LU Wk Pos: 20-30 LU Pos: >30 LU	Neg: <20 Units Wk Pos: 20-30 Units Mod. to Strong Pos: >30 Units	Neg: <20 Units Wk Pos: 20-30 Units Mod. to Strong Pos: >30 Units

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

CLSI (NCCLS) H18-A3 Sample storage conditions

CLSI (NCCLS) C24-A3 Appropriate Quality Control Practices

L. Test Principle:

Recombinant htTG and synthetic DGP, anti-IgG are each bound to different fluorescently 'colored' beads. These beadsets are mixed together and put into wells of a microwell plate under conditions that will preserve the antigens in their reactive state. Pre-diluted controls and diluted patient sera are added to separate microwells. If specific antibodies are present, they will bind to the antigen specific beads and free IgG will bind to the anti-IgG beads. Then an anti-human IgG fluorescent conjugate is added to each microwell. A second incubation allows the anti-human IgG fluorescent conjugate to bind to any patient antibodies that are bound to the antigen coated beads or the anti-IgG bead. The samples are then analyzed by the Luminex™ 100 or 200 flow analyzer. The flow analyzer can distinguish each beadset based on the

fluorescent spectrum as well as measure the fluorescent intensity of the conjugate on each bead. The fluorescent intensity on the bead is proportional to the amount of bound conjugate, which in turn is proportional to the amount of patient antibodies bound to the antigen coated beads or the anti-IgG bead. Each antibody can be semi-quantitated by comparing the fluorescent intensity of the patient sample with that of the corresponding Calibrator. The IgG control bead is also be used to ensure that false negative results due to operational errors are detected as described in Quality Control.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The intra-assay precision was determined by testing eighteen serum samples ten times on a single assay on two different lot numbers of microsphere beads. The inter-assay precision was determined by testing twenty two serum samples six times for six days. Results are summarized below.

Intra-assay:

	Anti-htTG IgG	Anti-DGP IgG
LU average ranges	26 - 153 LU	21 – 119 LU
CV% ranges	5 - 8%	4 – 6%

Inter-assay:

	Anti-htTG IgG	Anti-DGP IgG
LU average ranges	30 – 169 LU	21 – 130 LU
CV% ranges	7 – 17%	7 – 14%

b. *Linearity/assay reportable range:*

Not applicable.

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

There are no reference standards for htTG and DGP. The positive and negative controls are prepared in-house and arbitrary units are assigned during the development process.

Stability: The expiration date claims are one year for the QUANTA Plex™ Celiac IgG Profile kit and three months for the reconstituted IgG conjugate.

d. *Detection limit:*

The detection limit was determined by testing serially diluted low positive patient serum. Values did not decrease below 4 LU on further dilutions. The anti-htTG or anti-DGP antibodies were detectable at 4 LU.

e. *Analytical specificity:*

Interference by endogenous substances: A total of five serum samples were tested with the following interferents: 1000 mg/dL hemoglobin, 29.7 mg/dL bilirubin, 369 mg/dL cholesterol, 1016 mg/dL triglycerides and 17 µg/mL IgG on equal volumes of positive anti-htTG and anti-DGP. No or negligible interference was observed. The package insert states that grossly hemolyzed, lipemic, microbially contaminated, heat-treated samples or specimens

containing visible particulate should not be used in this assay.

Crossreactivity: The QUANTA Plex™ Celiac IgG Profile was tested for crossreactivity with other autoantibodies with 191 sera consisting of 10 with ulcerative colitis, 5 cirrhotic liver disease, 5 chronic hepatitis; 33 primary biliary cirrhosis or autoimmune hepatitis, 11 positive antinuclear antibody, 10 autoimmune thyroid disease, 4 systemic lupus erythematosus; 22 rheumatoid arthritis, 10 positive anti-CCP, 42 with high titer antibodies to various infectious diseases and 39 with known amounts of serum IgA. Positive reactions were observed on 2 samples for anti-htTG and 3 samples for anti-DGP IgG.

f. Assay cut-off:

The cut-off value of 20 LU for the assay was established from 278 asymptomatic blood donors. Age and gender were available for 39 samples and unavailable for the remaining 239 samples. The assay specificity was 100% (278/278) for anti-htTG IgG and 99.6% (277/278) for anti-DGP IgG. The cut-off value of arbitrary LU was chosen for the continuity of INOVA products. The value of the fluorescence that was assigned 20 LU was based on a non-parametric statistical analysis of the data.

2. Comparison studies:

a. Method comparison with predicate device:

Testing was performed on 954 samples (278 asymptomatic blood donors, 29 Celiac Disease (CD) patients, and 647 samples with other disease states or conditions. The 647 samples includes: 53 GI and liver diseases; 63 rheumatic and infectious diseases; 93 samples with autoimmune thyroid diseases, SLE, RA, 1st degree CD relative, defined IgA; 44 samples with CD related or suspected diseases and may or may not have antibodies to CD. The comparative study on anti-htTG IgG had 82.4% Positive Percent Agreement (PPA) (28/34); 96.8% Negative Percent Agreement (NPA) (891/920) and 96.3% Overall Agreement (919/954) (refer to table below).

Anti-htTG IgG		QUANTA Lite™ htTG IgG (Elisa)		
		Positive	Negative	Total
QUANTA Plex™ Celiac IgG Profile (Luminex)	Positive	28	29*	57
	Negative	6**	891	897
	Total	34	920	954

*24 of these samples were positive for at least 2 out of the 3 other serological markers (IgA anti-htTG, IgA anti-DGP, or IgG anti-DGP. Two samples were at 50 & 96 EU. One sample was an IgA deficient CD patient. The remaining two samples were not positive for any other CD serological marker tested and have no diagnosis.

**Three of these samples were known false positive on htTG IgG ELISA. Two samples were negative for a majority of other serological markers (IgA anti-htTG, IgA anti-DGP, and IgG anti-DGP) and were not IgA deficient. The 6th sample was a CD patient who was positive for both IgA anti-htTG and anti-DGP.

The comparative study on anti-DGP IgG had 86.9% PPA (133/153); 99.5% NPA (797/801) and 97.5% Overall Agreement (930/954) (refer to table below).

Anti-DGP IgG		QUANTA Lite™ Gliadin IgG II (Elisa)		
		Positive	Negative	Total
QUANTA Plex™ Celiac IgG Profile (Luminex)	Positive	133	4*	137
	Negative	20**	797	817
	Total	153	801	954

*Three of these samples were not positive for any other CD serological marker tested, and have no diagnosis. One sample was an initially diluted QC ELISA patient panel sample that is positive for both IgA anti-h-tTG and anti-DGP.

**Eight of these samples were negative for a majority of all other CD serological markers tested. Of those 8, two were average blood donors, 5 have no diagnosis, and 1 was a CD patient on a gluten free diet. Of the remaining 12 samples, most were less than 25 EU on the DGP IgG ELISA, with the highest being 36 EU.

b. Matrix comparison:

Both assays use serum as the matrix.

3. Clinical studies:

a. Clinical Sensitivity and specificity:

To determine the clinical sensitivity, 5 samples from IgA deficient celiac disease (CD) patients, the primary patient group this test is meant to help diagnose, were tested on both the QUANTA Plex™ Celiac IgG Profile and the corresponding ELISAs. The sensitivity for IgG anti-DGP and anti-h-tTG on IgA deficient CD patients were 100% and 80%, respectively. A further 29 IgA sufficient CD patients who were not on a gluten free diet (GFD) and 32 CD patients on a gluten free diet were also tested. The sensitivity for IgG anti-DGP on non-GFD IgA sufficient CD patients and GFD CD patients were 59% and 12% respectively. The sensitivity for IgG anti-h-tTG non-GFD IgA sufficient CD patients and GFD CD patients were 14% and 3% respectively. The new device % sensitivity was similar to the % sensitivity of the predicate ELISA devices.

To determine the clinical specificity, 487 patients who were not diagnosed with celiac disease were tested. This group included normal blood donors, relatives of celiac disease patients, patients with rheumatic, liver, gastrointestinal and infectious diseases, and patients with defined amounts of total IgA. The specificity for IgG anti-DGP and anti-h-tTG were 99.0% and 99.6% respectively (refer to table below).

Clinical Sensitivity and Specificity of QUANTA Plex™ Celiac IgG Profile

		Gliadin II IgG ELISA (predicate)	DGP IgG Luminex (new device)	h-tTG IgG ELISA (predicate)	h-tTG IgG Luminex (new device)
Patient Groups	# subjects	# Positive (%)	# Positive (%)	# Positive (%)	# Positive (%)
Celiac – IgA Deficient	5	5 (100%)	5 (100%)	4 (80%)	4 (80%)
Celiac – No GFD	29	19 (66%)	17 (59%)	2 (7%)	4 (14%)
Celiac – GFD	32	6 (19%)	4 (12%)	0 (0%)	1 (3%)
Other Diseases*	191	4 (2.1%)	3 (1.6%)	1 (0.5%)	2 (1.0%)
1 st Degree Relatives	18	1 (5.6%)	1 (5.6%)	0 (0%)	0 (0%)
Normal Blood Donors	278	3 (1.1%)	1 (0.4%)	0 (0%)	0 (0%)

*Includes patients with defined IgA levels and rheumatic, liver, gastrointestinal and infectious diseases.

b. Other clinical supportive data (when a. is not applicable):
Not applicable.

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

Expected values in the normal population should be negative.

N. Proposed Labeling:

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

O. Conclusion:

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