

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

A. 510(k) Number:

k063818

B. Purpose for Submission:

New Device

C. Measurand:

IgA Anti-human tissue transglutaminase (htTG) antibody

IgA 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 IgA 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:

II

3. Product code:

MVM, Autoantibodies, Endomysial (Tissue Transglutaminase)

MST, Antibodies, Gliadin

Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

The QUANTA Plex™ Celiac IgA Profile is a fluorescent immunoassay for the semi-quantitative detection of IgA anti-human tissue transglutaminase (htTG) and anti-deaminated gliadin peptide (DGP) antibodies, and the detection of an insufficient amount of human serum IgA. 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. Insufficient IgA indicates that there is not enough IgA to allow detection of IgA anti-htTG or anti-DGP.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use 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 4 different colored beadsets and each beadset is coated either purified htTG, DGP, anti-IgA, or IgA; positive and negative controls, Celiac calibrators (human serum IgA antibodies to htTG and DGP antigens); HRP sample diluent; fluorescent-labeled IgA conjugate (goat anti-human IgA alpha chain specific) and conjugate diluent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
QUANTA Lite™ htTG IgA
QUANTA Lite™ Gliadin IgA II
2. Predicate 510(k) number(s):
k011566 (IgA)
k052143 (IgA)
3. Comparison with predicate:

Similarities			
Item	New Device	Predicate Device	
	QUANTA Plex™ Celiac IgA Profile	QUANTA Lite™ htTG IgA	QUANTA Lite™ Gliadin IgA II
Antigen	Recombinant htTG and purified synthetic deaminated gliadin peptide	Recombinant htTG	Purified synthetic deaminated gliadin peptide
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 IgA Profile	QUANTA Lite™ htTG IgA	QUANTA Lite™ Gliadin IgA II
Intended use	For the semi-quantitative detection of IgA antibodies to recombinant human htTG and synthetic DGP in human serum	For the semi-quantitative detection of IgA antibodies to recombinant human htTG in human serum	for the semi-quantitative detection of IgA antibodies to synthetic DGP in human serum
Indications for Use	Aid in the diagnosis of both IgA	Aid in the diagnosis of celiac	Aid in the diagnosis of celiac

Differences			
Item	Device	Predicate	
	sufficient and IgA deficient celiac disease	disease and dermatitis herpetiformis	disease
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 IgA (alpha chain specific)	Horseradish Peroxidase, Goat anti-human IgA	Horseradish Peroxidase, Goat anti-human IgA
Assay washing step	None	Two steps	Two steps
Reading	Luminometer	Spectrophotometer	Spectrophotometer
Detection Method	Fluorescence	Colorimetric	Colorimetric
Cut-off	20.0 LU	20.0 units	20.0 units
Dynamic range	20 to >20,000 FU	200 to 300	200 to 300

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

None referenced.

L. Test Principle:

Recombinant hTg and synthetic DGP, anti-IgA and IgA are coupled to different fluorescently 'colored' beadsets. 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, a serum free control and diluted patient sera are added to separate microwells. If specific antibodies are present, they will bind to the antigen specific beads and free IgA will bind to the anti-IgA beads. Then an anti-human IgA fluorescent conjugate is added to each microwell. A second incubation allows the anti-human IgA fluorescent conjugate to bind to any patient antibodies that are bound to the antigen coated beads or the anti-IgA bead, and the IgA bead. The samples are then analyzed by the Luminex™ 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 IgA bound to the anti-IgA bead. Each antibody can be semi-quantitated by comparing the fluorescent intensity of the patient sample with that of the corresponding Calibrator. Comparing the fluorescent intensities of the anti-IgA bead and the IgA bead can

identify the selective IgA-deficient serum as described in the Calculation of Results. These two control beads can also be used to ensure that false negative results due to operator error such as no patient sample added to the well are detected.

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

Intra-assay				
	Anti-htTG IgA	Anti-DGP IgA	Anti-IgA	IgA
Mean LU	3.0 – 332.0	3.0 – 69.0	4.7 – 20.6	9.5 – 27.9
SD	0.8 – 13.8	1.2 – 7.0	0.2 – 1.4	0.3 – 1.2
CV%	2.2 – 9.3	3.2 – 12.0	2.1 – 7.4	1.1 – 5.1
Inter-assay				
Mean LU	2.0 – 484.0	3.0 – 99.0	0.9 – 22.1	9.9 – 29.1
SD	0.4 – 17.2	0.8 – 10.2	0.2 – 1.3	0.2 – 2.5
CV%	0.5 – 11.2	2.2 – 17.4	1.0 – 21.7	1.3 – 9.8

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 IgA Profile kit and three months for the reconstituted IgA 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 six serum samples were tested. One sample was used for each of the following interferents: 1000 mg/dL hemoglobin, 29.7 mg/dL bilirubin, 369 mg/dL cholesterol, 1016 mg/dL triglycerides and 5 µg/mL IgA 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 with other autoantibodies: The QUANTA Plex™ Celiac IgA

Profile was tested with 63 sera consisting of 10 RF and/or anti-CCP, 11 ANA IFA positive and 42 infectious disease samples positive for HCV, HSV, CMV, Toxoplasmosis, rubella or parvovirus. All samples were negative with the QUANTA Plex™ Celiac IgA Profile.

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 38 samples and unavailable for the remaining 240 samples. The assay specificity was 99.6% (277/278). 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:

Testing was performed on 954 samples (278 asymptomatic blood donors, 29 Celiac Disease (CD) IgA sufficient patients, and 647 samples with other disease states or conditions. The 647 samples includes: 53 GI and liver diseases; 63 infectious and rheumatic diseases; 10 autoimmune thyroid diseases; 4 SLE; 22 RA; 18 1st degree CD relative; 39 defined IgA samples; 44 CD [32 GFD, 7 suspected and 5 selected IgA deficient]; and 394 samples with no CD clinical symptoms but may or may not have antibodies to CD. The comparative study on anti-htTG IgA had a Positive Percent Agreement of 89.2% (174/195); Negative Percent Agreement 99.7% (757/759) and Overall Agreement 97.6% (931/954) (refer to table below).

Anti-htTG IgA		QUANTA Lite™ htTG IgA (Elisa)		
		Positive	Negative	Total
QUANTA Plex™ Celiac IgA Profile (Luminex)	Positive	174	2	176
	Negative	21	757	778
	Total	195	759	954

The comparative study on anti-DGP IgA had a Positive Percent Agreement of 81.4% (136/167); Negative Percent Agreement of 99.5% (783/787) and Overall Agreement of 96.3% (919/954) (refer to table below).

Anti-DGP IgA		QUANTA Lite™ Gliadin IgA II (Elisa)		
		Positive	Negative	Total
QUANTA Plex™ Celiac IgA Profile (Luminex)	Positive	136	4	140
	Negative	31	783	814
	Total	167	787	954

Quanta Plex Celiac IgA on Luminex 200 and IS.v.2.3 software

For this study, 96 samples were tested and results were compared to the Luminex 100. Results were as follows:

Anti-htTG IgA		Luminex 100		
		positive	negative	Total
Luminex 200	positive	40	0	40
	negative	0	56	56
	Total	40	56	96

Positive percent agreement 100% (40/40)

Negative percent agreement 100% (56/56)

Overall percent Agreement 100% (96/96)

Anti-DGP IgA		Luminex 100		
		positive	negative	Total
Luminex 200	positive	23	0	23
	negative	1	72	73
	Total	24	72	96

Positive percent agreement 96% (23/24)

Negative percent agreement 100% (72/72)

Overall percent Agreement 99% (95/96)

b. Matrix comparison:

Both assays use serum as the matrix.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

The study consisted of the following: 29 samples from active CD IgA sufficient patients and 494 samples not diagnosed with CD. The 494 samples include the following: 278 asymptomatic blood donors; 53 GI/liver disease; 42 infectious diseases (positive for HCV, HSV, CMV, Toxoplasmosis, rubella, or parvovirus); 11 ANA IFA Positive; 10 RF and/or anti-CCP Positive; 93 autoimmune diseases (10 thyroid disease, 4 SLE, 22 RA, 18 1st degree CD relative, 39 defined IgA sufficient CD patients); and 7 suspected CD. The clinical study on anti-htTG IgA were as follows: clinical sensitivity was 93.1% (27/29); clinical specificity was 97.8% (483/494) and overall agreement was 97.5% (510/523) (refer to table below).

Anti-htTG IgA		Diagnosis		
		Positive (CD with sufficient IgA and not on GFD)	Negative (Not diagnosed with CD)	Total
QUANTA Plex™ Celiac IgA Profile (Luminex)	Positive	27	11**	38
	Negative	2*	483	485
	Total	29	494	523

* Both samples were Elisa anti-htTG IgA negative.

** Ten of these samples were Elisa htTG IgA positive

The clinical study on anti-DGP IgA were as follows: clinical sensitivity was 65.5% (18/29); clinical specificity was 97.6% (482/494) and overall agreement was 95.6% (500/523) (refer to table below).

Anti-DGP IgA		Diagnosis		
		Positive	Negative	Total
QUANTA Plex™ Celiac IgA Profile (Luminex)	Positive	18	12**	31
	Negative	11*	482	492
	Total	29	494	523

*Ten of these samples were Elisa anti-DGP IgA negative.

**Ten of these samples were Elisa anti-DGP IgA positive.

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.